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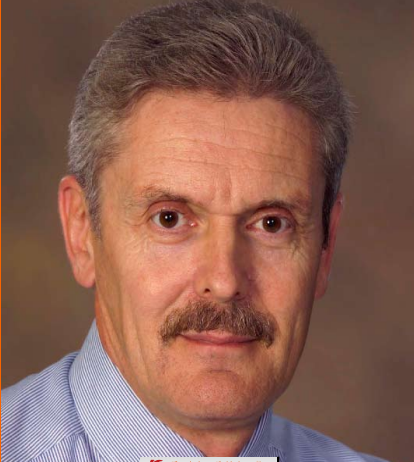


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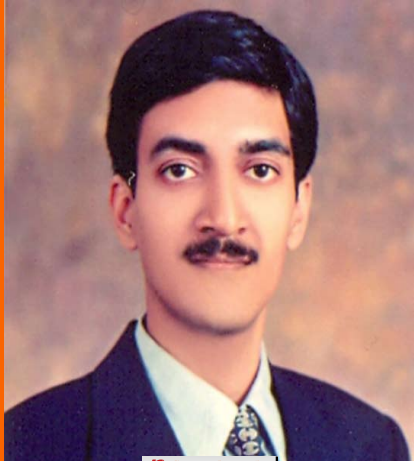
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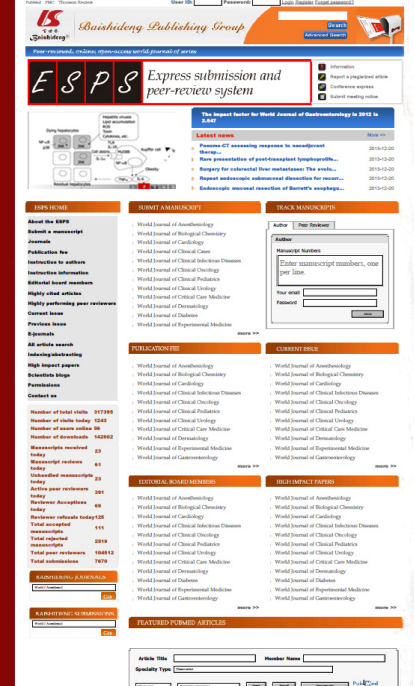


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Continuous quality improvement of colorectal cancer screening

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Abstract

Quality assurance is a key issue in colorectal cancer screening, because effective screening is able to improve primary prevention of the cancer. The quality measure may be described in terms: how well the screening test tells who truly has a disease (sensitivity) and who truly does not have a disease (specificity). This paper raises concerns about identification of the optimal screening test for colorectal cancer. Colonoscopy vs flexible sigmoidoscopy in colorectal cancer screening has been a source of ongoing debate. A multicentre randomised controlled trial comparing flexible sigmoidoscopy with usual care showed that flexible sigmoidoscopy screening is able to diminish the incidence of distal and proximal colorectal cancer, and also mortality related to the distal colorectal cancer. However, colonoscopy provides a more complete examination and remains the more sensitive exam than flexible sigmoidoscopy. Moreover, colonoscopy with polypectomy significantly reduces colorectal cancer incidence and colorectal cancer-related mortality in the general population. The article considers the relative merits of both methods and stresses an ethical aspect of patient's involvement in decision-making. Patients should be informed not only about tests tolerability

and risk of endoscopy complications, but also that different screening tests for bowel cancer have different strength to exclude colonic cancer and polyps. The authorities calculate effectiveness and costs of the screening tests, but patients may not be interested in statistics regarding flexible sigmoidoscopy screening and from an ethical point of view, they have the right to chose colonoscopy, which is able to exclude a cancer and precancerous lesions in the whole large bowel.

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Key words: Colorectal cancer; Cancer screening; Sigmoidoscopy; Colonoscopy; Standard of care; Ethical aspects; Clinical competence

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COMMENTARY ON HOT TOPICS

Colorectal cancer (CC) is a common cause of morbidity and mortality in which early detection is vital. From the United States comes a multicentre randomized study of colorectal screening with flexible sigmoidoscopy (FS)^[1]. The results of this study raise a number of important questions regarding the assessment of quality in screening tests and ethical issues.

A total of 77 445 participants of Schoen *et al*^[1] were randomly assigned to be screened for CC, and 77 455 to usual care (from 1993 to 2001). Participants in the intervention group were offered FS at baseline and at 3 or at 5 years. They were referred to their primary care physicians for decisions regarding diagnostic follow-up. A screening-detected cancer was defined as a CC diagnosed within 1 year after a positive FS and was considered to be posi-

tive, if a polyp or mass was detected. Cancers located in the rectum through the splenic flexure were defined as distal, and those in the transverse colon through the caecum were defined as proximal. Death from CC was the primary end point. Secondary end points included CC incidence, cancer stage, survival, harms of screening, and all-cause mortality. Participants in the control group only received endoscopy (FS or colonoscopy), if they asked for it, or if their physician recommended it.

A total of 86.6% of participants (67 071) underwent at least one FS screening, and 50.9% (39 440) underwent two screenings; at least one screening was positive for a polyp or mass in 28.5% of participants (22 083)^[1].

The study showed a reduction in the incidence of distal CC in the intervention group for each cancer stage, ranging from 19.8% for stage I cancers (50 fewer cases diagnosed) to 61.7% for stage IV cancers (66 fewer cases diagnosed). Mortality related to distal CC was also reduced for each stage, by 21.4% for stage I cancers (3 fewer deaths) to 60.7% for stage IV cancers (51 fewer deaths)^[1]. The number needed to screen with FS to prevent 1 death from CC was 871 and to invite to FS screening to prevent 1 CC was 282^[1].

Also the incidence of proximal CC was reduced by 14.4% to 20.7% in the intervention group for stages I, II, and III cancers (22, 34, and 25 fewer cases, respectively), but by only 2.0% (2 fewer cases) for stage IV disease^[1].

The study described by Schoen *et al*^[1] showed a reduction in the incidence of proximal CC, but FS was not success in identifying and removing all precursor lesions destined to develop into cancer in the whole colon and the authors did not show a reduction in mortality related to proximal CC^[1]. Although the study revealed that FS as compared with usual care may result in overall CC mortality, but much of the benefit in reducing CC in mortality from screening derived from its reduction in stage IV the disease, which has a much higher mortality than lower stages^[1].

Using colonoscopy as the screening method, Schoen *et al*^[1] calculated that they could increase the number of screening-detected cancers by approximately 16 percentage points (from < 25% to approximately 40% of CC diagnosed in participants assigned to FS). There is also evidence that colonoscopy with adenomas removal reduces incidence of CC^[2]. Moreover, it has an impact on the reduction of mortality from CC^[3-6], and in the first 10 years after polypectomy, reduces the risk to a level similar to that in a control group of patients with no adenomas^[3].

In the Schoen *et al*^[1] study, 28.5% of participants (22 083) underwent at least one positive endoscopy screening test for a polyp or mass. However, the authors did not mention, whether the second FS revealed only polyps? If this was the case and the second FS revealed a CC or large polyps then it is possible that the endoscopists' skills or bowel preparation may have an impact on the study results.

A high-quality examination ensures the detection of "all" neoplastic lesions - it may be related to an endosco-

pist's speciality^[5-7]. Patients who underwent colonoscopy performed by a gastroenterologist had the greatest reduction in risk for CC mortality^[5,6]. Also a reduction in death from proximal CC may be probably related to colonoscopy performed by a gastroenterologist^[5,6].

It could be argued that colonoscopy screening is more expensive than FS, but 50.9% participants (39 440) of the Schoen *et al*^[1] study, underwent two screenings FS (in 3-5 years). Moreover, there are no studies directly assessing the optimal interval for FS screening^[8], but there is a strongly and significantly lower risk of CC within 10 year after negative colonoscopy^[9]. Although, the ratio of the cost of FS screening to colonoscopy screening is unknown^[10], but diagnostic colonoscopy and diagnostic FS may cost £555 and £441 respectively (figures derive from the Trust's Service Line Reporting information April-September 2012 in The Pennine Acute Hospitals NHS Trust, United Kingdom). I think therefore a model-based economic analysis may easily find colonoscopy screening as less costly than FS screening.

Although colonoscopy has a slightly higher incidence of perforation than FS^[11], but the most common site of perforation during colonoscopy used to be the left colon^[11,12]. Schoen *et al*^[1] reported 0.0028% perforation for screening with FS (2.8 per 100 000 examinations), and nearly 40 times more perforations on repeat screening 0.1075% (107.5 per 100 000 examinations). The incidence of colonoscopic perforation could be very low 0.004% in diagnostic colonoscopy and could be as high as 0.02% in therapeutic colonoscopies, with individual series rates ranging from 0% to 0.86%^[13]. The national colonoscopy audit performed in the United Kingdom, reported rate 0.04% perforations (1:2511 procedures)^[14]. Nonetheless, the audited adult patients who underwent diagnostic or therapeutic colonoscopy could have an even higher risk of complications than screening individuals, because they were symptomatic patients (two perforations occurred in patients with inflammatory bowel disease)^[14].

Colorectal cancer is the third most common in incidence and the fourth most common cause of cancer death worldwide^[15]. An effective screening programme plays a key role to cope with the growing problem of CC. So far, the United Kingdom study has been the only study to show a significant 31% reduction in CC mortality from one-time screening with FS^[16]. It also found a significant reduction in the CC incidence (by 23%)^[16]. Another study performed in Italy showed an 18% reduction in incidence of CC, but FS in this study did not cause significant reduction in mortality^[17]. In Schoen's study comparing FS with usual care, after an average of nearly 12 years, participants in the screening group had a 21% reduction in the incidence of CC and a 26% lower rate of CC mortality than participants in the usual care group. Also a reduction of mortality by 50% and incidence by 29% related to distal CC was noticed.

Despite this great result, the doctors and health authorities are in an ethical dilemma over the optimal screening for CC. Colonoscopy provides a more complete examina-

tion than FS and a patient may not be interested in statistics regarding FS, and ask, if it is better for him to have FS or a complete colonoscopy.

When the patients will be totally informed about the limitations and benefits of FS and colonoscopy, they may be interested to make a decision themselves and choose a more sensitive endoscopy test which is able to exclude a cancer and precancerous lesions in the whole large bowel. Very experienced doctors do not need much more time to complete colonoscopy in most cases, when the top of the endoscope is in the area of splenic flexure. Furthermore, colonoscopy without sedation is common in many European countries and Asia^[18,19]. Therefore the cost of colonoscopy and FS may not differ widely, if endoscopists offer really good skills. In the future, every individual may be involved in the decision-making, and the doctors should be interested in the patient's preference regarding the screening test, because patients have the right to make their own choice^[20].

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Inhibition of apoptosis in the management of nonalcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in the developed world. The pathogenesis of NAFLD is multifactorial, involving obesity, insulin resistance, inflammation and oxidative stress. Accordingly, several treatments targeting these pathways have been evaluated in patients with NAFLD but have either shown limited efficacy or an unfavorable safety profile. On the other hand, increased hepatocyte apoptosis also appears to be implicated in the development and progression of NAFLD and recent pilot studies suggest that inhibition of apoptosis might represent a useful approach in this disease. However, several issues pertaining both to the efficacy and safety of this new class of agents remain unresolved and larger studies are required to clarify the role of this therapeutic modality in the management of NAFLD.

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Key words: Apoptosis; Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Fatty liver; Carcinogenesis; Cirrhosis; Caspase

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COMMENTARY ON HOT TOPICS

Nonalcoholic fatty liver disease (NAFLD) is a burgeoning health problem and is recognized as the main cause of chronic liver disease in the developed world^[1,2]. It affects approximately 34%-46% of the general adult population in Western countries^[3,4]. Moreover, the prevalence of NAFLD is substantially higher in obese and diabetic patients, reaching 70%^[5-7]. Given the worldwide growing epidemics of obesity and type 2 diabetes mellitus, the prevalence of NAFLD is expected to rise further in the following years^[1,5]. NAFLD covers a wide spectrum of histological abnormalities ranging from steatosis to the coexistence of steatosis with inflammation and a variable degree of fibrosis [nonalcoholic steatohepatitis (NASH)], to cirrhosis and even to hepatocellular carcinoma^[1,8]. Patients with NAFLD, particularly those with NASH, have increased all-cause mortality compared with the general population, with cardiovascular disease and liver-related disease being the leading causes of death^[9-11].

In light of the considerable prevalence of NAFLD and its associated increased mortality, there is a pressing need for identifying effective treatments for this disease. The pathogenesis of NAFLD is multifactorial, involving obesity, insulin resistance, inflammation, oxidative stress and increased hepatocyte apoptosis^[8,12]. Accordingly, several treatments have been evaluated in this population, including lifestyle changes and pharmacological agents targeting the underlying pathogenetic mechanisms, including insulin-sensitizing, weight-reducing, antioxidant and antiinflammatory agents^[1,12,13]. However, the evalu-

ated agents have either shown limited efficacy or have been associated with an unfavorable safety profile^[11,12,13]. Accordingly, current therapeutic approaches propose lifestyle modifications including diet and exercise as first line treatment in patients with NAFLD^[1]. However, diet and exercise are of limited efficacy and are characterized by low long-term adherence rates^[1].

In this context, novel agents targeting hepatocyte apoptosis might represent a useful tool in the management of NAFLD. Apoptosis is a physiological, highly organized and genetically programmed form of cell death which contributes to body homeostasis by removing aged and damaged cells. Thus, apoptosis represents a major protective defense mechanism against a number of harmful factors including viral attacks and carcinogens^[14]. However, aberrant hepatocyte apoptosis may induce hepatic injury and disease progression *via* up-regulation of inflammation and fibrosis^[14-16]. Indeed, hepatocyte apoptosis is increased in NAFLD and correlates with the severity of inflammation and fibrosis^[14-16]. Moreover, apoptosis is a main feature of NASH differentiating it from isolated steatosis and may also contribute to the progression from NASH to cirrhosis^[16-18]. In experimental models, increased apoptosis appears to contribute to progression to hepatocellular carcinoma (HCC) independently from other carcinogens (*e.g.*, inflammation)^[19]. It has been suggested that damaged hepatocytes become resistant to apoptotic death in more advanced NAFLD because of downregulation of proapoptotic molecules and upregulation of antiapoptotic mediators^[19-21]. As a result, damaged cells escape apoptosis and high proliferation rates are observed leading to HCC^[19-21].

Given the important role of apoptosis in NAFLD, a recently reported pilot study by Ratzu *et al*^[22] evaluated the safety and efficacy of inhibition of hepatocyte apoptosis in this disease. This phase II, randomized, double-blind, placebo-controlled, multicenter clinical trial evaluated GS-9450, an irreversible selective inhibitor of caspases 1, 8 and 9, in patients with NAFLD^[22]. Caspases are intracellular proteolytic enzymes that are key effectors of the apoptotic process^[14]. The study included 124 patients 18 to 75 years-old with biopsy-proven NASH and serum alanine aminotransferase (ALT) levels > 60 IU/L^[22]. Exclusion criteria included histological findings of cirrhosis, HCC, platelets < 75 000/mm³, neutrophils < 1500/mm³, hemoglobin < 11.0 g/dL, creatinine clearance < 70 mL/min (estimated with the Cockcroft-Gault equation), weight loss > 4% within 8 wk before screening, daily alcohol consumption > 30 g in males and > 20 g in females, drug-induced fatty liver and liver damage attributed to other liver diseases (*e.g.*, viral hepatitis, autoimmune hepatitis and hemochromatosis). Patients with type 2 diabetes mellitus were eligible for inclusion in the study if they were not insulin-dependent, they were not under treatment with glitazones for at least 6 mo before screening, the onset of diabetes was within the last 10 years and there were no signs of peripheral diabetic neuropathy or gastroparesis.

Patients were randomly assigned to receive GS-9450 1, 5, 10 or 40 mg or placebo once a day for 4 wk. All patients were required to follow a balanced lifestyle during the study. A follow-up of 4 wk followed the treatment period. The main efficacy endpoints were the change in serum ALT, aspartate aminotransferase (AST) and cytokeratin (CK)-18 fragment levels during the treatment period. CK-18 is a major cytoplasmic filament protein of the hepatocellular cytoskeleton that is cleaved mainly by caspase-3 during the apoptotic process leading to formation of CK-18 fragments^[15]. Thus, CK-18 fragment levels reflect the extent of hepatocyte apoptosis^[15].

Treatment with GS-9450 induced a significant, dose-dependent reduction in serum ALT levels whereas ALT levels did not change in the placebo group^[22]. This reduction occurred as early as the third day of treatment. In the group that received 40 mg GS-9450, at week 4, only 2 patients (8%) were nonresponders (*i.e.*, had a decrease in ALT levels of < 10% relative to baseline) whereas 35% of patients showed normalization of ALT values. A dose-dependent reduction was also observed in serum AST levels in patients who received GS-9450. Among patients who received the highest GS-9450 dose, the percent of patients who had normal AST levels increased from 20% at baseline to 48% at the end of the treatment period. Serum CK-18 fragments decreased only in patients who were treated with 10 and 40 mg GS-9450 but this decrease did not differ from the change in the placebo group^[22].

At 4 wk after treatment discontinuation, serum ALT levels returned to baseline levels in the groups that received 1, 5 and 10 mg GS-9450 but were lower than baseline in the 40 mg group^[22]. This rebound effect was apparent from the first week of the follow-up period^[22]. Serum AST levels increased within 1 wk of discontinuation of GS-9450 in all groups to modestly above baseline levels^[22].

There was no change in markers of insulin resistance (serum glucose and insulin levels, homeostasis model of insulin resistance), serum γ -glutamyl transpeptidase levels, lipids or weight during treatment with GS-9450^[22].

Regarding the safety of GS-9450, the majority of the adverse events recorded in patients treated with this agent were of mild to moderate severity and most were not attributed to GS-9450^[22]. No serious adverse events were recorded during treatment with GS-9450. Moreover, there were no notable differences in the frequency of adverse events between the groups assigned GS-9450 and placebo^[22].

Overall, the study of Ratzu *et al*^[22] suggests that GS-9450 dose-dependently lowers serum ALT levels and is well-tolerated in patients with NAFLD. Previous studies evaluating this agent have also reported promising results. In a phase I clinical trial, GS-9450 was well-tolerated when administered to healthy individuals^[23]. In a double-blind, placebo-controlled phase II trial in patients with chronic hepatitis C, a disease also characterized by increased hepatocellular apoptosis, GS-9450 reduced serum

ALT levels^[24]. Moreover, in a substudy of the latter trial, GS-9450 induced a moderate reduction in caspase-8 expression and a strong reduction in caspase-3 expression in peripheral T-lymphocytes^[25].

Besides GS-9450, a wide range of pan-caspase inhibitors has been evaluated in pilot studies yielding encouraging results. IDN-6556, an irreversible, broad-spectrum caspase inhibitor, attenuated hepatocellular apoptosis and hepatic inflammation and fibrosis in animal models^[26,27]. In humans, IDN-6556 was well-tolerated in a phase II clinical trial by both normal volunteers and patients with elevated transaminase levels and lowered transaminase levels in the latter^[28]. Moreover, in a phase I and II clinical trial, IDN-6556 reduced aminotransferase levels in patients with chronic hepatitis C or NASH^[29,30]. Another irreversible pan-caspase inhibitor, VX-166, reduced hepatocellular apoptosis, inflammation and fibrosis in experimental models but had a modest effect on ALT levels and markers of oxidative stress in animal models with established steatosis/steatohepatitis^[31,32].

However, there are some concerns regarding the safety of GS-9450 and caspase inhibitors in general. There is a potential risk of carcinogenesis when apoptotic mechanisms are inhibited given the key role of apoptosis in protecting against tumor development^[19,21]. The existing data regarding this possible association is meagre and controversial^[21]. GS-9450 might theoretically be safer than pan-caspase inhibitors since it acts primarily on hepatocytes and blocks the activity of specific caspases. However, most information about the safety of caspase-inhibitors is from experimental models and therefore it is difficult to reach definite conclusions about their safety in humans^[21]. The existing clinical studies are small and short in duration^[22,24,28-30]; accordingly, larger and long-term studies are required to evaluate the carcinogenic potential, if any, of caspase inhibitors.

Another concern regarding the safety of caspase inhibitors is ALT overshoot, *i.e.*, elevation of ALT levels three times the baseline value after discontinuation of treatment, which could result in acute hepatic failure^[29,30]. This adverse effect was observed in patients with chronic hepatitis C who were treated with the pan-caspase inhibitor IDN-6556 and could be due to massive apoptosis of hepatocytes, which escaped apoptosis during treatment, after the abrupt withdrawal of the drug^[30]. In the study by Ratziu *et al.*^[22], although ALT values increased after discontinuation of GS-9450 and in some patients exceeded baseline levels, they did not reach three times the initial values. Therefore, GS-9450 might be safer than pan-caspase inhibitors but this has to be further evaluated in larger studies. It has been suggested that the risk of ALT overshoot might be reduced by the gradual instead of sudden removal of the caspase inhibitor but this remains to be evaluated in future studies^[30]. On the other hand, the relapse of ALT levels after discontinuation of GS-9450 treatment suggests that long-term treatment will be necessary^[22], limiting the clinical significance of ALT overshoot.

In addition to these safety concerns, there are some limitations regarding the evaluation of the efficacy of GS-9450 in the study by Ratziu *et al.*^[22]. In this study, the change in serum ALT and CK-18 fragment levels was used to assess the efficacy of GS-9450^[22]. It is well established that both ALT and CK-18 fragment levels correlate with NAFLD severity^[1,10,15,33]. However, more than 60% of patients with NAFLD have normal ALT levels, implying that normal ALT levels do not exclude the presence of the disease^[1,4,33,34]. Moreover, the reduction in serum ALT levels correlates with the improvement in liver steatosis and inflammation but not fibrosis^[1,35]. On the other hand, liver biopsy is the gold standard for the diagnosis, staging, monitoring and evaluation of drug response in NAFLD^[1,3]. Given the short follow-up (4 wk), a second liver biopsy was not performed in the study by Ratziu *et al.*^[22]. Therefore, long-term studies that will evaluate the effects of GS-9450 on liver histology are needed before reaching definite conclusions on the efficacy of this agent.

In conclusion, despite its limitations, the pilot study by Ratziu *et al.*^[22] provides additional evidence that the inhibition of apoptosis might have a role in the management of NAFLD. Therefore, the efficacy and safety of this approach merits further evaluation in larger and longer-term studies. On the other hand, given that NAFLD has a multifactorial pathogenesis, a combination of agents targeting the multiple implicated mechanisms, including increased apoptosis, should be another focus of future studies. Finally, on the grounds of the strong genetic impact on NAFLD development and progression^[36,37], investigating related genes and polymorphisms might allow the identification of patients who are at higher risk for progression of NAFLD and/or who might experience greater benefits from the different therapeutic approaches.

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Tibetan herbal formula Padma Digestin modulates gastrointestinal motility *in vitro*

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RESULTS: Compared with the control treatment, the Padma Digestin extract had a procontractile effect on the antral smooth muscle strips. Padma Digestin decreased ACh sensitivity in cardia muscle strips and increased it in those from the antrum and pylorus. In the intestinal segments, spontaneous contractility was inhibited in both the duodenal and jejunal strips, whereas reactivity to ACh was inhibited in the jejunal strips only. In the colonic samples, Padma Digestin inhibited spontaneous and ACh-stimulated contractility at a low dose but seems to have increasing effects at a high dose.

CONCLUSION: Padma Digestin extract has region-specific effects on the contractility and excitability of gastrointestinal smooth muscle. Our results support the traditional use of Padma Digestin for maldigestion and functional gastrointestinal disorders.

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Key words: Tibetan Medicine System; Herbal; Gastrointestinal motility; Smooth muscle; Padma Digestin

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Abstract

AIM: To examine the effects of Padma Digestin on the smooth muscle motility of different gastrointestinal segments *in vitro*.

METHODS: The effects of the ethanolic extract of Padma Digestin (at 8.16 mg/mL or 81.6 mg/mL) on the contractility and susceptibility to acetylcholine (ACh) of muscle strips from the cardia, antrum, pylorus, duodenum, jejunum, ileum and colon of male Wistar rats were analyzed.

INTRODUCTION

Functional gastrointestinal disorders (FGDs) are characterized by various symptoms without underlying identifiable structural lesions or biochemical abnormalities^[1]. Symptoms such as abdominal pain and discomfort, bloating, flatulence, changes in stool consistency, and postprandial fullness are very common and have a great impact on

the quality of life of affected patients^[2,3]. The pathogenesis of FGDs is still unclear; however, different factors such as disturbed gastrointestinal motility, accommodation and hypersensitivity, side effects of pharmaceuticals, psychosocial status, changes in inflammatory status and helicobacter pylori infection are likely to be involved^[2,4-8].

In general practice, unspecific dyspeptic complaints are often addressed using symptom-based approaches such as proton pump inhibitor treatment, Helicobacter pylori eradication and dietary modifications^[8,9]. Given the high prevalence of FGDs, their impact on patient quality of life, and their socio-economic importance, safe and effective treatment options are urgently needed^[2,10,11]. Phytotherapeutics contain a wide variety of chemical substances in very small doses. They are known to act as so called multi-target drugs, which target and affect multiple different pathophysiological pathways simultaneously^[12-14].

Network models show that partial inhibition of multiple targets by synergistically acting agents can be more effective than complete inhibition of a single target^[12,15]. In herbal preparations, each chemical is usually present at a very low dose. The synergistic action of these chemicals allows them to be clinically effective, as well as minimizes the risk for side effects^[13,14]. Due to the joint activity of multiple herbal compounds, the resulting mechanism of action cannot be deduced from the known effects of each individual ingredient. Even though one might be able to predict which molecular pathways would be affected, the sum of these changes may not reflect the resulting effect of the mixture.

Due to their complex mechanism of action and overall favorable safety profile, herbal preparations seem to be especially well-suited for the treatment of multifactorial diseases. Various plants as well as herbal combination preparations have produced favorable outcomes in FGDs^[14,16-20]. The polyherbal preparation Padma® Lax has been shown to be effective in the treatment of dominant irritable bowel syndrome^[19,20], and the multimodal effects on intestinal motility have been identified as the mode of action of this formula^[21].

Padma Digestin® is a polyherbal formula produced in Switzerland according to the international pharmaceutical guidelines. The preparation is licensed as a drug (Swiss-medic No. 59375) and is available under the same name in various European countries. It is a modern representation of a formula from Traditional Tibetan Medicine (Tibetan name: Se'bru 5). Padma Digestin consists of five herbs, which have been used in this composition in the Himalayas for hundreds of years. In Europe, the formula has been used for more than 20 years for disturbed digestion with dyspeptic symptoms such as epigastric pressure, postprandial fullness, bloating, and flatulence as well as for lack of appetite, *e.g.*, in convalescence or old age. Traditionally, the formula has also been used for ailments of the lower abdomen and lower back including sexual dysfunction, recurrent cystitis or lower back pain. Some of the plants or chemical constituents that comprise Padma Digestin have previously been shown to influence

gastrointestinal motility^[22-24]. Despite the information on the individual ingredients, to our knowledge, there are no reports on the effects of the multicomponent formulation as a whole.

Therefore, the aim of the present study was to investigate the effects of Padma Digestin ethanolic extracts on different gastrointestinal segments regarding spontaneous contractile activity and susceptibility to acetylcholine (ACh) *in vitro*.

MATERIALS AND METHODS

Animals

After an overnight fast with free access to drinking water, 21 male Wistar rats (in-house breeding, Central Animal Facilities, University Hospital Berne, Switzerland) weighing 160 to 275 g were anesthetized with a mixture of ketamine and xylazine in a ratio of 1:1 (1 mL per kg body weight) (Dr. E Gräub AG, Berne, Switzerland). All of the procedures and subsequent animal care were in accordance with the guidelines of the Department of Agriculture of Berne, Switzerland, which provided the approval for this study.

Tissue preparation

Several gastrointestinal segments were analyzed: cardiac, antral, pyloric, duodenal, jejunal, ileal and colonic. Tissue samples were rapidly harvested and placed in cold modified Krebs-Ringer's bicarbonate buffer (118.3 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L MgSO₄, 1.2 mmol/L KH₂PO₄, 2.5 mmol/L CaCl₂, 25 mmol/L NaHCO₃, 0.026 mmol/L CaEDTA and 11.1 mmol/L glucose) (Sigma Chemicals, Buchs, Switzerland) saturated with carbogen (95% O₂ + 5% CO₂) (Carbagas, Berne, Switzerland). Different gut segments were excised from the same location in each animal and prepared as follows: circular muscle strips from cardiac (*n* = 12) and pyloric tissue (*n* = 9), muscle strips from the antrum (*n* = 44) in the circular axis and muscle strips from the duodenum (*n* = 22), jejunum (*n* = 24), ileum (*n* = 24) and colon (*n* = 36) in the longitudinal axis. The muscle strips were placed in organ bath chambers (5 mL) (Radonit Glass Technology Inc., Monrovia, CA, United States) filled with modified Krebs-Ringer's bicarbonate buffer maintained at 37.5 °C and aerated with carbogen.

Test substance

Padma Digestin is a multicomponent herbal preparation based on a classical formula composed of five herbs that originates from Tibetan Medicine. One capsule contains 204 mg pomegranate seeds (*Punica granatum* L.), 102 mg lesser galangal rhizome (*Alpinia officinarum* Hance), 25.5 mg long pepper fruit (*Piper longum* L./*Piper retrofractum* Vahl.), 12.75 mg cardamom seeds (*Elettaria cardamomum* Maton var. *Miniscula* Burkill), and 12.75 mg cassia bark (*Cinnamomum aromaticum* Nees). Padma Digestin was produced and supplied by Padma Inc., Hinwil, Switzerland. The mixture was extracted with 70% (v/v) ethanol

(EtOH) (B. Braun, Emmenbrücke, Switzerland). 250 mg/mL of the test substance was shaken for 30 min at 37 °C and centrifuged at 5000 *g*. Then, the supernatant was lyophilized with a yield of 20.4% (w/w). Just before use, the lyophilized extract was dissolved in 70% EtOH. One milliliter of this solution corresponded to the extract of 2126 mg Padma Digestin powder.

Study design

In the first set of experiments (9 animals), the *in vitro* effects of the ethanolic extract of Padma Digestin on the contractility and susceptibility to ACh (Sigma Chemicals, Buchs, Switzerland) were analyzed in colonic muscle strips, and EtOH was used as a control. Cardial, antral, pyloric, duodenal, jejunal and ileal tissue strips were collected from the same rats and used in preliminary experiments to assess the recording patterns and reproducibility of the *in vitro* contractile activity of different segments of the upper gastrointestinal tract (data not shown). In a second set of experiments (12 animals), the *in vitro* effect of the Padma Digestin ethanolic extract on the contractility and susceptibility to ACh was studied in cardial, antral, pyloric, duodenal, jejunal, ileal and colonic muscle strips and compared with EtOH, which was used as a control.

Experimental protocol

The proximal end of the muscle strip was fixed to a glass rod. The distal end was connected to a noncompliant force transducer system (Kulite Semiconductors Products Inc, NJ, United States) for continuous recording of the contractile activity. The muscle strips were stretched stepwise to their optimal point of tension-length relationship and allowed to equilibrate for a period of 60 to 90 min in the organ bath chamber with repetitive changes of buffer (Figure 1). First, the baseline contractility without Padma Digestin or EtOH was measured starting at the recording of spontaneous contractile activity. Then, the muscarinic receptor agonist ACh was added in increasing concentrations every six minutes. Preliminary experiments (data not shown) indicated that muscle strips from the stomach and the small intestine were less responsive to ACh than the colonic strips in this experimental setting. Therefore, colonic strips were treated at the concentrations of 20 mmol/L, 200 mmol/L and 2 μmol/L; however, two additional concentrations of 20 μmol/L and 200 μmol/L were used for all the other gastrointestinal segments. The muscle strips were then washed several times with Krebs-Ringer's modified solution until the spontaneous activity returned to stable. Next, 19.2 μL of the resuspended Padma Digestin extract was added to the organ chambers, resulting in a chamber concentration corresponding to 8.16 mg of the original raw powder per mL (low dose). This final concentration was chosen because it corresponds to 816 mg of active ingredients in 100 mL of water, which is the recommended single dose of Padma Digestin. At the same time 19.2 μL of 70% EtOH was added to the control muscle strips as a solvent control. After a superfusion time of 15 min, spontane-

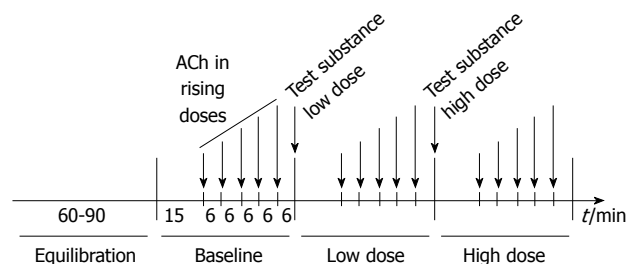


Figure 1 Experimental protocol. ACh: Acetylcholine.

ous contractility and excitability by ACh were recorded as described above. The strips were washed again thoroughly with Krebs-Ringer's solution to rinse away the test substances. Once the spontaneous activity returned to stable, 192 μL of resuspended Padma Digestin extract corresponding to a final chamber concentration of 81.6 mg/mL raw powder (high dose) or 192 μL of 70% EtOH (control) was added. The high dose corresponds to 10 times the recommended single dose of Padma Digestin. The recordings were repeated as described above. At the end of the experiment, the length of each muscle strip was measured, and the tissue was blotted dry and weighed to determine the cross sectional area (CSA).

Statistical analysis

Spontaneous contractile activity was checked visually. Muscle strips without useable recordings were excluded from the study. Contractile activity was calculated as the total area under the curve (integrated contractile activity) using the AcqKnowledge software (Biopac Systems, Inc, Goleta, CA, United States). For each reading, 5-min intervals were analyzed. The contractile activity values were normalized to CSA, which was calculated using the following formula: $CSA (mm^2) = [tissue\ wet\ weight\ (mg)] / \{ [tissue\ length\ (mm)] \times [tissue\ density\ (mg/mm^3)] \}$. The value for smooth muscle tissue density was taken from the literature^[25] as 1.05 mg/mm³. The results were expressed as % CSA ± SE and related to baseline activity. Student's *t* test was used to compare the effects of Padma Digestin and EtOH. *P* values < 0.05 were considered significant.

RESULTS

Gastric segments

Neither EtOH nor the Padma Digestin extract had an effect on the spontaneous contractile activity of the circular cardial strips (Figure 2A). In the antral strips, EtOH treatment reduced spontaneous contractility to 88% ± 2% and 83% ± 2% of the native control at the low and high doses, respectively. This inhibition was almost completely mitigated by the Padma Digestin extract (95% ± 2% and 99% ± 2% of the native control). A similar effect was observed in the pyloric strips; however, the increase in spontaneous contractility compared with the solvent control did not reach statistical significance (Figure 2A).

The ACh-stimulated contractile response in the circular cardial strips was reduced by EtOH, and this re-

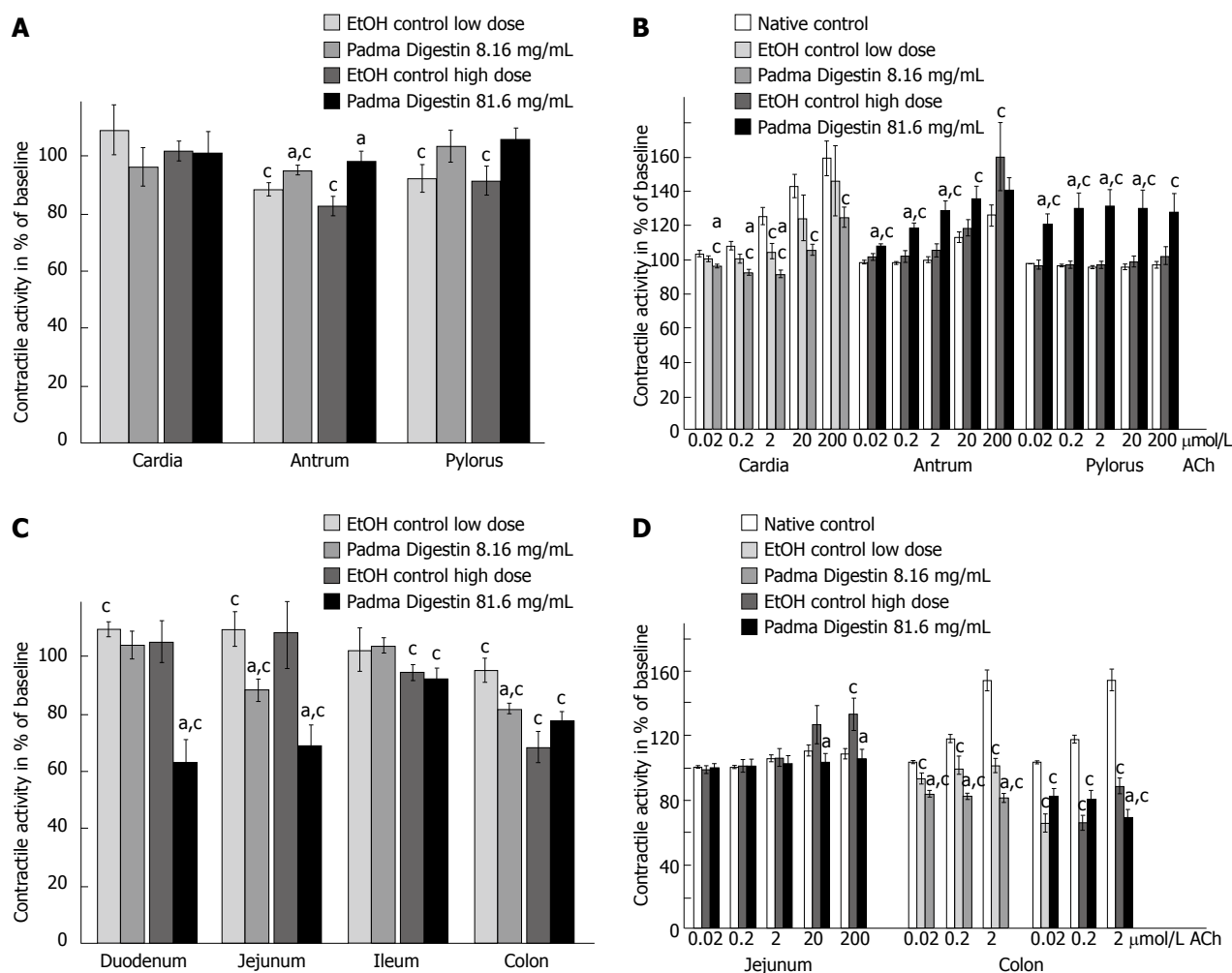


Figure 2 Effect of the Padma Digestin extract. A: Spontaneous contractility of gastric smooth muscle strips; B: Contractility of stomach smooth muscle strips stimulated by acetylcholine; C: Spontaneous contractility of intestinal smooth muscle strips; D: Contractility of intestinal smooth muscle strips stimulated by acetylcholine. EtOH: Ethanol, ^a $P < 0.05$ vs EtOH control; ^c $P < 0.05$ vs baseline.

duction was statistically significant at 2 $\mu\text{mol/L}$ and 20 $\mu\text{mol/L}$ ACh in the high dose and at 2 $\mu\text{mol/L}$ ACh in the low dose (Figure 2B). The Padma Digestin extract further decreased susceptibility to ACh and this effect was statistically significant in 20 mmol/L and 200 mmol/L as well as in 2 $\mu\text{mol/L}$ ACh in the low dose. In the antral and pyloric strips, EtOH increased the contractile response but only at a few concentrations of ACh (Figure 2B, low dose not shown). Compared with the EtOH control, the Padma Digestin extract enhanced the ACh-stimulated procontractile activity of the antral and pyloric strips (Figure 2B).

Intestinal segments

Low dose EtOH enhanced the spontaneous contractile activity of the duodenal and jejunal muscle strips; while in the ileal (high dose EtOH) and colonic (low and high dose EtOH) strips, the spontaneous contractility was reduced (Figure 2C). Compared with EtOH treatment, superfusion with the Padma Digestin extract strongly inhibited the spontaneous contractile activity in the duodenal (high dose), jejunal (low and high dose) and colonic strips

(low dose) (Figure 2C). The procontractile activity of ACh in the duodenal strips was inhibited by the solvent EtOH and more so by the Padma Digestin extract, albeit without reaching statistical significance (data not shown). In the jejunal strips, EtOH increased ACh susceptibility at the high dose and with 20 and 200 $\mu\text{mol/L}$ of ACh. This increase was abolished by the Padma Digestin extract (Figure 2D). Neither EtOH nor the Padma Digestin extract had an effect on the ileal strips (data not shown).

In the colonic strips, the pro-contractile effect of ACh was significantly inhibited by EtOH and was even further inhibited by the Padma Digestin extract at the low (all ACh concentrations) and the high (2 $\mu\text{mol/L}$ ACh) dose. With 0.02 $\mu\text{mol/L}$ and 2 $\mu\text{mol/L}$ ACh, the high dose of the preparation increased the contractility compared to solvent alone; however, this effect was not statistically significant (Figure 2D).

DISCUSSION

Functional gastrointestinal disorders, characterized by various gastrointestinal symptoms without identifiable

structural lesions, are multi-factorial conditions, i.e. they can be caused by multiple factors. They are difficult to treat due to the broad spectrum of symptoms, as well as the complex and ill-understood etiology. Because current standard treatment strategies are yielding unsatisfactory results, there is a growing interest in complementary methods^[26,27]. Previous studies have reported encouraging results on the use of phytotherapeutics for chronic functional gastrointestinal disorders^[9,16,20], and the influence of several herbal preparations on gut motility^[21,28-30].

The results of our *in vitro* study show that Padma Digestin can modulate gut motility in a region-specific manner. While in cardiac segments, Padma Digestin extract inhibited ACh-stimulated contractility compared with EtOH, it had a procontractile effect on the spontaneous and ACh-stimulated contractility of the antral and pyloric segments. Antral and pyloric motility are essential for gastric emptying and are reduced by different factors such as EtOH consumption or psychogenic stress^[31-34]. The Padma Digestin extract prevented EtOH-mediated motility suppression. Our results suggest that Padma Digestin could reduce epigastric pressure and postprandial fullness by improving gastric emptying.

In segments of the small bowel, the control solvent EtOH enhanced the spontaneous contractile activity in the duodenal and jejunal strips and inhibited the contractile activity in ileal strips at the high dose. This finding differs from the results reported in earlier studies^[32,35], possibly because in contrast to the single dose administration used in the present study, Palasciano *et al.*^[32] analyzed the effect of chronic EtOH administration. We analyzed longitudinal muscle strips in our study, whereas Lu *et al.*^[35] studied circular smooth muscle preparations, which are known to show different contractility patterns and sensitivity to neurotransmitters or other substances.

Compared with the EtOH control, the Padma Digestin extract inhibited the spontaneous contractility of duodenal and jejunal strips with little effect on ACh susceptibility^[36].

Different components of the Padma Digestin formula, such as pomegranate seeds^[22], piperine^[24,37], cardamom^[23,38] and cassia cinnamon^[39], are known to inhibit small bowel motility. This inhibition and especially the reduction in the susceptibility to ACh stimulation is thought to have spasmolytic effects^[22,37-39], thus relieving the abdominal symptoms of FGD. The finding that there is reduced motility in the duodenum and jejunum leads to a prolonged contact of nutrients with the small intestinal mucosa. This might increase their luminal absorption^[40] and is in line with the experiences and observations of Traditional Tibetan Medicine, where the formula is also used in malnutrition.

In the colonic strips, the inhibitory effect of EtOH on contractility is well-documented by earlier studies^[21,41]. The results we obtained with the Padma Digestin extract suggest a biphasic effect on colonic smooth muscle. While the low dose inhibited the spontaneous as well as the ACh-stimulated contractility compared with the solvent control, the high dose seems to have had a positive effect

on the spontaneous contractile activity and the contractility stimulated with 0.02 $\mu\text{mol/L}$ and 0.2 $\mu\text{mol/L}$ ACh, albeit without statistical significance.

Similar to Padma Digestin, different inhibitory and excitatory effects of other herbal substances have been shown in different parts of the stomach and intestine^[30,42]. A possible molecular mechanism may be the interaction of Padma Digestin with transient receptor potential (TRP) channels, which are known to influence smooth muscle activity. Various substances contained in the ingredients of Padma Digestin act on different TRP channels. Cinnamaldehyde, a component of cassia bark, has procontractile effects on rat urinary bladders *in vitro*, acting *via* TRP ankyrin 1 (TRPA1)^[43]. Pungent substances such as piperine and gingerols, contained in long pepper and lesser galangal, are known agonists of TRPA1^[44,45], which is involved in colonic smooth muscle contractions^[46]. Piperine may also exert contractile effects *via* TRP vanilloid 1 (TRPV1)^[43,47]. On the other hand, piperine also seems to have inhibitory effects on upper gastrointestinal motility, which may be due to either desensitization after prolonged activation of TRPV1^[48] or other receptors such as the cannabinoid 1 receptor^[24]. Piperine has been shown to have opposing effects on gastrointestinal motility at low and high doses^[49]. While lower doses lead to desensitization and seem to act *via* TRP channels, higher doses are thought to have nonspecific direct actions on the smooth muscle. Other TRP-influencing substances found in Padma Digestin plants are the flavonoid galangin^[6,8,10] and gingerols contained in lesser galangal^[50,51].

The effects of Padma Digestin shown in the present study are likely to occur *via* different mechanisms. Herbal medicines and especially polyherbal formulations are thought to act on multiple target pathways simultaneously^[13,15]. This type of multicomponent mechanism of action is especially well suited for the treatment of multifactorial, chronic diseases^[12] such as functional gastrointestinal disorders, where safe and effective treatment options are needed^[10]. Padma Digestin might be one such option with its diverse effects on gastrointestinal motility. Further studies may elucidate its other modes of action that are clinically relevant, as well as molecular mechanisms of this complex phytotherapeutic compound.

In summary, the results demonstrated a region-specific effect of Padma Digestin on the motility of the rat gastrointestinal tract *in vitro*. Padma Digestin may have a positive effect on functional gastrointestinal disorders by facilitating gastric emptying and intestinal nutrient absorption and by relieving muscular spasms. Thus, our data are in favor of the traditionally prescribed use of Padma Digestin for maldigestion and suggest a potential benefit of this herbal preparation in the treatment of functional disorders of the upper gastrointestinal tract in particular.

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COMMENTS

Background

Functional gastrointestinal disorders are widely spread among Western populations. They are defined by symptoms such as abdominal pain and discomfort, bloating, flatulence, changes in stool consistency, and postprandial fullness without any identifiable organic or structural cause. Although not life threatening the condition has a great impact on quality of life in affected patients and thus, e.g., by doctors visits or sick days from work, has also a socioeconomic relevance.

Research frontiers

Various factors are known to play a role in the development of functional gastrointestinal disorders. Because of the complex causes of the disease there is no accepted and effective standard therapy but treatment mostly follows a symptoms oriented trial and error method. Safe and effective treatment options are urgently needed. Some herbal medicines such as the formula Padma Digestin from Tibetan Medicine are traditionally used in functional dyspeptic symptoms but up to now their modes of actions are not known.

Innovations and breakthroughs

It was found that the herbal formula Padma Digestin has region-specific effects on contractility and sensitivity to stimulants of gastrointestinal smooth muscle. The effects shown here are known to promote gastric emptying and intestinal absorption and suggest a positive effect in functional dysmotility of the upper gastrointestinal tract. The results thus support the traditional use of Padma Digestin in maldigestion and functional gastrointestinal disorders.

Applications

The study suggests that by modulating stomach and gut smooth muscle motility the herbal formula Padma Digestin might be a much needed treatment option in functional gastrointestinal disorders.

Terminology

Padma Digestin is a classical herbal formula from the Tibetan Medicine System (Tibetan name: Se'bru 5). It is composed of five ingredients: pomegranate seeds, long pepper, cassia bark, cardamom seeds, and lesser galangal. The components of complex herbal formulas such as used in Tibetan Medicine achieve their effects synergistically and according to a multi-target mode of action.

Peer review

The investigation has profound pharmacological and therapeutic implications. The study is simple using the *in vitro* gastric tissue model and it has yielded convincing results. The authors have used "mixture" of phytochemicals. Further studies are needed to pin-point the exact molecular mechanisms for the observed effects on gastrointestinal motility.

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- 3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

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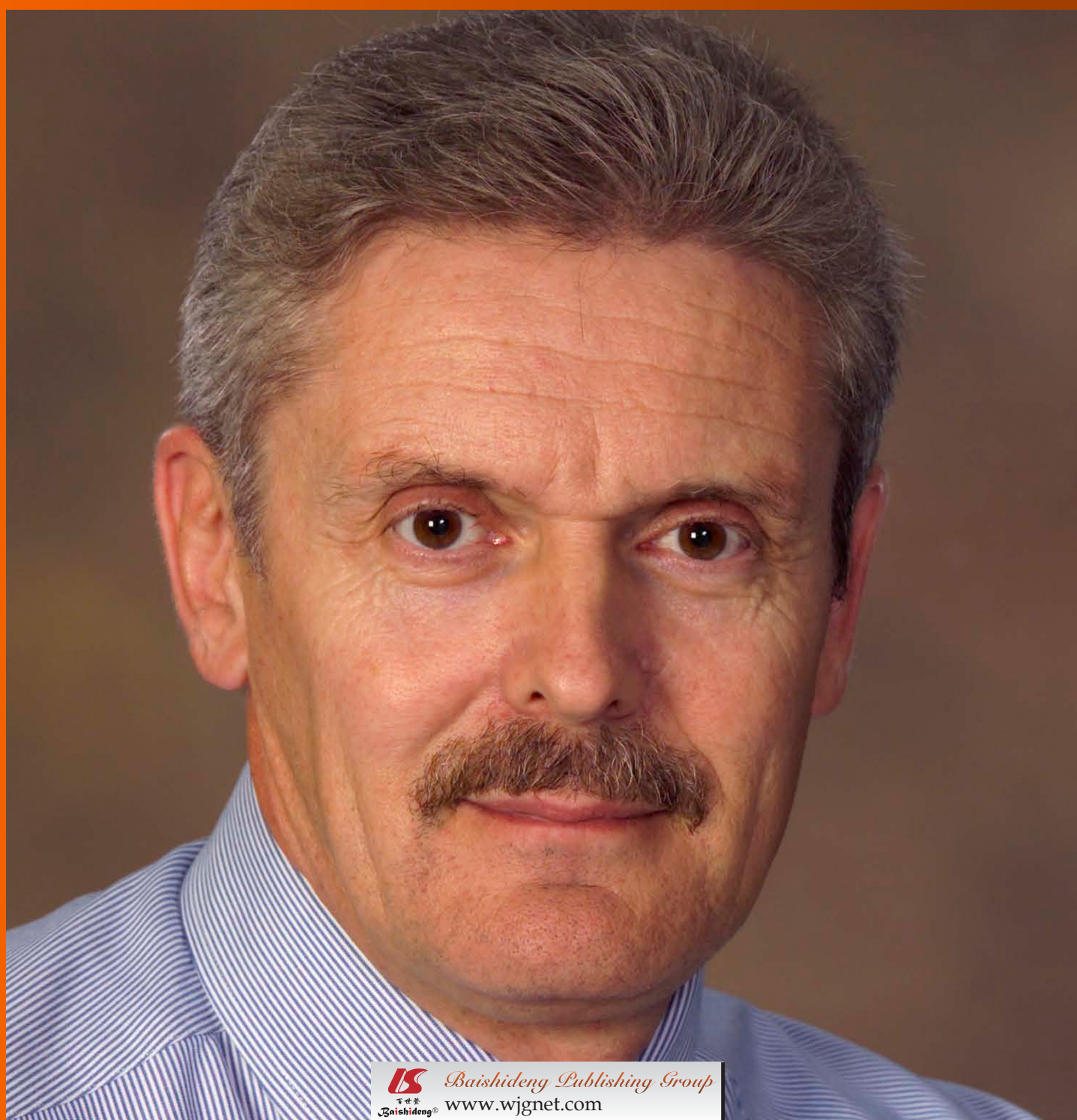
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Management practices of hepatitis C virus infected alcoholic hepatitis patients: A survey of physicians

Ashwani K Singal, Habeeb Salameh, Anjna Singal, Sarat C Jampana, Daniel H Freeman, Karl E Anderson, Don Brunder

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Abstract

AIM: To survey gastroenterologists and hepatologists regarding their current views on treating hepatitis C virus (HCV) infected alcoholic hepatitis (AH) patients.

METHODS: A sixteen item questionnaire was electronically mailed to gastroenterologists and hepatologists. A reminder was sent after 2 mo to increase the response rate. Participation of respondents was confidential. Accessing secured web site to respond to the questionnaire was considered as informed consent. Responses received on the secured website were downloaded in an excel sheet for data analysis.

RESULTS: Analyzing 416 responses to 1556 (27% response rate) emails, 57% respondents (56% gastroenterologists) reported HCV prevalence > 20% amongst AH patients. Sixty nine percent often treated AH and

46% preferred corticosteroids (CS). Proportion of respondents with consensus (75% or more respondents agreeing on question) on specific management of HCV infected AH were: routine HCV testing (94%), HCV not changing response to CS (80%) or pentoxifylline (91%), no change in approach to treating HCV infected AH (75%). None of respondent variables: age, specialty, annual number of patients seen, and HCV prevalence could predict respondent to be in consensus on any of or all 4 questions. Further, only 4% would choose CS for treating HCV infected AH as opposed to 47% while treating HCV negative AH.

CONCLUSION: Gastroenterologists and hepatologists believe that AH patients be routinely checked for HCV. However, there is lack of consensus on choice of drug for treatment and outcome of HCV positive AH patients. Studies are needed to develop guidelines for management of HCV infected AH patients.

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Key words: Survey; Alcoholic hepatitis; Hepatitis C virus; Alcoholic liver disease

Core tip: Alcoholic hepatitis (AH) carries about 40%-50% mortality amongst patients with severe disease. Physicians usually shy away from treating AH in presence of concomitant hepatitis C virus (HCV). We surveyed gastroenterologists and hepatologists to assess their practice patterns on treating HCV infected AH patients. We found that although, physicians agree on screening for HCV in these patients, there is lack of consensus on treatment approach. There was no agreement on choice of drug and response to corticosteroids or pentoxifylline amongst HCV infected AH patients. Guidelines are needed on treating AH in presence of HCV.

Singal AK, Salameh H, Singal A, Jampana SC, Freeman DH,

Anderson KE, Brunder D. Management practices of hepatitis C virus infected alcoholic hepatitis patients: A survey of physicians. *World J Gastrointest Pharmacol Ther* 2013; 4(2): 16-22 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v4/i2/16.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v4.i2.16>

INTRODUCTION

Hepatitis C virus (HCV) infection and alcohol-related liver disease (ALD) either alone or in combination are responsible for more than two third of all patients with chronic liver disease in the United States^[1]. Alcoholic hepatitis (AH), a distinct entity amongst patients with ALD, occurs in about 35% of heavy drinkers^[2]. In its severe form, this disease is associated with significant morbidity, one month mortality of 30%-50%^[3-8], and huge economic burden^[9]. HCV, the most common bloodstream infection in the United States, affects approximately 3.9 million individuals with a higher prevalence amongst alcoholics^[10]. Prevalence of HCV infection is higher amongst alcoholics with liver disease and correlates with its severity^[11-20].

Alcohol and HCV together, present in about 10%-15% of patients with chronic liver disease in the United States^[21], act synergistically resulting in higher prevalence of cirrhosis and hepatocellular carcinoma^[22-24], cause more severe and rapid progression of fibrosis^[25,26], and have poorer response to antiviral therapy compared to when either of these agents is present alone^[27,28]. However, data on the interaction of HCV and AH are scanty. In a single center retrospective study of 76 patients with AH, presence of concomitant HCV infection emerged as an independent risk factor for a poor outcome at 6 mo (Cox proportional hazard ratio 8.45, $P = 0.01$) after controlling for patient demographics, disease severity at admission, and treatment^[29]. Similar data have been reported on the in-hospital mortality in the large national inpatient database analyses^[30]. In our retrospective study, we also observed that AH patients were less likely to be treated with specific treatments such as corticosteroids or pentoxifylline in the presence of concomitant HCV infection compared to patients without HCV despite similar disease severity (27% *vs* 54%, $P = 0.05$)^[29]. This practice of physicians may reflect absence of guidelines for managing AH patients who are concomitantly infected with HCV. To further understand current practice, we conducted survey of gastroenterologists and hepatologists regarding their practice patterns on treating HCV positive AH patients.

MATERIALS AND METHODS

Survey questionnaire

A 16 item multiple choice questionnaire was designed with the aim to assess the responses to specific questions on the management of HCV infected AH patients. Questions specific to management of HCV infected AH patients were: (1) do you routinely test patients with

acute AH for HCV infection; (2) does the presence of concomitant HCV infection change the way you treat AH; (3) which treatment option do you prefer for patients with acute AH and concomitant HCV infection; (4) in your opinion, does HCV infection alter the clinical outcome in patients with AH; (5) in your opinion, does HCV affect the response to treatment of AH with corticosteroids; and (6) in your opinion, does HCV affect the response to treatment of AH with pentoxifylline. In order to avoid bias, we mixed these questions with other questions on respondent characteristics and management of AH in general. These questions were: (1) what is your age; (2) what is your primary area of specialty; (3) how many patients of acute AH on an average do you see per year; (4) what percentage of your patients with acute AH are admitted to the hospital; (5) what percentage of those admitted are admitted through the emergency room; (6) among your patients with acute AH, what percentage have needed liver biopsy to establish diagnosis; (7) what percentage of your patients have associated HCV infection; (8) how often do you treat acute alcoholic patients with corticosteroids or pentoxifylline; (9) if you decide to treat, what drug you prefer; and (10) which of the following would make you chose pentoxifylline rather than corticosteroids. Questions were purposely kept simple so as to be able to complete the survey within 5-10 min.

Study population

Gastroenterologists and hepatologists who are members of the American gastroenterological association or American association for study of liver diseases members and involved in clinical patient care were randomly selected for the study. Physicians known to be involved with seeing only pediatric gastrointestinal diseases were also excluded. Similarly surgeons and paramedical staff (pathologists, radiologists, microbiologists and virologists) were excluded. As physicians could be members for both the associations, duplicate entries were excluded from the Microsoft excel sheet.

Administration of questionnaire

The questionnaire was placed on a secure website of the University of Texas Medical Branch intranet. Potential respondents were emailed a request letter explaining the reason and aim for the survey and the mail. The emails were sent out using the merge email option from the Microsoft Outlook Express. Using the individual email address and linking to the last name, it was assured that the email goes out as a personal email. The link to the website was included in the email request letter. Clicking to the link and taking the survey was considered as an informed consent of the respondent to take part in the survey. After completing the survey questions, responses were submitted to the central website by hitting the submission tab. Once in the website, responses were de-identified. To increase the response rate, a maximum of three additional reminder

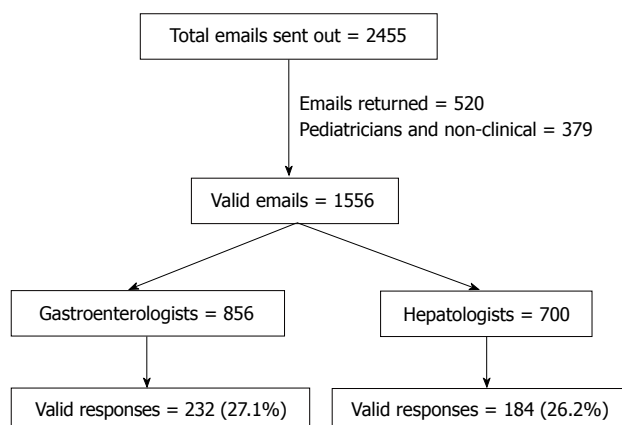


Figure 1 Attrition diagram of valid email responses analyzed for this study.

e-mails were sent at an interval of 1 mo each.

Statistical analysis

Responses on the website for each question were transmitted into a Microsoft excel sheet. If a particular question was not answered, it was assumed that the respondents did not know the answer to that question and was recorded as DNK. Responses were analyzed using the Statistical Analysis Software 9.2 (Cary Inc. Englewood NJ, United States). χ^2 student's *t*-tests were used for analyzing categorical and continuous variables respectively. Logistic regression analyses model was built to assess respondent variable for consensus on specific questions related to management of HCV infected AH. Consensus was defined as 75% or more respondents answering on a particular response option. *P* value < 0.05 was considered to be of statistical significance. The study was approved by the Institutional Review Board at the University of Texas Medical Branch, Galveston, TX, United States.

RESULTS

Respondent characteristics

There were 416 responses to 1556 valid emails with a response rate of 27% (Figure 1). Proportion of gastroenterologists was higher than hepatologists (56% *vs* 44%, *P* = 0.028) with about 75% of respondents < 55 years of age. About 72% reported seeing < 20 patients of AH per year. More than half responded to seeing over 75% of these patients as hospital admissions and through the emergency room.

Physician practice patterns for treating alcoholic hepatitis

About 15% reported need for liver biopsy for diagnosis, 68% would treat AH often with higher proportion reporting use of corticosteroids compared to pentoxifylline (47% *vs* 37%, *P* < 0.0001) and 14% using combination of both. The most common reason for choosing pentoxifylline over corticosteroids was presence or concern of infection or sepsis (72%) and 23% also reported concomitant HCV as a reason for choosing pentoxifylline.

Practice patterns on treatment for HCV infected alcoholic hepatitis patients

About 80% would screen AH patients for HCV infection with a higher proportion reporting HCV prevalence $\geq$ 20% compared to < 20% (49% *vs* 37%, *P* = 0.015). The poll was divided on the question of worse outcome of AH in the presence of concomitant HCV infection (*P* = 0.51). Majority responded that presence of HCV does not change approach to treatment policy and response to corticosteroids or pentoxifylline. However, only 3% would chose corticosteroids for treating HCV positive AH patients (Table 1).

On specific questions related to management of HCV infected AH patients, there was consensus ($\geq$ 75% agreement on particular question) amongst respondents for screening of AH patients for HCV (341/363, 94%), concomitant HCV does not change treatment response to pentoxifylline (297/326, 91%), concomitant HCV does not change treatment response to corticosteroids (262/329, 80%), and no change in treatment approach to AH in the presence of concomitant HCV infection (249/332, 75%) (Table 1). On logistic regression analysis, none of the respondent variables of age ($\leq$ 45 years *vs* > 45 years), specialty (gastroenterology *vs* hepatology), number of patients seen per year ($\leq$ 20 *vs* > 20), and prevalence of HCV ($\leq$ 20% *vs* > 20%) could predict the respondents to be in consensus on any of these 4 questions (Table 2). Further, only less than half of respondents were in agreement on all the four specific questions with lack of prediction by any of the respondent variables (Table 3).

DISCUSSION

About 14% of patients with chronic liver disease have combined alcohol abuse and HCV, and one-third of alcoholics with clinical symptoms of liver disease have been infected with HCV, which is four times the rate of HCV infection found in alcoholics who do not have liver disease^[13,31,32]. Prevalence of HCV amongst patients with AH varies from 8% to 22.2%^[16,19,33] and varies dependent on geographical areas and explains variation in the prevalence rates in our survey.

There was a lack of consensus on the outcome of AH in the presence of HCV infection. Our initial retrospective study showed HCV to be a strong predictor of mortality at 6 mo amongst AH patients^[29]. These data have been confirmed using larger VA and Nationwide Inpatient Sample databases showing higher in-hospital mortality amongst HCV infected AH patients compared to AH patients without HCV infection^[30,34]. Studies are suggested looking at the improvement of existing scoring systems with incorporation of HCV into the model. Further, with the availability of non-interferon based regimens for treatment of HCV infection, it would be worthwhile assessing feasibility of these options to improve outcome of HCV infected AH patients.

We found in a previous study that patients with concomitant AH and HCV received specific treatment for

Table 1 Respondent variables and responses on each question *n* (%)

Question	<i>n</i> (%)	DNK	<i>P</i> value
Age (yr)			0.190
< 35	85 (21)		
35-44	114 (27)		
45-54	112 (27)		
55-64	105 (25)		
Specialty			0.028
Gastroenterology	232 (56)		
Hepatology	184 (44)		
Patients (<i>n</i> per year)		23 (5)	< 0.001
< 10	141 (34)		
11-20	160 (38)		
≥ 20	92 (23)		
Percent admitted to hospital		39 (9)	< 0.001
< 25%	46 (12)		
26%-50%	74 (18)		
51%-75%	94 (23)		
> 75%	163 (38)		
Of admitted, percent through ER		44 (12)	< 0.001
< 50%	81 (19)		
51%-75%	69 (16)		
> 75%	222 (53)		
Percent needing liver biopsy for diagnosis		48 (12)	< 0.001
< 25%	318 (76)		
26%-50%	29 (7)		
> 50%	21 (5)		
Test AAH patients for HCV		53 (13)	< 0.001
Yes	341 (82)		
No	22 (5)		
Percent positive for HCV		58 (14)	0.015
< 20%	156 (37)		
≥ 20%	202 (49)		
Treatment for AH			< 0.001
Often	283 (68)		
Rarely	114 (27)		
Never	19 (5)		
Drug preference for treatment			< 0.001
Corticosteroids	197 (47)		
Pentoxifylline	150 (37)		
Combination	60 (14)		
No preference	9 (2)		
What makes you choose			> 0.05
Pentoxifylline			
Infection or sepsis	300 (72)		
GI bleeding	161		
Renal failure	125		
Hepatitis B	139		
Hepatitis C	94 (23)		
Change in treatment policy with HCV		84 (20)	< 0.001
Yes	83 (20)		
No	249 (60)		
Treatment preference with concurrent HCV		79 (19)	< 0.001
Corticosteroids	14 (3)		
Pentoxifylline	114 (27)		
Either	21 (5)		
Same as without HCV	180 (44)		
No treatment	8 (2)		
Does HCV alter outcome of AAH		80 (19)	0.510
No	174 (42)		
Worse	161 (39)		
Better	1 (0.2)		
Does HCV affect treatment response with CS		87 (21)	< 0.001
No	262 (63)		
Worse	65 (16)		

Better	2 (0.4)		
Does HCV affect treatment response with PTX		90 (22)	< 0.001
No	297 (71)		
Worse	3 (0.7)		
Better	26 (6)		

ER: Emergency room; HCV: Hepatitis C virus; AH: Alcoholic hepatitis; CS: Corticosteroids; GI: Gastrointestinal; PTX: Pentraxin; AAH: Acute alcoholic hepatitis.

AH less often compared with patients with AH alone for all the patients (28% *vs* 57%, $P = 0.014$) and for severe disease (41% *vs* 83%, $P = 0.0066$)^[29]. This is also reflected by our survey where only 3% would chose corticosteroids for treating AH in the presence of HCV infection in contrast to 38% choosing this option for treating AH in general. The presence of HCV infection is not considered a contraindication for using steroids^[35], and fear of using corticosteroids may be based on possible harmful effect of corticosteroids on the HCV replication^[36-41]. An *in vitro* study showed lack of effect of steroids on the HCV RNA level^[42]. Data in HCV transplanted patients have shown association of steroids use with risk for increased recurrence, worse disease, and progression of fibrosis^[43]. However, these effects are seen with bolus doses used for treatment of acute cellular rejection or when steroids are rapidly tapered after using them for long duration (6 mo or more)^[44]. In AH, steroids are used for a short period of 1 mo with slow taper later. Randomized studies are suggested comparing slow to rapid taper of steroids on the HCV RNA level and clinical outcomes.

Similar confusion and lack of consensus was also seen when choosing Pentoxifylline, a drug with excellent safety profile. Pentoxifylline has been used on long-term basis in cirrhotics including those with HCV cirrhosis^[45]. Not only, the drug was found to be safe but was also beneficial for reducing the liver disease complications especially hepatorenal syndrome^[46-48]. This is reflected by the 27% of the surveyed clinician who chose Pentoxifylline as a treatment option although there was general consensus that HCV does not affect AH treatment response.

To the best of our knowledge, this is the first study addressing current management of concurrent AH and HCV infection. Another strength of the study is limiting survey to gastroenterologists and hematologists who are usually involved to make decisions on management of severe AH. Lack of availability of data on gender and geographical area of respondents limited evaluation of these variables in the survey. Another limitation is keeping the survey anonymous which did not allow us to administer the survey again to check for reliability of the answers. However, this helped us to increase the response rate. Finally, some respondents did not answer to some of the questions and could have resulted in selection or non-response bias. However, we think that missing answers could be due to lack of sufficient AH population in clinical practices of respondents such as in China where HBV is endemic and or Middle East and Saudi Arabia where

Table 2 Effect of respondent variables on proportion of respondents being in consensus on each of the four specific questions related to management of hepatitis C virus infected alcoholic hepatitis patients

Respondent variable	Routine screening for HCV			HCV does not change treatment approach			HCV does not affect response to corticosteroids			HCV does not affect treatment response to pentoxifylline		
	n (%)	P value	OR (95%CI)	n (%)	P value	OR (95%CI)	n (%)	P value	OR (95%CI)	n (%)	P value	OR (95%CI)
Age (yr)		0.2			0.7			0.8			0.75	
< 35	68 (92)		1	51 (76)		1	54 (83)		1	59 (91)		1
35-44	85 (90)		0.8 (0.3-2.5)	66 (73)		1.3 (0.6-3)	69 (77)		0.6 (0.3-1.3)	79 (89)		0.9 (0.3-2.8)
45-54	99 (96)		2.1 (0.5-8)	73 (78)		0.9 (0.4-2)	75 (81)		0.7 (0.3-1.6)	85 (93)		1.7 (0.5-6)
≥ 55	88 (97)		2.5 (0.6-11)	58 (72)		1.4 (0.6-3)	63 (79)		0.6 (0.3-1.5)	73 (91)		1.3 (0.4-4.2)
Specialty		0.37			0.5			0.2			0.55	
GE	183 (93)		1	135 (73)		1	135 (77)		1	160 (92)		1
HP	157 (95)		1.5 (0.6-3.7)	113 (77)		0.8 (0.5-1.4)	126 (83)		0.7 (0.4-1.2)	136 (90)		1.3 (0.6-2.7)
Patients (n/yr)		0.29			0.6			0.2			0.38	
< 30	299 (93)		1	221 (75)		1	232 (81)		1	259 (91)		1
≥ 30	41 (98)		2.9 (0.4-22.0)	27 (71)		1.3 (0.6-2.7)	29 (73)		1.6 (0.7-3.3)	37 (95)		0.5 (0.1-2.3)
HCV prevalence		0.48			0.3			0.2			0.81	
< 20%	148 (95)		0.7 (0.3-1.8)	104 (72)		0.8 (0.5-1.3)	119 (83)		1.4 (0.8-2.5)	129 (91)		1.1 (0.5-2.4)
≥ 20%	188 (93)		1	143 (77)		1	141 (77)		1	166 (91)		1

HCV: Hepatitis C virus; GE: Gastroenterology; HP: Hepatology. Also shown are results of logistic regression analyses for each respondent variable given as odds ratio (OR) with 95% confidence interval (CI).

Table 3 Effect of respondent variables on proportion of respondents being in consensus on all four questions related to management of hepatitis C virus infected alcoholic hepatitis patients

Respondent variable	Consensus on all four questions related to management of HCV infected AH patients		
	n (%)	P value	OR (95%CI)
Age (yr)			
< 35	37 (44)		1
35-44	52 (46)		0.9 (0.5-1.8)
45-54	58 (53)	0.2	1.1 (0.6-3.1)
≥ 55	44 (43)		1.9 (0.5-4.3)
Specialty			
GE	125 (56)		1
HP	84 (47)	0.37	1.4 (0.97-2.1)
Patients (n/yr)			
< 30	180 (52)		1
≥ 30	22 (49)	0.29	1.1 (0.6-2.1)
HCV prevalence			
< 20%	71 (46)	0.48	0.9 (0.6-1.4)
≥ 20%	97 (48)		1

HCV: Hepatitis C virus; GE: Gastroenterology; HP: Hepatology; AH: Alcoholic hepatitis. Also shown are results of logistic regression analysis for each respondent variable given as odds ratio (OR) with 95% confidence interval (CI).

alcohol is not often used due to religious reasons. There is no minimum acceptable response rate in a survey^[49]. But we feel that 27% of response rate from a randomly selected group of gastroenterologists and hepatologists is adequate for the study results and avoid non-response bias.

In summary, our study showed a dissociated opinion amongst gastroenterologists and hepatologists on management of AH in the presence of concomitant HCV infection especially on the choice of drug and outcome of AH. Our findings suggest a clear need for studies to assess the response to treatment with corticosteroids amongst HCV infected AH patients and compare to AH patients without HCV infection in order to develop

guidelines for management of AH patients who are also infected with HCV.

COMMENTS

Background

Recent data have shown worse outcome of AH in presence of concomitant hepatitis C virus (HCV) infection. Further, many physicians consider HCV to be a relative contraindication for treating alcoholic hepatitis (AH) especially with corticosteroids. Findings of lack of consensus on managing HCV infected AH patients as found in this survey analysis imply need for development of guidelines on management of these patients.

Research frontiers

There are two research frontiers: high prevalence of HCV in AH patients; lack of consensus amongst gastroenterologists and hepatologists on managing HCV infected AH patients.

Innovations and breakthroughs

Lack of data on managing HCV infected AH patients dictates need for studies in this group of patients.

Applications

Need for well designed studies aiming to develop guidelines for management of HCV infected AH patients.

Peer review

Statistical tests using logistic regression analysis showing that none of respondent variables predicted respondent to be in consensus on questions related to managing HCV infected AH patients.

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Standard triple versus levofloxacin based regimen for eradication of *Helicobacter pylori*

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and the LBT groups on intention-to-treat (ITT) analysis (69% vs 80%, $P = 0.425$) and (79% vs 87%, $P = 0.513$) by per-protocol (PP) analysis respectively. Ulcer recurrence in the STT and LBT groups on ITT analysis was (20% vs 14%, $P = 0.551$) and (9% vs 6%, $P = 1.00$) by PP analysis. Compliance and side effects were also comparable between the groups. A complete course of STT costs Indian Rupees (INR) 1060.00, while LBT costs only INR 360.00.

CONCLUSION: *H. pylori* eradication rates and the rate of ulcer recurrence were similar between the STT and LBT. The LBT is a more economical option compared to STT.

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Key words: *Helicobacter pylori*; Eradication; Peptic perforation; Levofloxacin regime; Randomized control trial; Standard triple therapy

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Abstract

AIM: To compare the eradication rates for *Helicobacter pylori* (*H. pylori*) and ulcer recurrence of standard triple therapy (STT) and levofloxacin based therapy (LBT).

METHODS: Seventy-four patients with perforated duodenal ulcer treated with simple closure and found to be *H. pylori* infected on 3 mo follow up were randomized to receive either the STT group comprising of amoxicillin 1 g *bid*, clarithromycin 500 mg *bid* and omeprazole 20 mg *bid* or the LBT group comprising of amoxicillin 1 g *bid*, levofloxacin 500 mg *bid* and omeprazole 20 mg *bid* for 10 d each. The *H. pylori* eradication rates, side effects, compliance and the recurrence of ulcer were assessed in the two groups at 3 mo follow up.

RESULTS: Thirty-four patients in the STT group and 32 patients in the levofloxacin group presented at 3 mo follow up. *H. pylori* eradication rates were similar with STT

INTRODUCTION

Several studies have proved that the eradication of *Helicobacter pylori* (*H. pylori*) decreased the recurrence of ulcer in patients who had undergone simple closure for perforation^[1,2]. A proton pump inhibitor with two antibiotics, amoxicillin and clarithromycin, is the commonly used regimen for *H. pylori* eradication^[3]. Although it has been the recommended first line therapy for a long period of time^[4-6], recent studies report unacceptably low eradication rates^[7-9]. This is attributed to the development of resistance to clarithromycin. Levofloxacin based therapy

(LBT) has been recently reported to have higher and consistent eradication rates as a first line therapy in *H. pylori* eradication^[10-12]. LBT represents a better alternative to clarithromycin therapy as it meets the criteria set for *H. pylori* treatment: effectiveness, simplicity and safety.

Limited studies have been reported comparing the eradication rates for *H. pylori* between standard triple therapy (STT) and LBT^[10]. Hence, this study was done to compare the efficacy of STT to LBT as a first line therapy for the eradication of *H. pylori* and the prevention of ulcer recurrence in patients with perforated duodenal ulcer following simple closure.

MATERIALS AND METHODS

The study was conducted in the Department of Surgery, JIPMER, Puducherry, a tertiary care hospital in south India between September 2009 and August 2011, over a period of 23 mo. The study was conducted as an open-label prospective randomized trial. The study was cleared by the Institute Research Council and the Ethics Committee.

Patients

All consecutive patients who presented to the hospital with perforated duodenal ulcer were considered eligible for the study. Those patients who were found to have gastric ulcer perforation, upper gastro intestinal disorders, re-perforations or who had undergone any definitive surgery for perforated peptic ulcer were excluded from the study. At 3 mo follow up, following simple closure of the perforated duodenal ulcer, the patients found to be positive for *H. pylori* infection were included in the study.

Diagnosis of *H. pylori* infection

All recruited patients were subjected to upper gastro intestinal endoscopy using a video endoscope (EG 3400; Pentax, Montvale, NJ, United States) under topical anesthesia using 2% lignocaine viscous. Four gastric mucosal biopsies (two each from the gastric corpus and the antrum) were taken using standard endoscopic biopsy forceps. Two of these were used for urease test and the other two for histology after Giemsa staining. All endoscopies were done by an experienced endoscopist.

Diagnosis of *H. pylori* was made by urease test and histology by Giemsa stain. Urease test was done using a urea solution prepared and standardized in our institute^[13]. The solution was observed at room temperature until 24 h of inoculation with two gastric mucosal biopsies. The change of color of the solution from yellow to pink was considered as a positive test for *H. pylori* infection. Histology was done by Giemsa stain of the biopsies for identification of *H. pylori*. If either or both the tests were positive, the patient was considered to have a positive *H. pylori* status. If both the tests were negative, the patient was considered to be negative for *H. pylori* infection.

Treatment regimes

Patients positive for *H. pylori* were randomized into two groups using the sealed envelope technique and computer generated random numbers. One group received the STT regimen while the other received LBT. The STT comprised of amoxicillin 1 g *bid*, clarithromycin 500 mg *bid* and omeprazole 20 mg *bid* and the LBT comprised of amoxicillin 1 g *bid*, levofloxacin 500 mg *bid* and omeprazole 20 mg *bid* for 10 d each.

Follow up

Follow up of these patients was done at 3 mo following therapy. Upper gastrointestinal endoscopy was done at the follow up visit to assess the eradication rates of *H. pylori* for both the groups. Urease test and histology were done on the gastric mucosal biopsies to diagnose *H. pylori* status. The presence or absence of ulcer in these patients was also noted in the follow up endoscopy. Patients who were found to have a positive *H. pylori* status, as evidenced by either a positive urease test or a positive histology, were considered as a treatment failure. The compliance to the regimens and the side effect profile were studied through a questionnaire in the patients' own language, handed over at the time of follow up.

Assessment of outcome

The primary outcome of the study was eradication of *H. pylori* infection. The secondary outcomes were ulcer recurrence, side effects, compliance and the cost of the regimen.

Statistical analysis

Study design is an open-label prospective randomized superiority trial. The sample size calculations were done based on our study with the eradication rates for STT^[14]. A sample size of 35 in each group was determined for the power of the study to be 0.80. Statistical analysis for the observations were done using Graphpad Instat Software version 3 (Graphpad, San Diego, CA, United States). Fisher's exact test was used to analyze the statistical significance of the *H. pylori* eradication rates, ulcer recurrence rates, side effects of, and compliance to either regimen.

RESULTS

At 3 mo follow up, 130 patients presented for index endoscopy. Seventy four of these 130 patients were diagnosed to have *H. pylori* infection by urease test and/or histology. These 74 patients were randomized into two groups to receive either the STT ($n = 39$) or the LBT ($n = 35$).

In the STT group, 2 patients were non-compliant and 3 were lost to follow up. In the LBT group, 1 patient was non-compliant and 2 patients were lost to follow up. Sixty-six patients presented for the 3 mo follow up, which included 34 patients in the STT group and 32 patients in the levofloxacin group. These 66 patients were included in the per-protocol analysis, as shown in Figure 1. The eradication rates for *H. pylori*, ulcer recurrence rates,

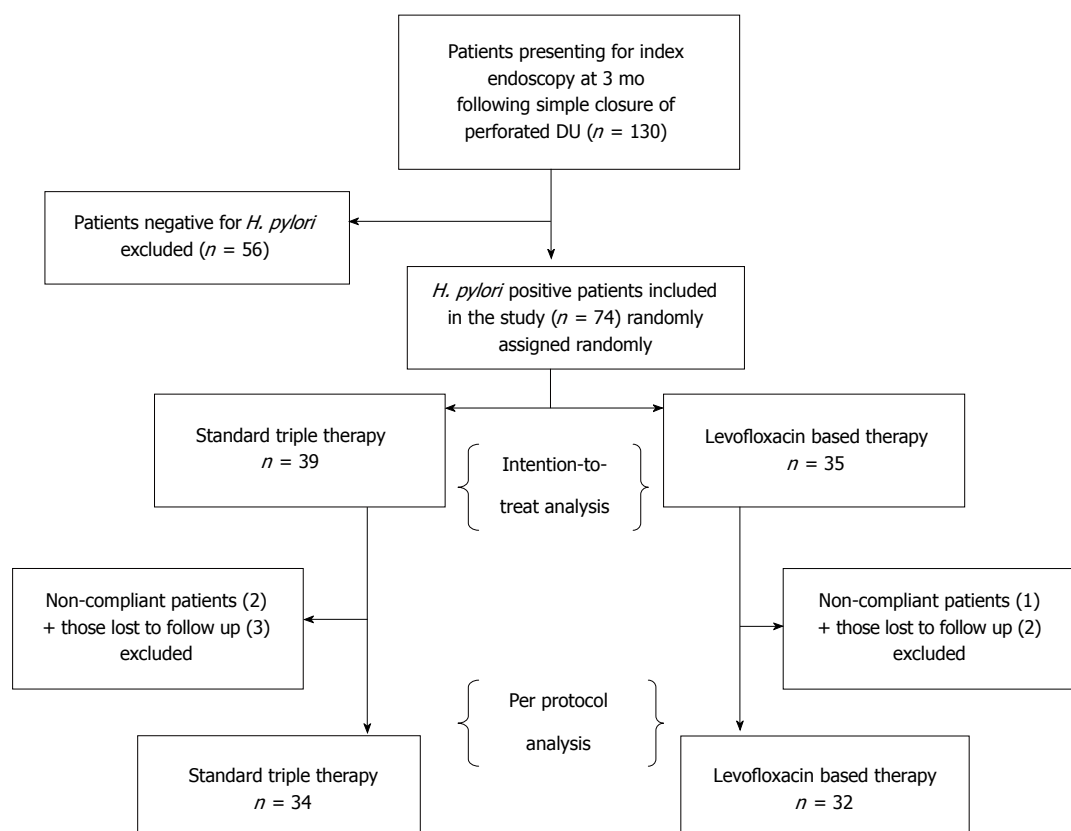


Figure 1 Schematic representation of the design of the study. DU: Duodenal ulcer; *H. pylori*: *Helicobacter pylori*.

side effects and compliance to the two treatment regimes were analyzed in these patients. The patients in the STT group were in the age group of 19-65 years, with a mean and standard deviation of 38 ± 11.99 years, and the LBT group had patients between 20 and 75 years, with a mean and standard deviation of 43.00 ± 12.25 years. The mean of the ages of the patients in both the groups were statistically comparable ($P = 0.0822$). The gender distribution in the groups also did not show any significant difference with 37/39 (95%) in the STT and 34/35 (97%) in the LBT being male ($P = 1.00$). The eradication rates for *H. pylori* infection by intention-to-treat (ITT) analysis for STT group was 27/34 (79%) and for the LBT group it was 28/32 (87%). The difference was not significant (69% vs 80%, $P = 0.425$). There was no difference in the eradication rates by per protocol (PP) analysis either (79% vs 87%, $P = 0.513$). The rate of ulcer recurrence between the groups was also analyzed at the follow up endoscopy. There were only 3 cases with recurrent ulcer in the standard therapy arm and only 2 in the LBT arm. The rate of ulcer recurrence between the groups were not significant by ITT or PP analysis (20% vs 14%, $P = 0.551$; 9% vs 6%, $P = 1.00$, respectively). Both the groups showed good compliance to therapy (94% in the STT group and 97% in the LBT). There was no significant difference between the groups in both ITT and PP analysis ($P = 0.713$, respectively). Side effects of both the treatment regimes were analyzed (Table 1).

In the STT arm, diarrhea was the most common side effect, with a prevalence of 26%. Other side effects re-

ported were epigastric pain, bloating, nausea, vomiting and rashes, in the decreasing order of frequency. In the LBT arm, the most common side effect complained of was nausea and vomiting (22%), followed by diarrhea, bloating, epigastric pain and rashes. There was no significant difference between the side effect profiles of the two groups. The cost of the complete course of STT was 1060.00 Indian Rupees (INR) and 360.00 INR for the LBT.

DISCUSSION

H. pylori has been identified as the most important risk factor for peptic ulcer disease^[15,16]. Significant decrease in the rate of ulcer recurrence was reported in patients with perforated peptic ulcer following simple closure and eradication of *H. pylori*^[2,15]. However, the choice of the optimal regimen for *H. pylori* eradication is debated^[17]. The STT, using amoxicillin, clarithromycin and omeprazole, is one of the widely used regimens for *H. pylori* eradication^[17]. Different studies have shown different rates of eradication with this regimen, ranging between 76% and 90%^[18]. The emergence of resistance of the organism to clarithromycin has urged researchers to look for alternatives to the STT. The sequential therapy and levofloxacin based regimens are two of the newly recommended combinations^[18-22]. Levofloxacin based regimens were used as rescue regimens in case of failure with standard regimen^[23]. The efficacy of this combination as a first line regimen has not been studied exten-

Table 1 Comparison of side effects between the two regimens

Side effect	Standard triple therapy	Levofloxacin based therapy	P value ¹
Diarrhea	26%	19%	0.3096
Nausea/vomiting	12%	22%	0.0892
Bloating	18%	19%	1.0000
Epigastric pain	21%	12%	0.1871
Rashes	6%	3%	0.4977

¹Fisher's exact test.

sively. Data regarding the efficacy of this regimen in the Indian setting is lacking.

The present study showed an eradication rate of 79% and 69% with the STT and 87% and 80% with the LBT in PP and ITT analysis respectively. Gisbert *et al*^[10] studied the efficacy of LBT as the first line treatment of *H. pylori* and found eradication rates of 88% and 84% under PP and ITT analysis respectively, which are comparable to the results of our study. Schrauwen *et al*^[24] from the Netherlands found that therapy with the levofloxacin regimen was very effective in the eradication of *H. pylori*. There are no reports to date of STT being superior to the former. The resistance to fluoroquinolones, including levofloxacin, has been reported to be low in most populations across the globe^[25]. This is one reason that encourages the use of this drug in eradication regimens.

Bose *et al*^[2], in a study conducted in our institute, found that eradication of *H. pylori* infection decreases the rate of ulcer recurrence significantly. At 3 mo follow up, they found that ulcer recurrence in patients in whom *H. pylori* was eradicated was only 18.6% compared to a 70% in the non-eradicated patients ($P = 0.003$).

In the present study, ulcer recurrence rates in both groups were comparable under both PP and ITT analyses. The STT group had ulcer recurrence rates of 20% and 9% under ITT and PP analysis respectively, while the levofloxacin group had ulcer recurrence rates of 14% and 6% respectively. The ulcer recurrence rates observed at 3 mo follow up in the STT group were comparable to that seen in the earlier study from the same center^[2].

The side effect profile in both groups did not show any significant difference with regard to any of the symptoms. The more common side effects of the individual agents were chosen, assessed using a questionnaire and analyzed. Diarrhea (26%), followed by epigastric pain (21%), was the most reported side effect in the STT group. The levofloxacin group complained mostly of nausea and vomiting (22%), with diarrhea and bloating occurring in 19% of patients. The irritant effect of amoxicillin on the gastrointestinal tract and the alteration of the native gut flora by the high dosage can explain the high incidence of diarrhea in both treatment groups^[26]. One of the major side effects of clarithromycin therapy is mild to moderate epigastric pain. This can explain the relatively high incidence of this symptom in the STT group.

Both study groups had comparable compliance to treatment in our study. The STT group had compliance

rates of 94% and 87% in the PP and ITT analysis respectively, compared to 97% and 91% in the levofloxacin group by PP and ITT respectively. These compliance rates are comparable to those seen in western studies. Vaira *et al*^[20] reported a compliance rate of 94% in patients receiving STT. Gisbert *et al*^[10] reported similar adherence to treatment in his study among patients receiving both STT and LBT.

Clarithromycin is the costliest component of the STT. Replacing it with less expensive levofloxacin helps to reduce the cost of treatment significantly. The cost analysis done as a part of the present study has shown that a complete course of the STT costs 1060.00 INR compared to 360.00 INR of the LBT, as on August 1, 2011. This economical convenience also favors the use of the latter treatment regimen.

The results of the present study show a higher trend of eradication of *H. pylori* and prevention of ulcer recurrence in patients who received LBT when compared to those who were administered STT, but statistical analysis failed to show any significance in these differences.

Thus, from the observations of this study, we conclude that LBT is an equally effective alternative to the STT with regard to *H. pylori* eradication rates, prevention of ulcer recurrence, compliance and side effect profile. The lesser cost of the LBT makes it an economical option for treatment of *H. pylori*.

COMMENTS

Background

Standard triple therapy (STT), with a proton pump inhibitor and two antibiotics, amoxicillin and clarithromycin, is the commonly used regimen for *Helicobacter pylori* (*H. pylori*) eradication. Recent studies report low eradication rates due to the development of resistance to clarithromycin.

Research frontiers

Levofloxacin based therapy (LBT) has been recently reported to have higher and consistent eradication rates as a first line therapy in *H. pylori* eradication.

Innovations and breakthroughs

Limited studies have been reported comparing the eradication rates for *H. pylori* between STT and LBT.

Applications

LBT represents a comparable alternative to clarithromycin therapy as it meets the criteria set for *H. pylori* treatment: effectiveness, simplicity and safety.

Peer review

This is an interesting study comparing the use of clarithromycin and levofloxacin based eradication therapy for *H. pylori* infection.

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Interaction of insulin with prokinetic drugs in STZ-induced diabetic mice

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Abstract

AIM: To study the possible interactions of metoclopramide, domperidone and erythromycin in streptozotocin-induced diabetic mice treated with insulin by various parameters.

METHODS: Effects of the individual as well as combined drugs were studied in diabetic mice *via* estimation of the blood glucose and serum insulin levels, small intestinal transit (SIT), gastric emptying (GE), xylose absorption and glucose tolerance tests. Groups were given insulin 2 IU/kg *s.c.*, metoclopramide 20 mg/kg *p.o.*, domperidone 20 mg/kg *p.o.* and erythromycin 6 mg/kg *p.o.* individually and in combination. There were also normal and diabetic control groups. The first set of experiments was carried out to investigate the sub-chronic effect on blood glucose and serum insulin levels in diabetic mice of one week of daily dose administration of the tested drugs individually as well as the combination of insulin with each prokinetic drug. The other five sets of experiments were carried out to investigate

the acute effect of a single dose of each drug individually and in combination on blood glucose and serum insulin levels, SIT, GE, oral xylose absorption and glucose tolerance tests.

RESULTS: The study included the prokinetic drugs metoclopramide (20 mg/kg), domperidone (20 mg/kg) and erythromycin (6 mg/kg), as well as insulin (2 IU/kg), which was individually effective in decreasing SIT, enhancing GE and increasing xylose absorption significantly in diabetic mice. Erythromycin tended to decrease blood glucose level and increase serum insulin level after 1 wk of daily administration in diabetic mice. Erythromycin potentiated the effect of insulin on blood glucose level and serum insulin level whereas other prokinetic agents failed to do so after repeated dose administration in diabetic mice. Metoclopramide or erythromycin in combination with insulin significantly decreased SIT, in diabetic mice, to lower levels than with insulin alone. Administration of prokinetic drugs along with insulin antagonized the action of insulin on xylose absorption. These combinations also increased the rate of glucose absorption from the gut.

CONCLUSION: The present study suggests that prokinetic drugs could potentially improve glycemic control in diabetic gastroparesis by allowing a more predictable absorption of nutrients, matched to the action of exogenous insulin. The use of prokinetics, such as erythromycin, may be interesting in the clinic in decreasing the need for insulin in diabetic patients. The dose of insulin may be safely decreased with erythromycin in chronic treatments.

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Key words: Streptozotocin; Gastrointestinal motility; Insulin; Prokinetic drugs; Intestinal absorption

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INTRODUCTION

Diabetes mellitus is the most common cause of gastroparesis and disturbed gastric and small intestinal intestine motility. Gastroparesis is a syndrome characterized by delayed gastric emptying (GE) in the absence of mechanical obstruction of the stomach. The cardinal symptoms include postprandial fullness (early satiety), nausea, vomiting, bloating, or epigastric pain. Symptoms attributable to gastroparesis are reported by 5% to 12% of patients with diabetes^[1,2]. There is an association between self-reported glycemic control and psychological distress, and the development of gastrointestinal symptoms in diabetics^[3]. Impaired function of the gastrointestinal tract related to diabetes mellitus results from diabetic autonomic neuropathy, impaired sensory innervation and the direct effect of persistent hyperglycemia^[4]. Once established, diabetic gastroparesis tends to persist, despite amelioration of glycemic control. Thus, GE and symptoms are stable over ≥ 12 years follow-up, despite improved glycemic control^[5]. Gastroparesis affects nutritional state and, in diabetics, it also has deleterious effects on glycemic control and secondary effects on organs, leading to increased mortality^[6]. First-line treatment includes restoration of nutrition and medication using prokinetic drugs. Therefore, searching for therapeutic interventions with prokinetic drugs that will improve the specific alterations associated with diabetic gastroparesis represents the most important aim of the present study. Prokinetic drugs have been used for gastroparesis in diabetic patients for a relatively long time and some data about the interactions with insulin in the clinic should be available. It seemed of interest to investigate the possible drug-drug interactions, which may develop from co-administration of insulin and the prokinetic drugs metoclopramide, domperidone and erythromycin. Also, the study aims to highlight the possibility that prokinetics might increase the hypoglycemic effect of insulin.

Prokinetic drugs, commonly used to treat delayed GE, have variable effects on small intestinal motility, and little is known about their effects on glucose absorption. The prokinetic drugs act primarily through neurons since peristalsis is based on neural reflexes. Dopamine antagonists such as metoclopramide and domperidone^[7] are used in this study. Motilides such as erythromycin enhance peristalsis by acting on motilin receptors^[8].

In the present study, streptozotocin (STZ)-induced diabetic mice were treated with insulin and prokinetic agents (metoclopramide, domperidone, erythromycin) individually and in combination. Acute and subchronic studies were carried out to determine whether the prokinetic drugs could improve the blood glucose level and

neuropathy changes in diabetic conditions treated with insulin. This was achieved by measuring some of the biochemical parameters affected by persistent hyperglycemia *via* estimation of blood glucose and serum insulin levels. The acute study were carried out to determine the effect of the test drugs on the gastrointestinal tract motility represented by small intestinal transit (SIT) and GE, in the knowledge that all of the prokinetic drugs used produce acute actions on the gut. The rate of GE is an important determinant of carbohydrate absorption and thus of the blood glucose profile^[9]. Oral xylose absorption and glucose tolerance tests were used as representative indices of carbohydrate absorption changes.

MATERIALS AND METHODS

Drugs and reagents

Insulin (Regular insulin, Novonordisk, Denmark), Metoclopramide (Memphis Pharmaceutical Co., Cairo, Egypt), Domperidone (El Kahira Pharmaceutical Co., Cairo, Egypt) and Erythromycin ethylsuccinate (Abbott Laboratories, Cairo, Egypt) were obtained. Glucose Reagent Kit (Biomérieux, France), Insulin IRMA Kit IM3210 (Immunotech Beckman coulter, Czech Republic), STZ (Sigma Aldrich Chemie, Germany), Phloroglucinol (Sigma chemical Co., United States) and *D*-xylose (Acros Organics, United States) were used. Insulin was diluted with normal saline solution to obtain a suitable strength for injection. Hydroxypropylmethylcellulose (1%) was used as vehicle to administer prokinetic drugs. The other reagents were the highest grade of commercially available products.

Animals

Healthy adult male albino mice weighting between 20-30 g were used in the present study. They were obtained from the animal house of the research department of Kahira pharmaceutical company, Cairo, Egypt. All animals were fed a standard pellet chow and had free access to water. They were maintained under controlled laboratory conditions (temperature, humidity) throughout the study. New groups of mice were recommended for each test carried out. Animals were sacrificed under mild ether anesthesia. Experiments were conducted in accordance with the guidelines set by the animal health research ethics training initiative, Egypt.

Drug treatments

Control groups received equal volumes of vehicle through corresponding routes. Groups were given insulin 2 IU/kg *s.c.*, metoclopramide 20 mg/kg *p.a.*, domperidone 20 mg/kg *p.a.* and erythromycin 6 mg/kg *p.a.* individually and in combination. There were also normal and diabetic control groups. The doses were selected based on the earlier reports, recommended clinical doses and prior pilot experiments^[10-12]. Metoclopramide, domperidone or erythromycin in the dose mentioned above were given alone 15 min before the administration of insulin/ve-

hicle. Insulin was given 50 min before determination of blood glucose and serum insulin levels. Six main sets of experiments were carried out. The first set of experiments was carried out to investigate the subchronic effect on blood glucose and serum insulin level in STZ-induced diabetic male mice of one week of a daily dose of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin. The other five sets of experiments were carried out to investigate the acute effect of a single dose of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin on blood glucose and serum insulin level, SIT, GE, oral xylose absorption and glucose tolerance tests.

Experimental induction of diabetes

Diabetes mellitus was induced in overnight fasted mice by a single intraperitoneal injection of freshly prepared solution of STZ (100 mg/kg body weight) in 0.1 mol/L cold citrate pH 4.5^[13,14]. The animals were allowed to drink 5% glucose solution to overcome STZ-induced hypoglycemia^[15]. The control mice were injected with citrate buffer alone. Two weeks after STZ injection, blood samples were collected by tail venopuncture of the mice and used for the estimation of blood glucose levels using an advanced Glucometer ACCU-CHEK (Roche, Germany)^[16,17]. Overnight fasted mice with blood glucose level above 200 mg/dL were selected and used in the present study.

Measurement of blood glucose and serum insulin levels

Blood was collected from the retro-orbital venous plexus according to the method of Cocchetto *et al.*^[18]. Blood was collected into Wassermann tubes using heparinized microhematocrit capillaries. Blood glucose level was measured using advanced a Glucometer ACCU-CHEK. Serum was separated by centrifugation at 11000 *g* for 2 min and serum glucose level was determined immediately using a glucose kit^[19]. There was no significant difference in glucose levels between the two methods. The remaining amount of serum was kept frozen at -20 °C for insulin determination. Serum insulin was estimated by immunoradiometric assay (IRMA)^[20] using an insulin IRMA Kit. This estimation was done 2 min before drug/vehicle administration and 50 min after insulin/vehicle administration.

Small intestinal transit

The passage of a charcoal meal through the gastrointestinal tract of mice was used as parameter for intestinal motility^[10,21]. Overnight fasted mice were treated with test prokinetic drug orally 45 min and/or insulin subcutaneously 30 min before administration of charcoal meal (0.3 mL of a 5% suspension of charcoal in 2% hydroxypropylmethylcellulose solution). After 20 min, animals were killed by cervical dislocation just after mild ether anesthe-

sia. The abdomen was opened, the charcoal marker was identified in the small intestine and tied immediately to avoid movement of marker. The entire intestine was removed by cutting at the pyloric and ileocaecal ends and then washed in water. The distance the meal had traveled through the intestine as indicated by the charcoal was measured and expressed as percent of the total distance from the pylorus to the caecum. $SIT = (\text{distance travelled by charcoal} / \text{total length of the small intestine}) \times 100$.

Gastric emptying

GE was determined by the phenol red method^[12,22]. The test prokinetic drug was given alone 45 min before administration of phenol red meal. Insulin (*s.c.*) was injected 30 min before the administration of the meal. A solution of 1.5% hydroxypropylmethylcellulose containing 0.05% phenol red as a marker was given intragastrically (0.5 mL/mouse) to overnight fasted mice. Fifteen minutes later, animals were sacrificed by cervical dislocation just after mild ether anesthesia. The abdominal cavity was opened, the cardiac and pyloric ends of the stomach were clamped, and the stomach was then removed and washed with normal saline. The stomach was cut into pieces and homogenized with 25 mL of 0.1 mol/L NaOH. The suspension was allowed to settle for 1 h, 5 mL of the supernatant was then added to 0.5 mL of 20% trichloroacetic acid (w/v) and centrifuged at 3000 *g* for 20 min. 4 mL of 0.5 mol/L NaOH was added to 1 mL of supernatant. The absorbance of this pink colored liquid was measured using a spectrophotometer at 560 nm (Model: Shimadzu 150-20). Phenol red recovered from animals that were sacrificed immediately after administration of the test meal was used as a standard (0% emptying). $GE (\%) = 100 - (x / y \times 100)$, x = absorbance of phenol red recovered from the stomach of animals sacrificed 15 min after test meal, y = mean ($n = 5$) absorbance of phenol red recovered from the stomach of control animals (killed at 0 min following test meal).

Oral D-xylose loading test

This test measures intestinal carbohydrate absorption by calculating the plasma concentration of *D*-xylose after ingestion of a known amount of *D*-xylose^[23,24]. The test prokinetic drug was given alone 45 min before administration of xylose. Insulin (*s.c.*) was injected 30 min before the administration of xylose. A 30% solution containing *D*-xylose (0.8 g/kg body weight) was administered by oral gavage to overnight fasted mice. After 60 min of xylose administration, blood samples were drawn from the retro-orbital venous plexus and centrifuged at 11000 *g* for 2 min. Plasma xylose concentrations were measured using a colorimetric assay. The assay involved incubation for 4 min at 100 °C of 20 μ L of plasma with 1 mL of colored reagent containing 1 g phloroglucinol in 200 mL glacial acetic acid and 20 mL concentrated HCl, followed by

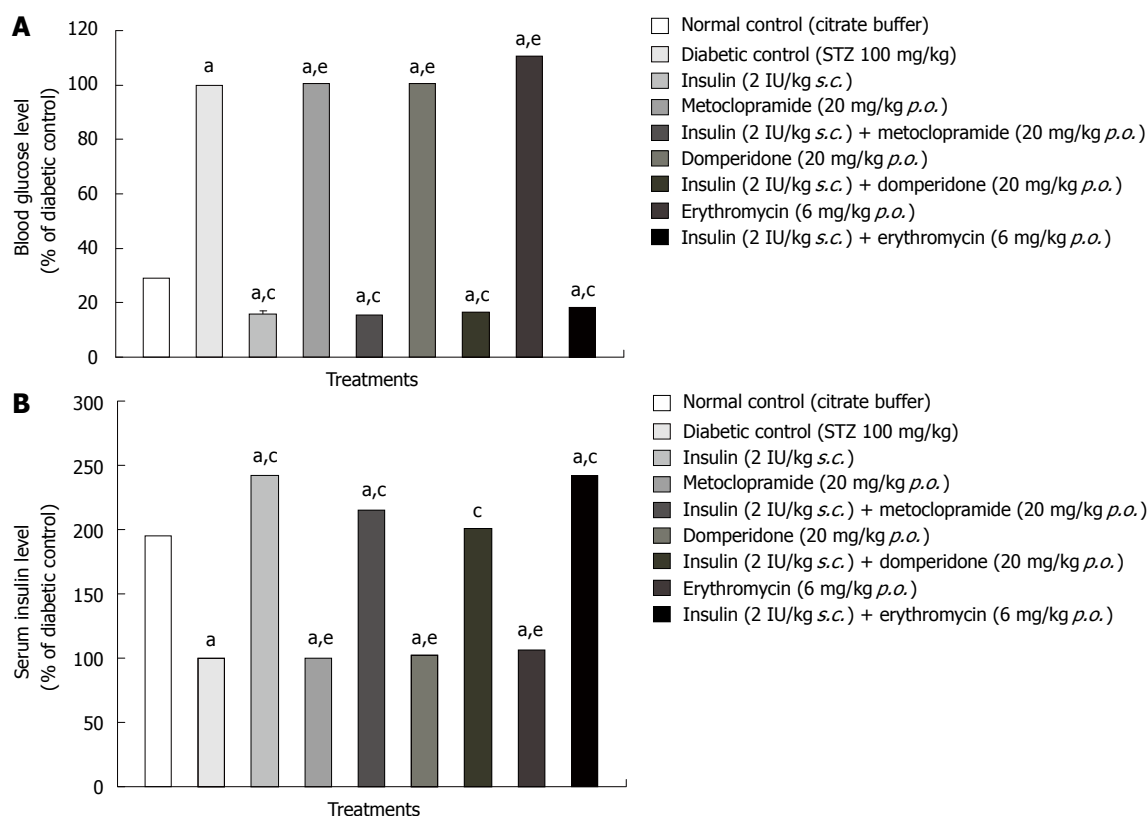


Figure 1 Acute effect in streptozotocin-induced diabetic mice of single doses of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin. A: Blood glucose level; B: Serum insulin level. Values represent the mean $\pm$ SE of eight mice per group. Significantly different from the normal control value at $^aP < 0.05$; Significantly different from the diabetic control value at $^cP < 0.05$; Significantly different from insulin value at $^eP < 0.05$.

reading of the absorbance at 554 nm using spectrophotometer (Model: Shimadzu 150-20).

Oral glucose tolerance test

The oral glucose tolerance test (OGTT) was used to evaluate intestinal absorption. The test was carried out according to the method of Stümpel *et al.*^[25] and Badole *et al.*^[26]. After the mice were fasted for 12 h, the test compound was administered half an hour before glucose loading. A 50% glucose solution (2.5 g/kg of body weight) was orally administered, and blood was taken from the tail vein at 0, 30, 60 and 120 min afterward. Blood glucose concentrations were determined immediately using an Accu-chek (Roche Diagnostics, Germany). The difference between the value of the diabetic control group and the diabetic treated groups represent the amount of glucose absorption from the intestine affected by the different drugs used in this study in addition to other factors. The extent of absorption of glucose was estimated using the total area under the curve, which represents blood glucose level from t_0 to t_{120} . AUC_{total} is calculated using the trapezoidal rule from t_0 to t_{120} .

Statistical analysis

All data were expressed as the mean $\pm$ SE with 6 to 10 mice per group. Statistical analysis was performed using two way analysis of variance (ANOVA) followed by

Tukey-Kramer multiple comparisons test. For all the statistical tests, the level of significance was fixed at $P < 0.05$.

RESULTS

Effects of insulin and prokinetic drug individually or combined on blood glucose and serum insulin levels in STZ-induced diabetic mice

Figure 1 show the antihyperglycemic effect of insulin against STZ-induced diabetic mice. Acute administration of insulin (2 IU/kg) significantly ($P < 0.05$) decreased blood glucose level to 45.37 ± 4.57 mg/dL and increased serum insulin level to 1.96 ± 0.10 μ IU/kg in diabetic mice close to hypoglycemic value. The acute administration of a single dose of metoclopramide (20 mg/kg), domperidone (20 mg/kg) or erythromycin (6 mg/kg) individually did not affect blood glucose level or serum insulin level in diabetic mice. Acute administration of metoclopramide, domperidone or erythromycin did not affect the action of insulin on blood glucose and serum insulin level (Figure 1). Erythromycin tended to decrease blood glucose level and increase serum insulin level after one week of daily dose administration in diabetic mice. Daily dose administration of insulin (2 IU/kg) for one week significantly ($P < 0.05$) decreased blood glucose level to 45.94 ± 2.60 mg/dL and increased serum insulin level to 2.01 ± 0.02 μ IU/kg in diabetic mice close

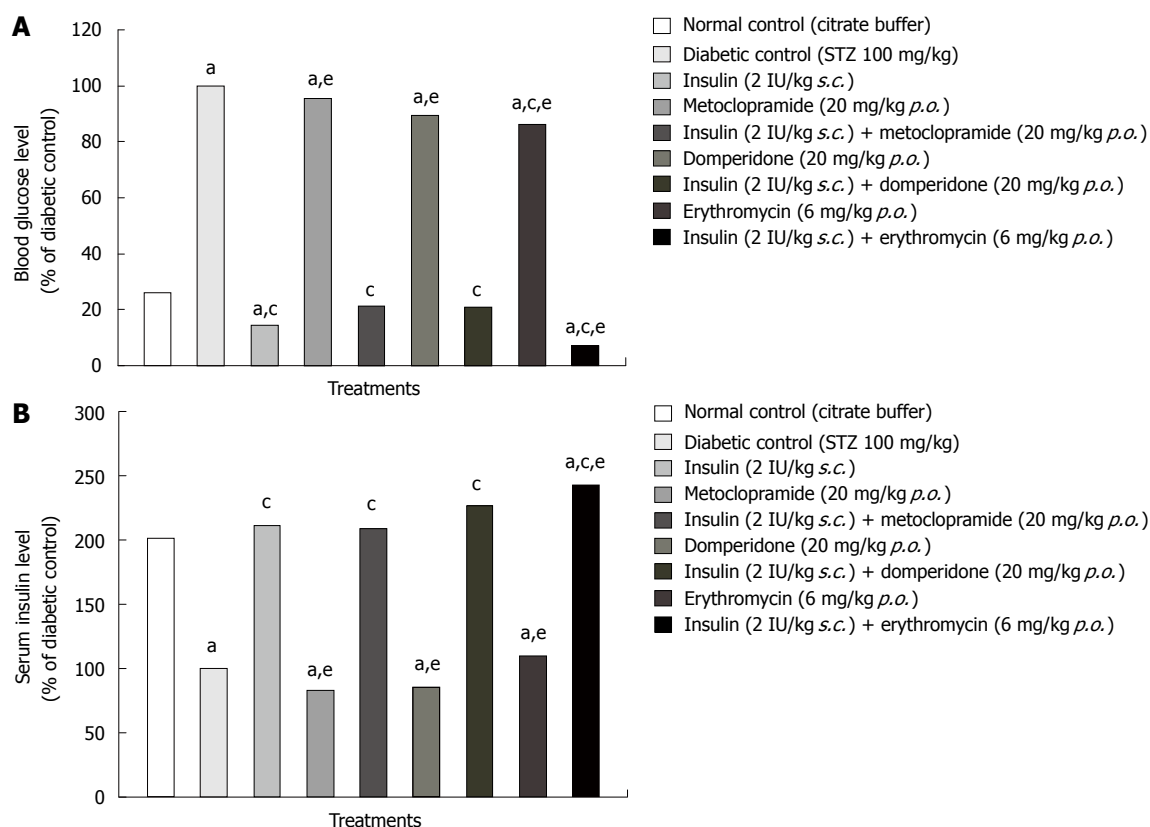


Figure 2 Subchronic effect in streptozotocin-induced diabetic mice of one week of daily administration of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin. A: Blood glucose level; B: Serum insulin level. Values represent the mean $\pm$ SE of eight mice per group. Significantly different from the normal control value at $^aP < 0.05$; Significantly different from the diabetic control value at $^cP < 0.05$; Significantly different from insulin value at $^eP < 0.05$.

to hypoglycemic value. There is no interaction between insulin and the test prokinetic, metoclopramide or domperidone, on blood glucose level and serum insulin level after one week of daily administration in diabetic mice. On the other hand, combination of insulin and erythromycin significantly ($P < 0.05$) decreased blood glucose level to 22.9 ± 1.91 mg/dL and increased serum insulin level to 2.18 ± 0.12 μ IU/kg (Figure 2).

Small intestinal transit

The normal control value of the SIT was $56.61\% \pm 2.58\%$ of the total length of the small intestine. Induction of diabetes in mice significantly ($P < 0.05$) increased SIT to $76.90\% \pm 6.12\%$. Insulin (2 IU/kg) significantly ($P < 0.05$) decreased SIT in diabetic mice to $61.05\% \pm 3.85\%$ as compared to diabetic control group. The test prokinetic drugs, metoclopramide (20 mg/kg), domperidone (20 mg/kg) and erythromycin (6 mg/kg), significantly ($P < 0.05$) decreased SIT in the diabetic mice to $50.04\% \pm 2.42\%$, $48.70\% \pm 4.53\%$ and $43.05\% \pm 3.50\%$ respectively. Either metoclopramide or erythromycin in combination with insulin significantly ($P < 0.05$) decreased SIT in diabetic mice and this effect was less than that of insulin alone. Domperidone did not affect the action of insulin on SIT in diabetic mice (Table 1).

Gastric emptying

The normal control value of the GE was $72.50\% \pm 1.68\%$ of the total amount of the phenol red meal given. The high blood glucose level in diabetic control mice delayed GE significantly ($P < 0.05$) to $55.23\% \pm 9.30\%$. Insulin (2 IU/kg), metoclopramide (20 mg/kg), domperidone (20 mg/kg) or erythromycin (6 mg/kg) in the doses employed increased GE significantly ($P < 0.05$) to $95.87\% \pm 2.41\%$, $76.38\% \pm 7.67\%$, $90.92\% \pm 4.92\%$ and $84.77\% \pm 2.11\%$, respectively, compared with diabetic control mice. Administration of prokinetic drugs (metoclopramide, domperidone or erythromycin) along with insulin (2 IU/kg) did not affect the action of insulin on GE (Table 1).

Oral D-xylose absorption test

The normal control value of serum D-xylose concentration was 1.63 ± 0.10 mg/mL after 60 min of D-xylose administration (0.8 g/kg). The amount of xylose absorbed from the GIT significantly ($P < 0.05$) decreased in the diabetic mice, to 0.606 ± 0.030 mg/mL, as compared to normal control group. Insulin (2 IU/kg), metoclopramide (20 mg/kg), domperidone (20 mg/kg) and erythromycin (6 mg/kg) individually increased xylose absorption to 1.64 ± 0.16 mg/mL, 0.989 ± 0.030 mg/mL, 1.162 ± 0.030 mg/mL and 1.469 ± 0.030 mg/mL, respectively. Administration of prokinetic drugs, along with insulin

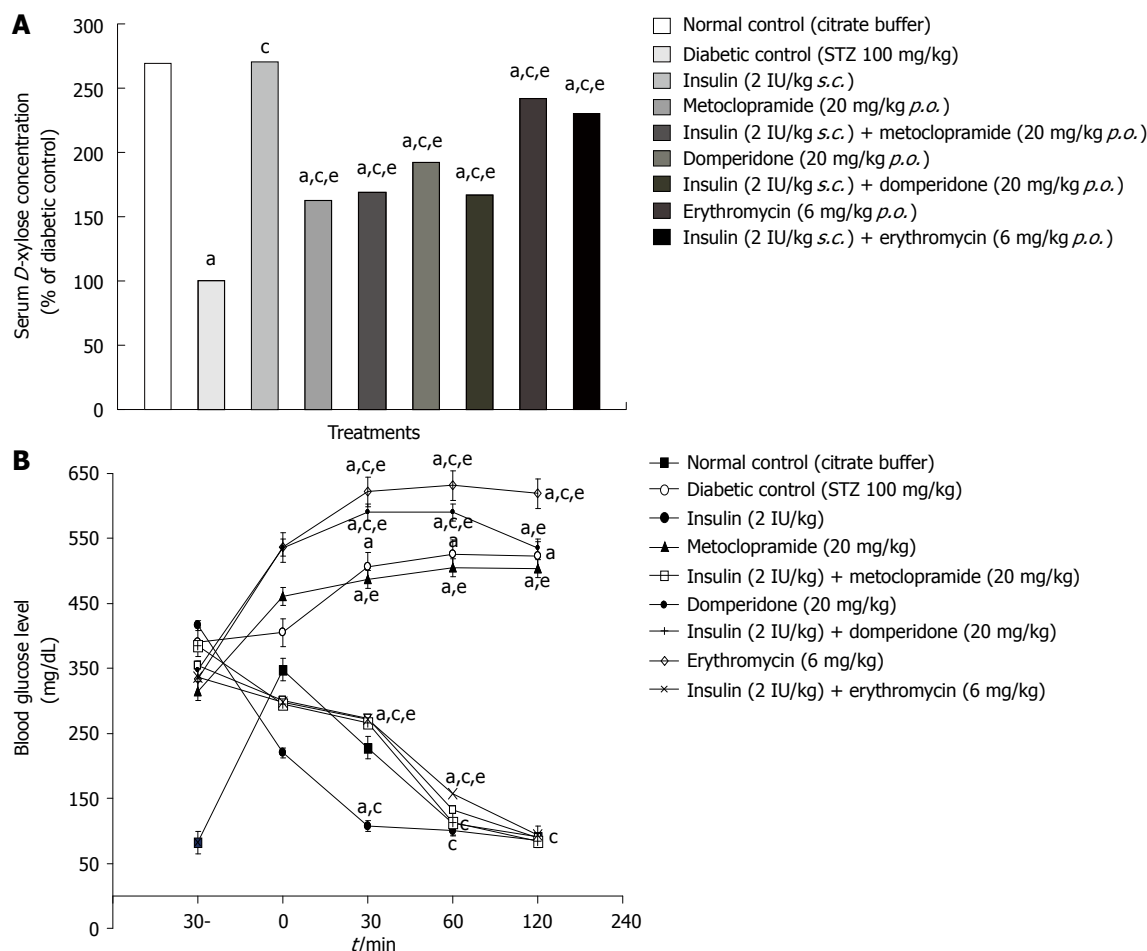


Figure 3 Effect on oral D-xylose absorption and tolerance test in streptozotocin-induced diabetic mice of single doses of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin. A: Oral D-xylose absorption test; B: Oral D-glucose tolerance test. Values represent the mean $\pm$ SE of eight mice per group. Significantly different from the normal control value at $^aP < 0.05$; Significantly different from the diabetic control value at $^cP < 0.05$; Significantly different from insulin value at $^eP < 0.05$.

antagonized the action of insulin (2 IU/kg) on xylose absorption (Figure 3A) in diabetic mice.

Oral D-glucose tolerance test

The OGTT can be used to evaluate blood glucose homeostasis and also indirectly evaluate glucose absorption. As shown in Figure 3B, glucose load (2.5 mg/kg) in normal mice produced rapid increase in blood glucose levels at 30 min and returned to baseline values within 120 min. In contrast, STZ-induced diabetic mice demonstrated basal hyperglycemia (399 ± 14 mg/dL) which remained above 400 mg/dL during all time points determined. The peak increase in serum glucose concentrations in diabetic mice was observed after 60 min of glucose treatment, while that of normal mice observed after glucose loading, indicating delayed glucose homeostasis in diabetic mice. STZ significantly ($P < 0.05$) increased the area under the curve (Figure 4). Insulin (2 IU/kg) significantly ($P < 0.05$) decreased blood glucose level to 107.16 ± 8.51 mg/dL and 100 mg/dL after 30 and 60 min of glucose loading, respectively, and the effects persisted until 120 min (Figure 3B). The area under the curve was significantly reduced to 226.53 ± 12.28 mg/dL after 120 min (Figure 4).

Metoclopramide (20 mg/kg) did not affect blood glucose level where BGL was 487.5 ± 13.6 mg/dL and 505.50 ± 14.55 mg/dL after 30 and 60 min of glucose loading, respectively. Domperidone (20 mg/kg) and erythromycin (6 mg/kg) produced significant ($P < 0.05$) increases in blood glucose level to 590 ± 13 mg/dL, 590.8 ± 17.4 mg/dL and 622.00 ± 23.11 mg/dL, 631.50 ± 21.48 mg/dL after 30 and 60 min of glucose loading, respectively (Figure 3B). In addition, domperidone and erythromycin significantly ($P < 0.05$) increased the area under the curve (Figure 4). Administration of metoclopramide, domperidone or erythromycin along with insulin significantly ($P < 0.05$) increased blood glucose levels as compared to insulin treated values (Figure 3B). Combination of insulin with metoclopramide, domperidone or erythromycin significantly ($P < 0.05$) increased the area under the curve as compared to insulin treated value (Figure 4).

DISCUSSION

Findings of the present investigation revealed that STZ-induced diabetes resulted in a significant increase in SIT and a significant decrease in GE. Abnormalities in GE and small intestinal motor functions were also reported

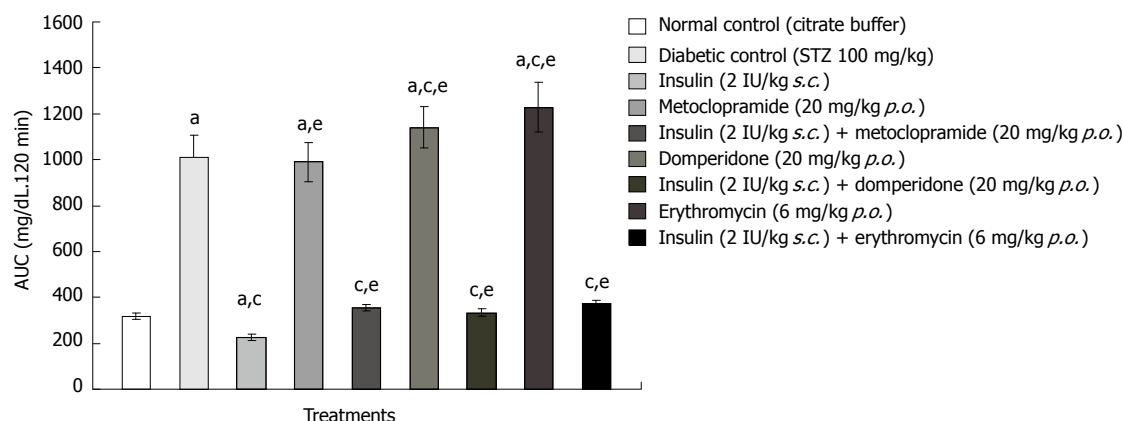


Figure 4 Effect on the area under the curve of blood glucose concentrations during oral glucose absorption test in streptozotocin-induced diabetic mice of single doses of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin. Values represent the mean $\pm$ SE of eight mice per group. Significantly different from the normal control value at $^aP < 0.05$; Significantly different from the diabetic control value at $^cP < 0.05$; Significantly different from insulin value at $^eP < 0.05$. AUC: Areas under the curve.

in diabetic mice^[27,28]. Increased intestinal transit may be partially due to increased cholinergic and decreased beta-adrenergic receptor activities in diabetic animals^[29]. The delay in GE could be partially attributed to the decrease in the number of myenteric neurons in the stomach as a result of diabetes^[30-32]. Similarly, the increase in intestinal transit could be mediated through the same mechanism. All of the stomach's smooth muscle cells have the ability to produce electric depolarizations "slow waves" from resting potential. These rhythmic contractions are thought to originate in the non-smooth muscle pacer cells in the interstitial cells of Cajal (ICCs)^[33]. GE is delayed because the number of ICCs is markedly diminished in diabetes^[34]. Data of the current study showed that insulin-induced hypoglycemia significantly attenuated SIT and accelerated GE in diabetic mice. These results are partly in agreement with earlier reports^[35-38]. The obtained results might be partially due to the direct effect of insulin and not only due to the antidiabetic effect of insulin in decreasing blood glucose level, thereby leading to decreased SIT. This effect could be due to counter-regulation of hypoglycemia. The actions of insulin on the stomach could be mediated via an insulin stimulant effect on the vagus nerve, as reported by Quigley *et al.*^[39].

Data from the present investigation showed that STZ-induced diabetes resulted in a significant decrease in xylose absorption, in agreement with the finding of Fuessl^[40]. The decrease in xylose absorption could be mediated the decreased rate of GE, which resulted from elevation in BGL, as reported by the present study. This explanation coincides with that given by Rayner *et al.*^[9] and Chapman *et al.*^[41].

Recent studies have shown that modifications of systemic glycemia in OGTT reflect the activity of the intestinal glucose transporter SGLT1^[42]. In the present study, STZ-induced diabetic mice demonstrated basal hyperglycemia, which remained above 400 mg/dL during all time points determined. The capacity of the small intestine to absorb glucose increases in experimentally induced diabetic animals as a consequence of the enhanced

activity and abundance of SGLT1, as shown by Fedorak *et al.*^[43], suggesting SGLT1 as a potential target for glycemic control in diabetic animals. STZ-induced diabetic mice exhibited severe hyperglycemia with increased Na^+ dependent glucose uptake activity, compared with normal mice^[44]. Acute insulin-induced hypoglycemia increased xylose absorption and glucose absorption from the GIT in the diabetic mice. In the present study, the effects of insulin on the intestinal absorption of sugar did not differentiate between an effect of insulin on the absorption capacity of the mucosa and other factors that may affect total sugar absorption. Some studies have shown that insulin causes increased Na^+ dependent glucose carrier activity in the small intestine, which leads to increased glucose absorption^[45]. Some studies reported that insulin-induced hypoglycemia accelerates gastric emptying in type 1 diabetes^[37]. The decreased time for movement of sugar from stomach to the small intestine, in addition to its therapeutic effect, decreases the rate of glucose production and increases the rate of glucose utilization by cells^[46].

By studying the prokinetic drugs individually in the current study, domperidone (20 mg/kg *p.o.*) was found to be the most effective agent in the diabetic mice both when compared to diabetic control group and to the other prokinetic drugs. Domperidone, metoclopramide and erythromycin significantly ($P < 0.05$) decreased small intestine transit and accelerated GE in STZ-induced diabetic mice. Similar results have recently been reported from other studies^[47-51]. The inhibitory effect of domperidone on SIT is probably mediated *via* its action on dopamine since it is a dopamine antagonist^[52]. Dopamine has an indirect inhibitory effect through inhibition of cholinergic transmission in the myenteric plexus, which regulates the gastrointestinal tract^[53]. It could be suggested that metoclopramide produces its action through inhibition of presynaptic and postsynaptic D_2 receptors, stimulation of presynaptic excitatory 5-HT₄ receptors and/or antagonism of presynaptic inhibition of muscarinic receptors, in accordance with the conclusions of Valenzuela *et al.*^[54]. The action of erythromycin is prob-

Table 1 Effects of insulin and prokinetic drugs alone and in combination on small intestinal transit and gastric emptying in diabetic mice

Treatments	SIT (%)	GE (%)
Normal control (citrate buffer)	56.61 ± 2.58	72.50 ± 1.68
Diabetic control (streptozotocin 100 mg/kg)	76.90 ± 6.12 ^a	55.23 ± 9.30 ^a
Insulin (2 IU/kg s.c.)	61.05 ± 3.85 ^c	95.87 ± 2.41 ^{ac}
Metoclopramide (20 mg/kg p.o.)	50.04 ± 2.42 ^{ac}	76.38 ± 7.67 ^{ac}
Insulin (2 IU/kg s.c.) + Metoclopramide (20 mg/kg p.o.)	54.30 ± 3.46 ^c	94.91 ± 1.01 ^{ac}
Domperidone (20 mg/kg p.o.)	48.70 ± 4.53 ^{ac}	90.92 ± 4.92 ^{ac}
Insulin (2 IU/kg s.c.) + Domperidone (20 mg/kg p.o.)	62.60 ± 3.07 ^c	92.87 ± 1.14 ^{ac}
Erythromycin (6 mg/kg p.o.)	43.05 ± 3.50 ^{ac}	84.77 ± 2.11 ^c
Insulin (2 IU/kg s.c.) + Erythromycin (6 mg/kg p.o.)	49.14 ± 4.57 ^{ac}	90.86 ± 3.20 ^{ac}

Values represent the mean ± SE of eight mice per group. Significantly different from the normal control value at ^a*P* < 0.05; Significantly different from the diabetic control value at ^c*P* < 0.05; Significantly different from insulin value at ^{ac}*P* < 0.05. SIT: Small intestinal transit; GE: Gastric emptying.

ably mediated *via* its agonistic activity to motilin receptors, which accelerates GE^[8,33].

Metoclopramide significantly increased xylose absorption but did not affect glucose absorption in STZ-induced diabetes. These findings are in agreement with those of Kuo *et al.*^[55]. The action of metoclopramide is mediated through increased plasma concentrations of glucagon like peptide-1 and glucose dependant insulinotropic polypeptide, which are responsible for delay in glucose absorption, although this action does not affect rate of xylose absorption. Domperidone (20 mg/kg) and erythromycin (6 mg/kg *p.o.*) significantly increased xylose absorption and glucose absorption in the diabetic mice as compared to diabetic control group. The effect of erythromycin could be mediated through the action of erythromycin on motilin receptors in the GIT. The action of erythromycin is probably mediated *via* its agonistic activity to motilin receptors, which accelerates GE and increases the rate of sugar absorption.

Combination of domperidone (20 mg/kg), metoclopramide (20 mg/kg) or erythromycin (6 mg/kg) with insulin (2 IU/kg) decreased the amount of xylose absorbed from the GIT when compared to insulin given alone in the diabetic mice. This indicates antagonistic interaction between each two drugs on xylose absorption. It is difficult to explain this action satisfactorily on the basis of the limited information available on the two drugs in this respect. Combination of insulin with metoclopramide, domperidone or erythromycin increased glucose absorption from the intestine when compared to the effect of insulin alone in the diabetic mice. These results suggest that combination of prokinetic drugs with insulin may lead to increased Na⁺ dependent glucose carrier activity in the small intestine. In addition to treating symptoms, prokinetic drugs could potentially improve glycemic control in diabetic gastroparesis by allowing a more predictable absorption of nutrients, matched to the action of

exogenous insulin.

The present study suggested that prokinetics may increase the hypoglycemic effect of insulin. Erythromycin tended to decrease the blood glucose level and increase the serum insulin level after one week of daily administration in STZ-induced diabetic mice. Erythromycin at a dose of 6 mg/kg *p.o.* potentiated the effect of insulin on blood glucose and serum insulin levels after one week of daily administration in diabetic mice where other prokinetic agents failed to do so after repeated administration. Similar results have been reported by Ueno *et al.*^[56]. The action of erythromycin on insulin could be mediated *via* its action as a motilin agonist. It is to be noted that motilin controls cyclic release of insulin through vagal cholinergic muscarinic pathways, as reported by Suzuki *et al.*^[57]. Itoh *et al.*^[58] found that there are no motilin receptors in the pancreas. Therefore, the action of erythromycin on insulin secretion is probably mediated *via* vagal-cholinergic muscarinic pathway stimulation linking to serotonergic receptors. This is a common mechanism in the stimulatory effect of motilin on muscle contraction in the stomach and on pancreatic polypeptide secretion from the endocrine pancreas^[59,60].

The action of erythromycin on gastrointestinal motility in the present study is contradictory to some studies which claim that active motilin receptors do not exist in rodents, and that they only exist as pseudogenes^[61,62]. However, the present results are in agreement with other studies^[63-65]. It is suggested that the action of erythromycin is mediated by binding to central motilin receptors which might be involved in regulation of gastric motility in diabetic rats.

This study dealt with an important issue concerning the use of prokinetic drugs in insulin-treated diabetic individuals. Not all diabetic patients develop gastrointestinal motility disorders or gastroparesis. However, the use of prokinetic drugs might be also relevant in these patients. Erythromycin potentiates the effect of insulin on blood glucose levels and serum insulin levels after repeated administration in diabetic mice. This seems to suggest that the use of prokinetic drugs, such as erythromycin, might be useful in the clinic for decreasing the need of insulin.

In conclusion, combination of insulin with metoclopramide, domperidone or erythromycin increases glucose absorption. This leads to the suggestion that such prokinetic drugs may guard against the risk of severe hypoglycemia associated with diabetic mice treated with insulin. The present study suggests that the use of prokinetic drugs, such as erythromycin, may be useful in the clinic for decreasing the need for insulin in diabetic patients. Use of erythromycin may allow the dose of insulin to be safely decreased in chronic treatments.

COMMENTS

Background

Diabetes mellitus is the most common cause of gastroparesis and disturbed gastric and small intestine motility. Gastroparesis is a syndrome characterized by delayed gastric emptying (GE) in the absence of mechanical obstruction of the stomach. Prokinetic drugs have been used for gastroparesis in diabetic pa-

tients for a relatively long time and some data about the interactions with insulin in the clinic should be available.

Research frontiers

Searching for therapeutic interventions with prokinetic drugs that will improve the specific alterations associated with diabetic gastroparesis was the most important aim of the present study. The research focus was to study the possible effects of metoclopramide, domperidone or erythromycin combined with insulin on different parameters in streptozotocin (STZ)-induced diabetic mice and to highlight the possibility that prokinetic drugs might increase the hypoglycemic effect of insulin.

Innovations and breakthroughs

Diabetic gastroparesis has been managed most successfully with drugs that stimulate GE. Previously prokinetic agents have generally been prescribed in order to relieve symptoms associated with diabetic gastroparesis. The prokinetic drugs metoclopramide, domperidone and erythromycin are all reported to reduce disturbance of gastrointestinal motility. This study deals with an important issue concerning the use of prokinetic agents in insulin-treated diabetic individuals. Not all diabetic patients develop gastrointestinal motility disorders or gastroparesis, but the use of prokinetic drugs might be also useful in these patients. In the present study, erythromycin potentiated the effect of insulin given on blood glucose levels and serum insulin levels after repeated administration in diabetic mice. Combination of insulin with metoclopramide, domperidone or erythromycin increased glucose absorption from the intestine.

Applications

The present study suggests that prokinetic drugs could potentially improve glycemic control in diabetic gastroparesis by allowing a more predictable absorption of nutrients, matched to the action of exogenous insulin. The use of prokinetic drugs, such as erythromycin, may be useful in the clinic for decreasing the need for insulin in diabetic patients. Use of erythromycin may allow the dose of insulin to be safely decreased with in chronic treatments.

Terminology

Gastroparesis is a syndrome characterized by delayed GE in the absence of mechanical obstruction of stomach. Prokinetic drugs enhance gastrointestinal motility by increasing the frequency of contractions in the small intestine or making them stronger, but without disrupting their rhythm. They are used to relieve gastrointestinal symptoms such as abdominal discomfort, bloating, constipation, heart burn, nausea, and vomiting.

Peer review

This manuscript deals with an important issue concerning the use of prokinetic drugs in insulin-treated diabetic individuals. Different prokinetic agents are used alone or in combination with insulin in STZ-induced diabetic mice in order to determine whether there exists any interaction between these drugs in different parameters. The study is interesting and the design is adequate.

REFERENCES

- tients for a relatively long time and some data about the interactions with insulin in the clinic should be available.
- ## Research frontiers
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Steroid ulcers: Any news?

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Abstract

Steroid ulcers, although a common feature in experimental studies, seldom develop in clinical practice, as observed by the meta-analyses carried out in the 90s. Corticosteroids alone become ulcerogenic only if treatment lasts longer than one month and the total administered dose exceeds 1000 mg. On the other hand concomitant intake of non-steroidal anti-inflammatory drugs results in a synergistic, highly damaging effect on the gastroduodenal mucosa. Thus, despite the survival of the steroid ulcer myth in the medical culture, pharmacological protection against steroid-induced peptic ulcers is a rare necessity while the best prophylactic strategy still remains to be determined.

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Key words: Corticosteroids; Peptic ulcer; Proton pump inhibitors

Core tip: Although the myth of steroid ulcers still survives among general practitioners, the incidence of ulcers in patients receiving corticosteroids is so low that concomitant gastric protection is not necessary except in patients on long-term, high-dose steroids or taking concomitant non-steroid anti-inflammatory drugs.

STERIOD ULCERS

The ability of corticosteroids to promote the development of peptic ulcers is an old concept, mainly based on the evidence provided by experimental and pharmacological studies, still widely accepted by clinicians.

A recent survey carried out in the Czech Republic has shown that 82% of physicians believe that cortisone is ulcerogenic^[1].

From an experimental point of view, this has been clearly established and corticosteroids are known to inhibit the biosynthesis of gastric cytoprotective prostaglandins, while suppressing as well the production of gastric damaging leukotrienes.

In animal studies both gastric mucus production and gastric bicarbonate secretion are impaired by steroid administration, which results in a weakening of gastric mucosal defences^[2]. In addition steroids impair both angiogenesis and epithelial repair mechanisms in experimental ulcers^[3-5].

However, for reasons not quite clear, the real incidence of steroid-induced ulcers in the clinical setting is much lower than what could be expected on the basis of the experimental data.

In the past three different meta-analyses have been performed^[6-8], the most recent and larger dating back to 1994^[8] clearly indicates that steroid-treated patients have a relative risk for gastric and duodenal ulcers not significantly different from untreated controls. Unfortunately the quality of the clinical studies employed to perform the analyses was rather poor (no double blind conditions, no information about concomitant medications, no distinction between gastric and duodenal ulcers), which did not allow firm, definitive conclusions.

Thus, in spite of the “definitive” review articles ap-

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peared on the subject^[2,8] the myth of steroid ulcers still survives, also because, during the last twenty years or so, neither additional, perspective clinical studies nor other meta-analyses have been carried out. Admittedly, due to the low incidence of steroid ulcers in the clinical setting, it would be hard to conduct studies in this area unless an extremely large number of patients are recruited. This has not been done and most likely won't ever be done due to the lack of both sufficient interest by the scientific community and commercial motivation by the drug industry.

All in all, it would seem that corticosteroids rather than directly cause ulcers can, in keeping with experimental studies^[3-5], hamper the healing process of ulcers caused by other agents, namely anti-inflammatory drugs (NSAIDs)^[2,9].

Epidemiological studies have proved that NSAIDs are significantly more ulcerogenic than steroids but that the association of the two types of drugs has a truly synergic and lethal effect, increasing of 3 to 6 times the relative risk^[10,11]. Yet corticosteroids themselves can become ulcerogenic if treatment lasts for more than one month, with a total intake higher than 1000 mg of prednisolone^[6]. Elderly people (aged more than 65 years) seem to be more exposed to the risk of developing peptic ulcers.

A recent retrospective study examining the risk of gastrointestinal bleeding with low-dose aspirin alone and in combination with other drugs, has shown that the risk is increased when a high dose (but not low/medium dose) of corticosteroids is co-administered^[12].

Pharmacological prevention of steroid ulcers in clinical practice does not seem, therefore, justified in the large majority of patients under corticosteroid treatment. Subjects undertaking a high-dose long-term steroid administration would deserve concomitant pharmacological "protection", but evidence-based information about the best therapeutic measures is wanting.

Proton pump inhibitors (PPI) are often prescribed but no controlled studies in this area are available.

Indirect evidence, obtained by analyzing sub-populations in NSAID-treated patients suggests that the prostaglandin derivative misoprostol might be effective in counteracting the possible gastric toxicity of cortisone^[13]. However this hypothesis, although consistent with the results of experimental studies on the effects of steroids on ulcer repair^[3] remains largely unproven.

The results of the recent above mentioned survey^[1] show that about 60% of gastroenterologists, compared with only 30% of the other physicians, refrain from prescribing any concomitant "gastroprotective" medication when low doses of steroids are employed. By contrast, when higher doses (*i.e.*, 1 mg/kg prednisone) are prescribed, more than 70% of gastroenterologists and about 90% of the other physicians also carry out empirical pharmacological prevention with PPI.

In conclusion, the body of knowledge on the possible ulcerogenic effects of steroid treatment in humans has

grown very little in the last years. The more recent clinical studies (and subsequent meta-analyses) available in the scientific literature date back to the 90s and the experimental studies performed in the last two decades have added precious little to what was already known in the past.

Due to the apparent lack of interest by clinical researchers, the myth of steroid ulcers, although based on a very weak and disputable clinical evidence, still survives. In daily practice development of peptic ulcers in steroid-treated patients remains a very infrequent event, for which pharmacological protection is seldom required and the most effective drug prevention is still undetermined.

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Inflammatory bowel disease: An archetype disorder of outer environment sensor systems

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Abstract

The pathogenesis of the two inflammatory bowel disease (IBD) phenotypes ulcerative colitis (UC) and Crohn's disease (CD) has remained elusive, thus frustrating attempts at defining a cure. IBD often presents as a complex inflammatory process wherein colon lesions (UC) or widespread ulceration and fissure (CD) might be accompanied by ancillary extra-intestinal manifestations involving the eye, skin, joints or liver, but also by full-blown "autoimmune" disorders from psoriasis and multiple sclerosis to rheumatoid arthritis; attempts at unraveling a link or a hierarchical order in these entities have proven almost fruitless. More recently, the input of genetics has suggested that the IBDs might be multi-organ inflammatory processes, elicited by a large number of low-penetrance susceptibility genes, with environmental factors needed to induce full-blown disease. At a noteworthy exception to this rule, the description of the nucleotide-oligomerization domain (*NOD*) gene mutations in CD came at the beginning of the 2000s: the *NOD-LRR* are part of a highly conserved microbial sensor system which respond to bacterial peptidoglycans by mounting an inflammatory response. At least in Caucasian patients, the prevalently loss-of-function mutation of *NOD* permitted to unexpectedly define CD as an immune deficiency state, and upon its recent description in apparently unrelated disorders such as the

Blau syndrome (a granulomatous pediatric syndrome), and perhaps in psoriasis and chronic obstructive pulmonary disorders, has contributed to revolutionize our view of IBD and CD in particular. The latter affection, together with psoriasis and chronic pulmonary disease can now be included into a newly identified category named "barrier organ disease", wherein a barrier organ is defined as a large mucosal or epithelial surface with an abundant metagenomic microbial population and an underneath reactive tissue, the whole structure being in contact with the outer environment and capable to react to it. Personalized treatments and empowerment of research across different disease phenotypes should be the advantages of this novel mindset.

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Key words: Inflammatory bowel disease; Psoriasis; Chronic pulmonary disease; Innate immunity; Nucleotide oligomerization domains

Core tip: With its extended mucosal surface in contact with the environment, an overly reactive lymphoid tissue underneath, and an extraordinarily abundant metagenomic flora, the gut is in the position to play a central role in the pathogenesis of both its core disorders (inflammatory bowel disease) and remote autoimmune or immunopathic diseases. The IBDs have been listed as a "barrier organ dysfunction". We hereby focus on psoriasis, a barrier organ dysfunction which is often co-morbid with IBD, sharing with it microbial receptor genetic polymorphisms, and response to therapy. This comprehensive mindset shall boost science and drive our medical choices for immunoinflammatory pathology.

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INTRODUCTION

Perturbation of the intestinal homeostasis might be derived from any cause of breach of the active^[1] and passive^[2] barrier defense mechanisms of the lining epithelial cells, allowing contact of gut luminal contents with the overly reactive lymphoid tissue underneath. Despite the accumulating results of years of research in the field, the tenet of the need for a “sealed” epithelium as a primary means to restrain undue gut mucosal inflammation is still informing most of the currently active research. Basically, a perturbed mucosal permeability is thought to represent one of the shared features of the two officially recognized phenotypes of the inflammatory bowel diseases (IBDs), Crohn’s disease (CD) and Ulcerative colitis (UC)^[3], even though significant elements of difference between the two phenotypes are emerging from modern investigation, as discussed below.

Indeed, currently available evidence seems to best be interpreted by seeing the IBDs not as a dichotomy, but rather as a collection of discrete entities that, though sharing several similarities, do appear distinct enough as to call for a conceptual process of “splitting” over one of “lumping up”. The conception of a genotype-serotype-phenotype axis might serve as the spine for such a way of thinking, as illustrated in a few examples that have recently been mastered in an authoritative review^[4]. Polymorphisms of the nucleotide-oligomerization domain 2/caspase activation recruitment domain 15 secondary to mutation of their coding loci (*NOD2/CARD15*) (see below) would preferentially be expressed in ileal CD^[5]; the *HLA-DRB1*0103* allele has been found to correlate with the severity of extra-intestinal disease in UC^[6]; a whole range of anti-glycan antibodies including the widely used anti-saccharomyces cerevisiae antibody (ASCA) have been correlated with different CD behaviors in widespread geographical areas^[7]; finally, racial factors conditioning for example the onset of peri-anal forms of CD have been described^[8]. In the lines to follow, this non-exhaustive list shall be implemented by our own attempt to introduce a concept of the IBDs as centrally conditioned syndromes, insofar as being often diagnosed as co-morbidities of variegated bone marrow anomalies, often affecting the innate arm of the immune response. We shall further discuss that this “splitting drift”, now somewhat prevailing in IBD nosography, can by contrast be mitigated by the breakthrough observation that the IBDs may be intertwined not only between themselves but also with other (apparently unrelated) disorders of remote systems (skin and respiratory organs) insofar as the gut, skin, and lungs are all lining territories between the outer and the inner environments (barrier organ diseases) (please see text below). We shall conclude by presenting evidence that this is not merely an academic exercise, but it can influence medical policies, and our decision making

at patient’s bedside.

AN UPDATED REAPPRAISAL OF THE PATHOPHYSIOLOGY OF IBD: CD

For several years, at the end of the last century, CD has been thought of as being derived from an unbalanced secretion of the pro-inflammatory cytokines tumor necrosis factor- α (TNF- α) and interferon (IFN)- γ , hence a Th-1 mediated disorder^[9]. However, clinical and genetic data accrued early following 2000, have contributed to place the emphasis on phenomena of defective innate immunity, originating the novel concept of CD as an immune deficiency state. Central to this hypothesis were the observations of defective neutrophil accumulation in CD patients^[10], with an impaired clearance of bacteria from tissues. The underlying problem appeared to be a defective macrophage which, though fully capable to form pro-inflammatory cytokines at the translational levels, erroneously trafficked them to premature lysosomal degradation^[11]. Some 5 years before these clinical findings, a keystone genetic insight into the underlying innate immunity defects of CD had been released. Basically, some 40% of CD patients of a Western origin in both the European and American continents were found to carry at least one of three disease-associated variants (*L1007fsinsC*, *G908R*, *R702W*) of *CARD15*, consistently identified as a major susceptibility gene for CD in the caspase recruitment domain^[12]. The intact *CARD15* encodes the NOD2 receptor, a member of a family of intracellular and host-specific cytosolic pattern-recognition receptors, containing a NOD and a leucine-rich repeat. Through this sequence, NOD2/*CARD15* recognizes muramyl-dipeptide (MDP) a component of bacterial cell wall. The ensuing signal transduction cascade then culminates in the serine phosphorylation of the cytoplasmic inhibitor I κ B (which then becomes ubiquitinated and tagged for proteosomal degradation) with following release and activation of NF- κ B (previously inactive as sequestered in the cytoplasmic dimer). Translocated to the nucleus, NF- κ B essentially de-represses the synthesis of several pro-inflammatory cytokines. CD-specific mutations of the *CARD* gene family hampers initial NOD-mediated recognition of MDPs, blunting the beginning of the response cascade^[13]. Whether such genetic faults in fact lead to loss or gain of function has long been debated, with any interpretation being conditioned by the need to account for the excess (not the diminution as would be expected) of inflammation found in CD patients. This question mark seems to be partly met by the hypothesis that TLR-2 driven inhibition of Th-1 responses seems to be lost in *CARD*-mutated CD patients^[14]. *NOD2* genes are also highly expressed in Paneth cells, where their mutations would cause decreased expression of antimicrobial peptides, including alpha-defensins^[15]. Not less importantly, the Familial Mediterranean Fever genes (*MEFV*) have been shown to co-localize with *NODs* on chromosome 16, sharing with the *NODs* the same protein superfamily that regulates

apoptosis, cytokine processing and inflammation^[16]. This might warrant envisaging a kind of a continuum between CD and autoinflammatory syndromes, providing arguments for the discussion below^[17]. The NODs have most recently been shown to act in synergy with cell autophagy systems, a highly conserved chain that normally effects disposal of cell debris and intracellular bacteria^[18]. Wild-type NOD proteins have been shown to serve as nucleating factors for the initiation of bacteria-induced autophagy, by recruiting the *ATG16-L1* gene product to coalesce on cell membrane at the point of bacterial contact. Cell bactericidal activity may be hampered both by a polymorphic NOD becoming incapable to accrue the autophagy proteins, and by mutations of the *ATG16-L1* products themselves^[19].

AN UPDATED VIEW ON THE PATHOPHYSIOLOGY OF UC

The demonstration of a role of innate immunity or of any of the immune effector limbs has been less immediate in the case of UC, lending limited help to the unraveling of its pathogenesis. A recent review^[20] of the cytokine profile of UC has come to list a vast array of cytokines spanning from TNF- α and interleukin (IL)-6 up to IL-33; the lymphocyte subsets Th5 and Th17 seem to be the prevalent functional targets of this cytokine plethora. Of note, a rather robust experimental evidence indicates that the UC epithelial lesions could be derived from the pore-forming effects of IL-13, a secretory product of NK-T cells; in coherence with this tenet, recent experimental evidence has shown that in biopsy specimens of pediatric patients with early UC, STAT6, a transduction element that responds to IL-13, is in a prevalently phosphorylated state^[21]. An interpretation of UC as a disease of increased permeability could be derived from the complex of these premises. If there is no convincing evidence for a role of innate immunity, UC could be most easily interpreted as a T-mediated disorder, considering its preferential response to the T cell modulatory drug cyclosporine^[22].

IBD AND SOME (IMMUNE) DISORDERS SHARE GENETIC POLYMORPHISMS: THE BASIS TO PERCEIVE IBD AS A SYSTEMIC "SYNDROME"

If defects of the innate immune response have convincingly been described in CD, they have also been shown to be under a bi-directional influence: CD-like pictures have been found to often accompany disorders of innate immunity, and, the specific immune abnormalities that are present in CD have been described in other unrelated disorders. The literature on such topics is rather exhaustive. Among the examples of the former instance, chronic granulomatous disease^[23] and glycogen storage disease type-1b^[24] have been mostly characterized. Both disorders

share an impairment of the respiratory burst, preventing neutrophil-mediated killing of bacteria and fungi. The underlying biochemical fault is the absence/malfunction of the nicotinamide adenine dinucleotide phosphate-oxidase enzyme complex. In-depth clinical and pathologic characterization of a subset of CGD patients has notably highlighted that their gut disease meets all of the criteria to make it indistinguishable from "idiopathic" CD^[25]. *CARD15* gene mutations have been found in some cases of early-onset sarcoidosis, and, more strikingly, have been found to be responsible for the Blau syndrome, characterized by a granulomatous inflammation expressing mainly as arthritis, uveitis, and skin rash; the role of such mutations in infectious disease has been more controversial, but an association in patients developing gastric lymphoma during *Helicobacter pylori* infection has been suspected^[26]; perturbed neutrophils but not mutated *CARD15* have been associated with the clinical expression of tuberculosis.

APPROACHING THE CONCEPTS: IBD-ALIKES AND IBD-SYNDROMES

These premises may generate the concept of an IBD-like picture, or of IBD-look-alikes, namely those conditions that mimic IBD, yet are still to be distinguished from so-called "idiopathic" IBD. The modes of this distinction must be conceived as being subject to the power of our diagnostic means, to the parameters being used, whether clinical, biochemical, endoscopic, histologic, and finally genetic; at which of these escalating grounds we decide to limit our escalation, and how high the bar is set within each ground.

To gain more insight into the issue, we searched the PubMed database mainly using IBD-likes, IBD-look-alikes, and IBD mimics as key words; the references of the articles that were retrieved were further manually scanned. This search disclosed an initial paper published in 1984^[27], enumerating and recommending the pathologic features to distinguish true IBDs from so-called "impostors". This report was followed in 2006^[28] by a rather extensive discussion of the IBD-like pictures that may accompany diverticular disease, the so-called segmental colitis associated with diverticular disease (SCAD). The authors emphasized that the contents of the several relevant articles were heterogeneous, with some of them finding morphologic features indistinguishable from CD or UC in a high proportion of the cases, others finding only a minority of such "IBD-alikes", and others denouncing a plethora of non specific lesions. As it is evident from the above paragraphs, a considerable literature has accumulated on this topic, and we have now attempted to identify a few common mechanisms in the genesis of these IBD-like diseases, gathering them in clusters.

We aimed to identify three main cluster mechanisms that are likely to generate an IBD-like picture: errors (often of pediatric relevance) of either of the two arms of the immune response; involvement of the gut from acquired

hematologic disorders or vasculitides; polymorphism of specific genes involved in the immune response, leading, through variegated pathways though, to the final common inflammatory imbalance that marks an IBD. We like to draw particular attention to the role attributed to the polymorphisms of pyrin, a protein domain coded for by chromosome 16 that, together with the TIR and CARD domains, is assumed to belong to the gene superfamily (often ill-defined as CATERPILLER)^[29] of innate immune detection of micro-organisms in mammals. It has been shown that carriers of some pyrin mutations may develop not only familial Mediterranean fever, but, notably, a UC-like disease that turns out to resist conventional treatment, whilst showing response to colchicines^[30].

We believe that from the above presented arguments it stems clear that a plethora of pictures that fit the requirements to be labeled as IBD may be derived from a vast array of factors; to signify this, the Greek-rooted term “syndrome” should be used to progressively replace the term “disease” and the acronym IBD. This change should adequately reflect the tapering of the list of the so-called “idiopathic” IBDs that is likely to occur as our causative knowledge becomes progressively refined^[31].

RE-POSITIONING IBD INTO A BROAD CATEGORY: THE BARRIER ORGAN DEFECTS

If *CARD* gene sequences turn out to be embedded into a highly conserved domain complex that govern the response of mammalian cells to micro-organisms (The TIR, CARD and PYRIN triad)^[32], it seems justified to expect that the clinical expressions of their eventual polymorphisms (*e.g.*, CD) will best be interpreted if contextualized.

Barrier organs and systems

The skin, gastrointestinal tract, and the respiratory epithelia constitute the main components of the barrier systems in the human body. Their structure mainly comprises an epithelial surface or a mucosa extensively interfacing the polluted environment, a basement membrane which, together with a wide array of cell-junction devices, serves to upgrade the sealing state of the system, and, typically, a highly reactive lymphoid tissue underneath, composed of antigen-presenting cells, often classified as dendritic cells, and various lymphocyte subsets capable to react with release of pro-inflammatory cytokines mostly including IL-1, TNF- α and IL-17. The surface of the barrier systems further harbors a huge variety of microbial species, which, at least in the case of the gastrointestinal tract, outweighs the number of somatic cells: this second cell universe within the body is sometimes called “metagenoma”^[33]. When working properly, for instance at the gut level, the sensor machinery that was labeled as CATERPILLER above, serves to maintain the balanced co-existence between the local immune system at the barrier organ and the bacterial flora (with genetically-

based mutations leading to undue inflammation up to the CD phenotype as discussed at the beginning); on the other hand, in the case of a full-blown outer infection, the CATERPILLERS will be called on the battleground to restrain a pathogen that has perforated the epithelial seals.

Two main phenotypes of barrier organ disease correlated with IBD

Representing an extended surface between the body and the outer world, being endowed with an overly reactive immune tissue, and harboring a large microbe metagenome, the skin shows a number of commonalities with the gut archetype. The development of its main disorder, psoriasis, is thought to initiate with a genetic/environmental breach of the sealing epithelium, causing substance loss and psoriatic plaques; this ends up with activating keratinocytes and inducing dendritic cells to produce pro-inflammatory mediators, fueled by degraded RNAs and DNAs as debris of massive cell wreck; in the presence of favoring genetic prerequisites and external factors (stress) the process may easily become chronic with the typical waxing-and-waning course that recalls IBD. Several observations of clinical and genetic orders suggest a link between IBD and psoriasis. Psoriatic skin lesions seem to occur seven times more frequently in CD patients than controls^[34]; ten percent of patients with CD had a first-degree relative with psoriasis^[35]; both disorders do respond well to the T-lymphocyte inhibitor drug cyclosporine. On genetic grounds, a susceptibility area for psoriasis, named *PSORS8* has been identified, in proximity with the CATERPILLER domain, on chromosome 16q21^[36].

The mucosae of the airways represent a second example of obvious analogy, in terms of contact with outer antigens, and functional anatomy of their reactive tissue. To this end, chronic obstructive pulmonary disease (COPD) has received most of the attention^[37]. Available studies have shown that COPD patients might be at an increased risk of developing CD^[38], whereas IBD patients are notoriously prone to exhibit respiratory manifestations^[39]. At a partial contrast with the generally shared opinion that smoking plays a crucial role in causing COPD, clinics do advise concern over several inconsistencies: (1) COPD severity varies widely irrespective of the number of pack years of smoking; and (2) full-blown COPD is found to develop only in 10%-20% of smokers^[40]. Such observations have advised some investigators to focus on genetic studies of COPD, and their efforts have not remained fruitless. Recently, a loss-of-function conformational mutation of NOD2 has been described in COPD patients^[41]. In this paper, non-COPD smokers were appropriately used as controls, in order to exclude a role of smoking per se in the distribution of the NOD2 variant (Figure 1).

CONCLUSION AND CAVEATS

We are excited and interested by seeing that part of our conclusive thoughts might coincide with those of other

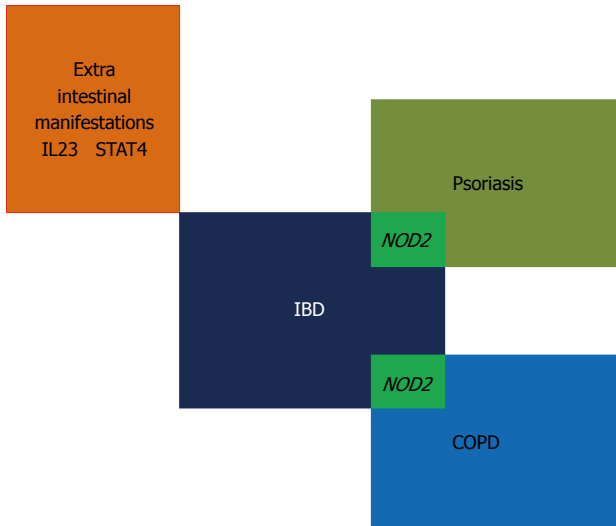


Figure 1 Morbid inflammatory bowel diseases galaxy. As barrier organ dysfunctions (see text) psoriasis and chronic obstructive pulmonary disease are supposed to overlap with inflammatory bowel disease (IBD) insofar as sharing mutations of nucleotide-oligomerization domain 2 (*NOD2*) genes; the so-called extra-intestinal manifestations of IBD might be mediated by interference with polymorphisms of the cytokine interleukin (IL)-23 or signal transduction systems 4 (STAT4); being not a topic in the present text, they are not further commented.

barrier disease researchers *e.g.*, psoriasis^[36], in reckoning that intensifying attempts at modulating surface bacterial flora and unifying research on shared mediators of inflammation can be successful and cost-effective.

Conceiving IBD as a polyfactorial condition may make us more conducive to perceive the disorder hiding behind a given IBD-like picture; the possibly dramatic clinical counterpart of this changed mindset might be the identification of the patient's personalized treatment, or even the only causative treatment (as for example in the case of pseudo-UC incited by the mutated pyrin gene) that can save lives and financial resources.

Caveats

The above data and statements must still be interpreted with caution: it may still take a while before genetic profiling reaches its wide-scale clinical application; as of today, the described anomalies of innate immunity do more readily apply to CD than UC; CD is an extraordinarily heterogeneous affection, as shown for example by failure to detect *NOD* mutations in the disease subtypes that have been characterized in the Far East^[42]. The latter observation has stimulated lively debate in the last several years. An interesting analysis of 2005^[43] has hypothesized that in populations traditionally exposed to highly contaminated environments, defects of the innate immune response may confer a disadvantage and get selected out. However, progressive environment decontamination and use of "aseptic" industrial distribution food enriched in chemical additives yet virtually "sterile", may favor loosening of tolerance originating from continuous stimulation of sensors, thus allowing emergence of unchecked gut inflammation. Thus, one may cast the hypothesis that

lifestyle rather than key genetic defects might drive the defective innate immune responses in Asians with IBD. With their accelerated pace of development, the Far East communities still represent a seminal area of interest and investigation, perhaps allowing to identify causative IBD factors and take preventive measures that in Western countries were not taken on time and got outdated.

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Are medical ethics universal or culture specific

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Abstract

In our society and culture where family is of utmost importance, sometimes I wonder how much of a doctor's duty is to the patient and how much is to the whole family. As a medical student, I remember being told by my professors that we should treat the patient as a whole and not focus on just one problem or organ system. Similarly when practicing medicine in Pakistan, one cannot treat the patient alone and ignore the family. How much should relatives' wishes be taken into account when dealing with a patient? Don't patients have a right to their medical information? When, how, and by whom can that right be waived? What role does culture play when debating medical ethics?

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Key words: Ethics; Culture; Pakistan

Core tip: This paper discusses to role of culture when debating medical ethics and whether cultural norms should be taken into account when applying the principles of medical ethics to a particular society. For example, in a society where the importance of family is paramount, how much should relatives' wishes be taken into account when dealing with a patient?

Ahmed F. Are medical ethics universal or culture specific. *World J Gastrointest Pharmacol Ther* 2013; 4(3): 47-48 Available

After graduating from the Aga Khan University Medical School in Karachi, Pakistan, I moved to the United States for postgraduate training in internal medicine and then gastroenterology and hepatology. Among the many things I learnt in those 10 years was that good medicine entails good communication with patients.

After completing my training in the United States, I began my practice as a gastroenterologist and hepatologist in my hometown of Karachi. One of the first patients I saw in my fledgling practice was a 65-year-old man with advanced esophageal cancer referred to me for palliative esophageal metal stenting. I spent a great deal of time explaining the nature of the disease to the patient and his family, as well as the somewhat limited options he had given the advanced state of his malignancy. I was greeted with blank stares and thought to myself "surely I am not the first one to explain these things to them and the doctor who sent him to me for a palliative procedure must have told them something". The patient and most of his relatives thanked me and left the consultation room. One son stayed back and then angrily asked me "Why did you tell him these things? He didn't know he has cancer! What right do you have to disclose this to him? As his family we know him best and know what is best for him and how much information he can handle." I was dumbfounded and mumbled some apologies. The patient never returned to me for esophageal stenting.

Sine this encounter, I have seen many patients who are unaware of their diagnoses - usually malignancies and occasionally chronic viral hepatitis. In the majority of these cases a relative will poke their head in the door before the patient enters to say that he/she does not know their diagnosis and please do not tell them. When I ask why, the answer I invariably get is: "The patient's spirit is too weak to absorb such news." I have learnt that arguing with such logic is futile. I have had only one rela-

tive ask me if they are doing the right thing in hiding the diagnosis from a patient. The daughter of a patient with stage 4 colorectal cancer asked what I would do if I were in her place and are they doing the right thing in hiding the true nature of her father's illness from him. She also said that I had recently returned from the United States and how are these issues handled in the West. As a doctor, I told her, I felt that it was my duty to tell patients the truth. I told her that if I were the patient, I think I would want to know if my days were numbered so that I could prepare myself, my family, and my affairs for my impending death. I also feel that patients probably know that they are dying and I don't see what avoiding the subject achieves. She listened to what I had to say but I doubt I changed her mind.

There are limits to my concessions to these demands of secrecy. I refuse to do endoscopic procedures in patients who are unaware of the indication and potential risk of the procedure. I take informed consent seriously. Secondly, I refuse to initiate interferon-based antiviral therapy in patients who are unaware of their diagnosis of chronic viral hepatitis. I was quite taken aback the first time I was asked to start a patient on a six month course of thrice weekly interferon injections without telling the patient the true reason for the treatment. When I posited this dilemma to the relative making the request, I was told that the family would handle it and come up with a story to tell the patient. Furthermore, the entire family has to be on the same page. If there is disagreement within the family, then I feel the patient should be told the truth and the family can work out their differences later. I also try not to deliberately lie to patients. At their relative's

requests I may be vague and not voluntarily divulge certain information but if asked directly I don't tell outright lies. What surprised me earlier on was how few of the patients would directly ask me about their diagnosis and life expectancy. As a result of needing self justification for dealing with patients this way, I tell myself that many of these patients themselves do not seem to want to know about their disease. I have yet to have a patient ask me directly what their disease is, saying they suspect their family is hiding something from them. However, I can not always be entirely sure of this. How does one know if a patient does not want to know the truth unless they explicitly say so?

In our society and culture where family is of utmost importance, sometimes I wonder how much of a doctor's duty is to the patient and how much is to the whole family. As a medical student, I remember being told by my professors that we should treat the patient as a whole and not focus on just one problem or organ system. Similarly when practicing medicine in Pakistan, one cannot treat the patient alone and ignore the family.

But I continue to feel conflicted about the issue^[1]. How much should relatives' wishes be taken into account when dealing with a patient? Don't patients have a right to their medical information? When, how, and by whom can that right be waived? By hiding a patient's diagnosis, are we doing good or harm to a patient? Finally, what role does culture play when debating medical ethics?

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Emerging causes of iron deficiency anemia refractory to oral iron supplementation

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Abstract

While oral iron supplementation is commonly used throughout many clinical setting, treatment with intravenous (IV) iron has historically been reserved for specific settings, such as chronic kidney disease, gynecologic issues, and anemia associated with cancer and its treatments. However, the use of IV iron has begun to gain popularity in the treatment of iron deficiency anemia (IDA) associated with two conditions that are being seen more frequently than in years past: patients who are status post gastric bypass procedure and those with inflammatory bowel disease (IBD). The Roux-en-Y procedure involves connecting a gastric pouch to the jejunum, creating a blind loop consisting of distal stomach, duodenum, and proximal jejunum that connects to the Roux limb to form a common tract. IDA occurs in 6%-50% of patients who have undergone a gastric bypass, the etiology being multifactorial. The proximal gastric pouch, the primary site of gastric acid secretion, is bypassed, resulting in a decreased ability to metabolize molecular iron. Once metabolized, most iron is absorbed in the duodenum, which is entirely bypassed. After undergoing bypass procedures, most patients significantly limit their intake of red meat, another factor contributing to post-bypass IDA. Chronic anemia occurs in approximately 1/3 of patients who suffer from IBD,

and almost half of all IBD patients are iron deficient. IBD leads to IDA through multiple mechanisms, including chronic intestinal blood loss, decreased absorption capabilities of the duodenum secondary to inflammation, and an inability of many IBD patients to tolerate the side effects of oral ferrous sulfate. In this study, we reviewed the charts of all patients who received IV iron at Sylvester Comprehensive Cancer Center/University of Miami Hospital Clinic from January 2007 to May 2012. The most common indications for IV iron were for issues related to cancer and its treatment (21.9%), IBD (20.1%), and gastric bypass (15.0%). Of the 262 patients who received IV iron, 230 received iron sucrose and 36 received iron dextran. While doses of 100, 200, 300, and 400 mg of iron sucrose were given, 100 and 200 mg were by far the most common dosages used, 122 and 120 times, respectively. The number of dosages of iron sucrose given ranged from 1 to 46, with a mean of 5.5 and a median of 4 doses. The average dose of iron dextran given was 870.5 mg, with 1000 mg being the most common dosage used. Most patients (22 of 36) who received iron dextran only received one dose. While patients with traditional indications for IV iron, such as gynecologic issues and kidney disease, still were represented in this study, we expect to see a continued increase in physicians using IV iron for emerging gastrointestinal indications, especially considering the increased safety of new low-molecular formulations.

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Key words: Anemia; Iron deficiency anemia; Intravenous iron; Gastric bypass; Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Chemotherapy-associated anemia

Core tip: Decreased absorption of oral iron leading to iron deficiency is a significant cause of anemia in several patient groups, including those status post gastric

bypass surgery and those with inflammatory bowel disease. In these patients, oral iron supplementation is unlikely to correct the deficiency. Intravenous iron is a safe, effective treatment strategy for overcoming the iron deficit seen in these patients, resulting in better outcomes and improved quality of life.

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INTRODUCTION

Intravenous iron has been available for medical use for over 60 years. Traditional indications for its use include medical conditions such as inflammatory bowel disease (IBD), chronic kidney disease (CKD), chronic inflammatory arthritis, congestive heart failure, pregnancy and postpartum state, and cancer, as well as orthopedic, cardiac, colorectal, and gynecologic procedures^[1]. The development of recombinant human erythropoietin (EPO) in the late 1980's led to a renewed interest in its use as combination therapy in the setting of such conditions as CKD and malignancies. In a randomized study of 132 hemodialysis patients, the use of intravenous (IV) iron as an adjunct to EPO led to a greater increase in hemoglobin (Hgb) levels, the need for fewer doses of EPO, and less adverse effects associated with EPO use^[2].

Iron dextran can lead to both local and systemic side effects. The most commonly reported local reactions include pruritus, pain, phlebitis, and muscle necrosis^[3]. A retrospective study of patients who received IV iron for CKD compared the safety of the dextran to the sucrose preparation^[4]. A total of 979 doses of dextran and 504 of sucrose were given, with 3 minor adverse events (AEs) occurring in the dextran group and 1 in the sucrose group. No serious AEs or anaphylactic reactions were reported in either group. Another retrospective study examined 619 patients who had received IV iron over a 2 year period^[5]. Overall, there were 32 reported AEs, but no serious AEs or anaphylactic reactions. Larger retrospective studies have shown the rate of serious AEs with iron dextran to range from 0.0002% to 0.032%, with rates of serious AEs due to iron sucrose much lower^[6]. The primary reason for the increased safety of iron sucrose is likely due to the fact that iron sucrose induces less sensitivity reactions than iron dextran does. A large safety review showed that sucrose induced 3.3 allergy episodes per million doses, while dextran led to 8.7 allergy events per million doses^[7].

The Roux-en-Y procedure, the most commonly used method of gastric bypass surgeries, involves connecting a gastric pouch to the jejunum, creating a blind loop consisting of distal stomach, duodenum, and proximal

jejunum that is connected to the Roux limb to form a common tract. A retrospective study of 150 patients who received gastric bypass found that 36.8% developed anemia^[8]. The mean time from operation to the development of anemia was 20 mo. Almost 50% had a low serum iron concentration. A more recent prospective study followed 348 patients who had undergone gastric bypass for a 10 year period and found that 54% developed anemia, while 47% were iron deficient, with iron deficiency being much more common in women than in men^[9].

While the etiology of iron deficiency anemia (IDA) in this population is often multifactorial, there are three causes that are cited most commonly: avoidance of red meat, diminished gastric acid secretion, and exclusion of the duodenum^[10]. Red meat is the primary source of iron in North America, with heme accounting for two-thirds of total body iron, while molecular iron accounts for the other third^[11]. Studies, as well as common experience, have shown that after patients undergo gastric bypass they are less able to tolerate the intake of red meat. One study of 69 patients found that 39% experienced emesis as a result of eating high fiber meats^[12]. Molecular iron must be solubilized in an acidic environment before it can be absorbed^[11]. In bypass procedures, the proximal gastric pouch, the primary site of gastric acid secretion, is bypassed. In a prospective study of eight patients who underwent a gastric bypass procedure, Behrns *et al*^[13] demonstrated a marked decrease in gastric acid secretion in the stomachs of patients after they had undergone bypass, compared to pre-procedure levels. As a result of this lack of parietal cells, molecular iron is unable to get optimally metabolized. Once metabolized, most iron is absorbed at the duodenal brush border after it has been reduced from its ferric to ferrous form by ferric reductase^[14]. However, in standard Roux-en-Y procedures, the duodenum is entirely bypassed, leading to marked decreased ability to absorb iron.

Other factors that may contribute to iron deficiency include gastritis involving the gastric pouch, esophagitis, and gastric ulcers^[15]. While patients are recommended to take multivitamin supplements after undergoing gastric bypass to prevent nutritional deficiencies, patients may still be at risk for the development of IDA. In a randomized, blinded, prospective study of 56 menstruating women who had recently undergone gastric bypass, Brolin *et al*^[16] found that twice daily ferrous sulfate, at a dose of 320 mg, was able to prevent iron deficiency. However, oral iron tablets are often difficult to tolerate, especially in patients who have undergone gastric bypass procedures, and there is still no consensus on the most effective method to limit the development of iron deficiency in this population.

Current guidelines recommend that patients who have undergone a malabsorptive procedure take 40-65 mg of oral iron daily to prevent the development of iron deficiency^[17]. However, these guidelines acknowledge that patients may have difficulty tolerating oral supplementation and do not account for the fact that many patients

may be iron deficient prior to undergoing the procedure. The guidelines also state that once iron deficiency has developed, patients may be refractory to oral iron, requiring IV iron as a means to replenish their iron stores.

Anemia occurs in approximately 1/3 of patients who suffer from IBD, and almost half of all IBD patients are iron deficient^[18]. Anemia in IBD is due to a combination of chronic intestinal blood loss, decreased absorption capabilities of the duodenum secondary to inflammation, the underlying inflammatory conditions that lead to anemia of chronic disease (ACD), and an inability of many IBD patients to tolerate the side effects of oral ferrous sulfate^[18]. When patients are in an active inflammatory state secondary to their IBD, successfully treating anemia in IBD is significantly more difficult, making control of IBD paramount to the management of anemia in IBD^[19].

Several randomized trials have evaluated the efficacy of iron versus oral supplementation in anemic patients with IBD. Lindgren *et al.*^[20] randomized 91 patients with IBD and anemia to receive oral iron sulfate or IV iron sucrose for 20 wk. The IV iron group tolerated the treatment better and saw a greater amount of patients increase their Hgb by > 2 g/dL (66% to 47%), have a resolution of their anemia (16% to 41%), and reach their reference Hgb level (42% to 22%). Another study randomized 200 patients with anemia and IBD to receive IV or oral iron in a 2:1 ratio^[21]. The study met its primary endpoint, which was to prove non-inferiority of IV iron in increasing Hgb levels over a 12 week course. Of note, that rate of discontinuation of therapy due to AEs was 7.9% in the oral group compared to 1.5% in the IV group.

New guidelines recommend IV iron as first line therapy for IDA in patients with IBD. Absolute indications for the use of IV iron include a hemoglobin < 10 g/dL, intolerance or inappropriate response to oral iron supplementation, severe disease activity, use of EPO, and patient preference^[22]. IV iron leads to a more rapid and prolonged response compared to oral therapy, and is better tolerated and leads to an improved quality of life. Furthermore, recent evidence has shown that oral iron can actually have a deleterious effect in patients with IBD, including an increase in oxidative stress, disease activity, and intestinal inflammation, as well as increasing the risk of colorectal cancer, as seen in animal models^[22]. IV iron is beneficial even in cases where the anemia is attributable to ACD, which is defined as ferritin > 100 µg/L and transferrin saturation < 16% in the setting of anemia^[23].

The combination of EPO plus IV iron has been shown to be an effective method to reduce the need for blood transfusion in patients with cancer who suffer from chemotherapy-induced anemia, as well as ACD. European Organization for Research and Treatment of Cancer (EORTC) guidelines recommend that patients receiving chemotherapy and/or radiotherapy who develop Hgb levels between 9-11 g/dL and display symptoms of anemia be considered for EPO treatment. Patients with Hgb < 9 g/dL will likely need blood transfusions, at least as initial treatment^[24].

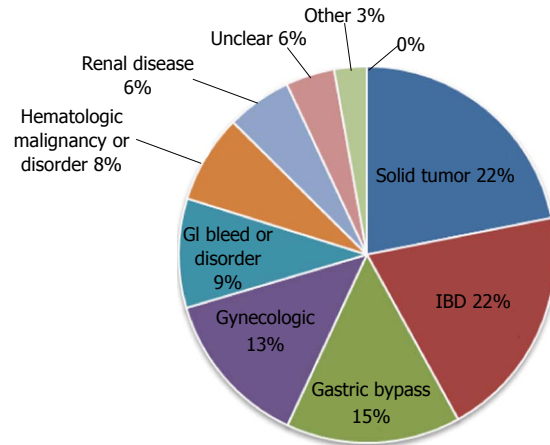


Figure 1 Indications for intravenous iron. IBD: inflammatory bowel disease; GI: Gastrointestinal.

In a randomized trial of 477 women with IDA secondary to heavy uterine bleeding, patients were randomized to receive either weekly IV iron or oral ferrous sulfate 325 mg three times a day for six weeks. Compared to those in the oral repletion group, more patients who received IV iron achieved a > 2 g/dL increase in Hgb (82% to 62%), a > 3 g/dL increase in Hgb (53% to 36%), and a correction of Hgb levels, defined as Hgb > 12 g/dL (73% to 50%), with no serious adverse effects reported in either group^[25].

RESEARCH

After obtaining approval through the University Institutional Review Board, we searched Intellidose, the electronic program that records medication administration, for all instances in which intravenous iron was administered at Sylvester Comprehensive Cancer Center/University of Miami Hospital Clinic from January 2007 to May 2012. We documented the type of iron used, number of administrations, and dosages. We then searched UChart, an electronic medical record used by the university, to ascertain the indications for IV iron based on the patients' known diagnoses.

A total of 262 patients received IV iron. Several patients had multiple indications for IV iron. The most common indications for IV iron were for issues related to cancer and its treatment (21.9%), IBD (20.1%), and gastric bypass (15.0%). Other indications included gynecologic issues (13%), a gastrointestinal bleed or disorder other than IBD (9%), and hematologic malignancies or disorders (8%) (Figure 1 and Table 1).

Of the 262 patients who received IV iron, 230 received iron sucrose and 36 received iron dextran. While doses of 100, 200, 300 and 400 mg of iron sucrose were given, 100 and 200 mg were by far the most common dosages used, 122 and 120 times, respectively. The number of dosages of iron sucrose given ranged from 1 to 46, with a mean of 5.5 and a median of 4 doses. The average dose of iron dextran given was 870.5 mg, with 1000 mg

Table 1 Specific diagnoses that led to administration of intravenous iron

Gastric bypass	43
Inflammatory bowel disease (Crohn's disease, Ulcerative colitis)	58
Gastrointestinal bleed (ulcers, arteriovenous malformation, hemorrhoids, diverticulosis, Cronkhite-Canada syndrome)	16
Gastrectomy (secondary to gastric cancer, Mucosa-associated lymphoid tissue lymphoma)	5
Celiac disease	3
Pernicious anemia	1
Colectomy secondary to Familial adenomatous polyposis	1
Bulimia	1
Small bowel resection- reason unclear	1
Solid tumor (secondary to chemotherapy, due to disease infiltration/progression)	63
Gynecologic (menorrhagia, fibroids, endometriosis)	39
Hematologic malignancy (Hodgkin's, Myelodysplastic Syndrome, Post-transplant lymphoproliferative disorder, myelofibrosis, paraproteinemia, 11 follicular lymphoma, Monoclonal gammopathy of undetermined significance, Acute myeloid leukemia, Chronic myeloid leukemia)	11
Lupus Anti-Coagulant on anti-coagulation, Antiphospholipid syndrome on anti-coagulation	4
Systemic lupus erythematosus	3
Anemia of chronic disease	1
Thalassemia	1
Sickle cell trait	1
Autoimmune hemolytic anemia	1
Jehovah's witness	4
Renal disease (polycystic kidney disease, chronic kidney disease end-stage renal disease)	16
Pregnancy	1
Rheumatoid arthritis	1
Skin wounds	1
Other indication, also on warfarin	5
Unclear on review of records (some have iron deficiency anemia as a diagnosis)	12

Note: Several patients had multiple indications for IV iron; all indications noted in chart are listed above. Data for 262 patients. Patients received treatment at Sylvester Comprehensive Cancer Center in Miami and Kendall.

being the most common dosage used. Most patients (22 of 36) who received iron dextran only received one dose.

CONCLUSION

While we expect IV iron to continue to be used for traditional indications, such as CKD and conditions associated with malignancies, we also expect to see a rise in its use for emerging indications, such as in patients status post gastric bypass procedures and in patients with IBD. Our study supports this claim, as 35% of the patients who received IV iron at our institution received it for one of these two emerging indications. Large studies have demonstrated the safety of iron dextran, and iron sucrose appears to be an even safer alternative. IV iron avoids many of the downsides of oral supplementation, such as decreased GI tolerance, absorption issues, and the ability to correct the deficiency with a short course of treatments, as opposed to long-term oral repletion. IV iron in combination with EPO has also been shown to decrease the need for blood transfusions. While oral iron remains front-line therapy for IDA, we expect to see IV iron used sooner in the course of treatment for GI-related deficiencies. This issue is likely to become more important in the future, as increasing numbers of patients undergo gastric bypass procedures and the prevalence of IBD continues to rise^[26].

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Association of *ITPA* polymorphism with outcomes of peginterferon- α plus ribavirin combination therapy

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METHODS: Patients who underwent Peg-IFN α + RBV combination therapy were enrolled ($n = 120$) and they had no history of other IFN-based treatments. Variation in hemoglobin levels during therapy, cumulative reduction of RBV dose, frequency of treatment withdrawal, and SVR rates were investigated in each *ITPA* genotype.

RESULTS: In patients with *ITPA* CC genotype, hemoglobin decline was significantly greater and the percentage of patients in whom total RBV dose was < 60% of standard and/or treatment was withdrawn was significantly higher compared with CA/AA genotype. However, SVR rates were equivalent between CC and CA/AA genotypes, and within a subset of patients with Interleukin 28B (*IL28B*) (rs8099917) TT genotype, SVR rates tended to be higher in patients with *ITPA* CC genotype, although the difference was not significant.

CONCLUSION: *ITPA* CC genotype was a disadvantageous factor for Peg-IFN α + RBV treatment in relation to completion rates and RBV dose. However, CC genotype was not inferior to CA/AA genotype for SVR rates. When full-length treatment is accomplished, it is plausible that more SVR is achieved in patients with *ITPA* CC variant, especially in a background of *IL28B* TT genotype.

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Key words: Chronic hepatitis C; Interleukin 28B; Inosine triphosphatase; Peginterferon; Ribavirin

Abstract

AIM: To analyze the association between inosine triphosphatase (*ITPA*) (rs1127354) genotypes and sustained virological response (SVR) rates in peginterferon (Peg-IFN) α + ribavirin (RBV) treatment.

Core tip: Inosine triphosphatase (*ITPA*) polymorphism at rs1127354 is significantly associated with hemoglobin decline and reduction of ribavirin (RBV) during peginterferon- α + RBV therapy. However, the effect of the *ITPA* gene single-nucleotide polymorphism on treatment outcome is still unclear. In this study, *ITPA*

CC genotype (rs1127354) was not inferior to CA/AA genotype for sustained virological response rates although CC genotype was a disadvantageous factor for the treatment in relation to completion rates and RBV dose. When full-length treatment is accomplished, the SVR rate tended to be higher in patients with the CC genotype, especially in a subset of patients with the favorable TT genotype (rs8099917) of Interleukin 28B.

Fujino T, Aoyagi Y, Takahashi M, Yada R, Yamamoto N, Ohishi Y, Nishiura A, Kohjima M, Yoshimoto T, Fukuizumi K, Nakashima M, Kato M, Kotoh K, Nakamuta M, Enjoji M. Association of *ITPA* polymorphism with outcomes of peginterferon- α plus ribavirin combination therapy. *World J Gastrointest Pharmacol Ther* 2013; 4(3): 54-60 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v4/i3/54.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v4.i3.54>

INTRODUCTION

Hepatitis C virus (HCV) genotype 1b accounts for around 70% of chronic hepatitis C in Japan^[1,2]. A sustained virological response (SVR) in eliminating HCV RNA by peginterferon (Peg-IFN) α + ribavirin (RBV) combination therapy is attained in 40%-50% of individuals with HCV-1b^[3-5]. Triple therapy using Peg-IFN α + RBV + telaprevir is anticipated to be effective for SVR in approximately 75% of patients with HCV-1b^[6-8]. It is known that polymorphisms located upstream of the Interleukin 28B (*IL28B*) gene, encoding for λ or type III interferon (IFN- λ), are major predictors of SVR in the Peg-IFN α -based combination therapies^[9-12]. Two single-nucleotide polymorphisms (SNPs), rs8099917 TT genotype and rs12979860 CC genotype, have been independently associated with a higher rate of SVR following Peg-IFN α -based combination therapies in individuals with HCV-1b infection. IFN- λ is believed to upregulate the JAK-STAT (Janus kinase-signal transducer and activator of transcription) pathway through interaction with a cellular transmembrane receptor, resulting in antiviral activity. In Japanese individuals, strong linkage disequilibrium is recognized between the two *IL28B* SNPs, rs8099917 and rs12979860, and 99% coincidence has been reported^[13].

The most important adverse events of Peg-IFN α -based combination therapies include RBV-induced hemolytic anemia, which is severe enough to require dose reduction of RBV in 10%-20% of patients, and which may affect overall efficacy^[3]. RBV-induced ATP depletion in red blood cells is believed to be a primary mechanism for RBV-induced hemolytic anemia. A genome-wide association study has shown a strong association between SNPs of the inosine triphosphatase (*ITPA*) gene in chromosome 20 and RBV-induced anemia in patients infected with HCV-1b^[14]. Two functional SNPs, a missense variant in exon 2 (rs1127354) and a splicing altering variant in intron 2 (rs7270101), independently reduce the expression of *ITPA*, leading to inosine deficiency and protection

against RBV-induced ATP depletion^[15-18]. Accordingly, the protective genotypes, rs1127354 CA and AA as well as rs7270101 AC and CC, are associated with decreased *ITPA* activity, which confers protection against RBV-related ATP depletion and hemolytic anemia. The Japanese have the AA genotype exclusively at rs7270101, therefore the CC genotype at rs1127354 is a major predictor of RBV-induced anemia during antiviral combination therapy in Japanese patients infected with HCV-1b^[18,19].

However, it is controversial whether *ITPA* (rs1127354) CC genotype, which induces heavier hemoglobin decline, affects therapeutic outcomes. From the standpoint of health economics, it is important to examine the significance of factors predicting viral response to antiviral treatments and therapeutic outcomes. In this study, Japanese patients infected with HCV-1b, who had experienced Peg-IFN α + RBV combination therapy, were retrospectively analyzed. Patients were divided into groups according to genotyping of *ITPA* rs1127354 and *IL28B* rs8099917. Our primary analysis was focused on the quantitative change from baseline in hemoglobin levels and platelet counts, cumulative reduction of RBV dose, frequency of treatment withdrawal, and estimation of treatment outcome.

MATERIALS AND METHODS

Study patients

This retrospective cohort study was performed in 120 patients with chronic HCV-1b infection who were treated with Peg-IFN α + RBV combination therapy at Kyushu Medical Center Hospital between January 2007 and December 2009. The patients met the following inclusion and exclusion criteria. Inclusion criteria were: (1) baseline serum HCV RNA levels > 5.0 log IU/mL; and (2) Japanese patients aged 20-65 years at study entry. Exclusion criteria were: (1) decompensated liver cirrhosis; (2) serum hepatitis B surface antigen; (3) hepatocellular carcinoma or its history; (4) autoimmune hepatitis, alcoholic liver disease, hemochromatosis, or chronic liver disease other than chronic hepatitis C; (5) chronic renal disease or creatinine clearance < 50 mL/min at baseline; (6) hemoglobin < 12 g/dL, neutrophil < 1500/ μ L or platelets < 100000/ μ L at baseline; and (7) history of receiving IFN-based treatment. All patients gave consent for analysis of SNPs in *ITPA* and *IL28B* genes. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of Kyushu Medical Center. Written informed consent was obtained from each patient.

Antiviral treatment

Peg-IFN α 2b (1.5 μ g/kg) or Peg-IFN α 2a (180 μ g) was injected subcutaneously once weekly. RBV (600-1000 mg/d) was administered after breakfast and dinner. The RBV dose was adjusted by body weight: 600 mg for < 60 kg; 800 mg for 60-80 kg; and 1000 mg for > 80 kg. As a standard combination therapy, Peg-IFN α and RBV were continued for 48 wk. Treatment duration was extended up to

Table 1 Baseline characteristics of patients

Baseline characteristics	<i>ITPA</i> polymorphism (rs1127354)		<i>P</i> value
	CA/AA (<i>n</i> = 37)	CC (<i>n</i> = 83)	
Age (yr)	61 ± 8	59 ± 11	NS
Gender: male/female	18/19	37/46	NS
HCV RNA (log IU/mL)	6.2 ± 0.6	5.9 ± 0.5	NS
Hemoglobin (g/dL)	13.4 ± 1.5	13.8 ± 1.7	NS
WBC ($\times 10^3/\mu\text{L}$)	4.7 ± 1.2	5.0 ± 1.5	NS
Platelet ($\times 10^4/\mu\text{L}$)	18.0 ± 6.0	18.0 ± 7.0	NS
AST (IU/L)	56.8 ± 34.9	58.2 ± 42.3	NS
ALT (IU/L)	65.5 ± 40.0	68.4 ± 56.8	NS
GGT (IU/L)	56.1 ± 52.3	55.3 ± 49.4	NS
AFP (ng/mL)	5.3 ± 4.0	24.2 ± 61.8	NS
Staging: F ₁₂ /F ₃₄	19/16	49/27	NS
<i>IL28B</i> : TT/TG + GG	29/8	53/30	NS

ITPA: Inosine triphosphatase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ -glutamyl transpeptidase; AFP: α -fetoprotein; NS: Not significant; HCV: Hepatitis C virus; *IL28B*: Interleukin 28B.

72 wk in some patients in whom HCV RNA first became undetectable after week 12 but before week 48. SVR was defined as undetectable serum HCV RNA for 24 wk after treatment completion. Rapid virological response (RVR) and early virological response (EVR) were defined as undetectable serum HCV RNA at 4 wk and 12 wk of Peg-IFN α + RBV treatment, respectively. The RBV dose was reduced by 200 mg in patients receiving 600 or 800 mg (by 400 mg in those receiving 1000 mg) when hemoglobin decreased to < 12 g/dL, and by another 200 mg when it was < 10 g/dL. RBV was withdrawn or stopped temporarily when hemoglobin levels decreased to < 8.5 g/dL. Dose of Peg-IFN α 2b (or Peg-IFN α 2a) was reduced by 50% when the leukocyte count decreased to < 1500/ μL , neutrophil count to < 750/ μL , or platelet count to < 80000/ μL ; Peg-IFN α 2b or Peg-IFN α 2a was withdrawn when the above measures were decreased to < 1000/ μL , < 500/ μL or < 50000/ μL , respectively.

Laboratory data

Hematological, biochemical, and virological parameters were determined by the clinical laboratory at Kyushu Medical Center. Serum HCV RNA concentrations were determined by the COBAS TaqMan polymerase chain reaction (PCR) HCV test (Roche Diagnostics, Tokyo, Japan). Genotyping for the *IL28B* (rs8099917) and *ITPA* (rs1127354) polymorphisms was performed by TaqMan SNP Genotyping Assays (Applied Biosystems, Branchburg, NJ, United States) that apply a PCR-based restriction fragment length polymorphism assay.

Statistical analysis

Statistical analysis was performed using JMP software (SAS Institute Inc., Cary, NC, United States). Differences between categorical variables were analyzed using Fisher's exact test or χ^2 test. Mann-Whitney *U* test was used for continuous variables. Multivariate analysis was used to identify factors independently associated with the achievement of SVR.

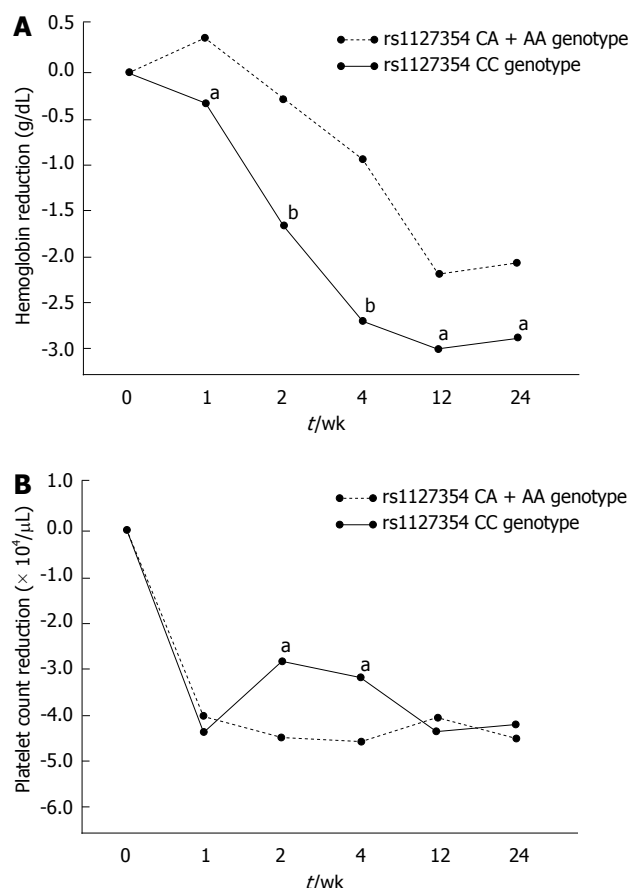


Figure 1 Chronological variation of hemoglobin levels (A) and platelet counts (B) in each inosine triphosphatase genotype at rs1127354. ^a*P* < 0.05, ^b*P* < 0.01 compared with CA/AA groups.

The OR and 95%CI were also calculated. *P* < 0.05 was considered to be statistically significant.

RESULTS

Association between *ITPA* deficiency and hemoglobin decline

Baseline characteristics of 120 enrolled patients are shown in Table 1. The study population included 83 patients with major (CC) genotype and 37 patients with minor (CA/AA) genotype of *ITPA* at rs1127354. Within listed items, no significant difference was seen between *ITPA* CC and CA/AA groups. Chronological variation of hemoglobin levels and platelet count during Peg-IFN α + RBV therapy is shown in Figure 1. As reported previously, hemoglobin decline was obvious in patients with *ITPA* CC genotype (rs1127354) and a significant difference was seen at week 1, 2, 4, 12 and 24 (Figure 1A), meaning that *ITPA* deficiency due to CA/CC genotype was associated with slower hemoglobin decline early in treatment. The greatest difference in mean hemoglobin reduction was found at week 4, while platelet reduction was temporally heavier in patients with *ITPA* CA/AA genotype at week 2 and 4 (Figure 1B). Leukocyte and neutrophil counts were equivalent between *ITPA* genotype CC and CA/AA

Table 2 Sustained virological response rates according to total ribavirin dose in each inosine triphosphatase genotype

<i>ITPA</i> genotype (rs1127354)	Patients with > 60% total RBV dose	Patients with < 60% total RBV dose	Total
CA + AA	48.3% (14/29)	12.5% (1/8)	40.5% (15/37)
CC	58.5% (31/53)	20.0% (6/30)	44.6% (37/83)

Each group includes patients in whom treatment was withdrawn. RBV: Ribavirin; *ITPA*: Inosine triphosphatase.

Table 3 Virological response according to classification by inosine triphosphatase and interleukin 28B single-nucleotide polymorphisms *n* (%)

Virological response	<i>IL28B</i> : TT		<i>IL28B</i> : TG + GG	
	CA + AA (<i>n</i> = 29) ¹	CC (<i>n</i> = 53) ¹	CA + AA (<i>n</i> = 8) ¹	CC (<i>n</i> = 30) ¹
RVR	3 (10.3)	10 (18.9)	0 (0.0)	4 (13.3)
RVR + EVR	18 (62.1)	35 (66.0)	1 (12.5)	8 (26.6)
SVR	13 (44.8)	29 (54.7)	2 (25.0)	8 (26.6)

¹Inosine triphosphatase (*ITPA*). SVR: Sustained virological response; RVR: Rapid virological response; EVR: Early virological response; *IL28B*: Interleukin 28B.

groups during treatment (data not shown).

Treatment outcome in each genotype of *ITPA*

As a result of hepatocellular carcinoma, therapeutic inefficiency, or adverse events, such as depression, appetite loss, easy fatigability, retinal hemorrhage, and hemolytic anemia, Peg-IFN α + RBV therapy was discontinued in 18 patients with *ITPA* CC genotype (21.7%) and 6 patients with CA/AA genotype (16.2%). Moreover, serious reduction of RBV administration (< 60% of scheduled total dose) was compelled in significantly more patients with CC genotype compared with the CA/AA genotype. The percentage of patients receiving < 60% total RBV dose, including patients with treatment interruption/withdrawal, was significantly higher for the CC genotype (37.3% *vs* 21.6%, *P* < 0.05). To investigate the influence of dose reduction of Peg-IFN on treatment outcome, we also analyzed the dose of Peg-IFN administered for each rs1127354 genotype, and > 70% of the expected total dose was administered to all patients with treatment completion (data not shown). SVR rates were analyzed according to the total RBV dose and *ITPA* genotype (Table 2). In the whole population, SVR rates were higher in *ITPA* genotype CC than CA/AA genotype (44.6% *vs* 40.5%), although the difference was not significant. SVR rates tended to be higher for the CC genotype than the CA/AA genotype in patients with > 60% total RBV dose (58.5% *vs* 48.3%) or < 60% total RBV dose (20.0% *vs* 12.5%), but there were no significant differences between the *ITPA* genotypes.

SVR, RVR and EVR rates were determined for *IL28B* (rs8099917) and *ITPA* (rs1127354) genotypes (Table 3). In a subset of patients with *IL28B* TT genotype, RVR, RVR + EVR and SVR showed higher rates in patients

Table 4 Comparison of profile between sustained virological response and non-sustained virological response patients

Factors	SVR (<i>n</i> = 54)	non-SVR (<i>n</i> = 66)	<i>P</i> value
Age (yr)	57 $\pm$ 12	61 $\pm$ 9	< 0.05
Gender: male/female	21/33	33/33	NS
Body mass index (kg/m ²)	23.5 $\pm$ 4.1	22.6 $\pm$ 3.3	NS
HCV RNA (log IU/mL)	5.9 $\pm$ 0.6	6.1 $\pm$ 0.6	< 0.05
Hemoglobin (g/dL)	13.7 $\pm$ 1.3	13.8 $\pm$ 1.8	NS
WBC ($\times 10^3$ /mL)	4.7 $\pm$ 1.3	5.1 $\pm$ 1.5	NS
Platelet ($\times 10^4$ /mL)	20 $\pm$ 7	17 $\pm$ 6	< 0.05
AST (IU/L)	46.2 $\pm$ 25.8	66.7 $\pm$ 47.1	NS
ALT (IU/L)	56.1 $\pm$ 33.3	75.1 $\pm$ 61.1	NS
GGT (IU/L)	39.8 $\pm$ 24.1	67.4 $\pm$ 61.2	NS
AFP (ng/mL)	8.3 $\pm$ 19.8	10.1 $\pm$ 24.2	NS
Staging: F _{1,2} /F _{3,4}	12/40	28/30	< 0.01
72 wk treatment: +/-	10/44	14/52	NS
Ribavirin dose (%) ¹	90 $\pm$ 35	76 $\pm$ 41	NS
<i>ITPA</i> : CC/CA + AA	38/16	45/21	NS
<i>IL28B</i> : TT/TG + GG	44/10	38/28	< 0.01

¹Percentage of ribavirin administration to the scheduled total dose of full-length treatment (48 or 72 wk). SVR: Sustained virological response; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ -glutamyl transpeptidase; AFP: α -fetoprotein; *ITPA*: inosine triphosphatase; NS: Not significant; HCV: Hepatitis C virus; *IL28B*: Interleukin 28B.

Table 5 Multivariate analysis for predictive factors associated with SVR

Factors	Category	95%CI	<i>P</i> value
HCV RNA (log IU/mL)	$\geq$ 6.0: 1.0 < 6.0: 3.94	1.42-10.95	0.008
<i>IL28B</i> (rs8099917)	TG + GG: 1.0 TT: 3.46	1.18-10.10	0.023

HCV: Hepatitis C virus; *IL28B*: Interleukin 28B; SVR: Sustained virological response.

with *ITPA* CC genotype compared with CA/AA genotype, although the difference was not significant. In a subset of patients with *IL28B* TG/GG genotype, SVR rates were equivalent between CC and CA/AA genotypes.

When background of SVR and non-SVR patients was compared, there was a significant difference in age, HCV RNA concentrations, platelet counts, staging, and *IL28B* SNPs, but not in *ITPA* SNPs (Table 4). Table 5 shows the result of multivariate analysis for predictive factors associated with SVR. The multivariate analysis proved that viral load (HCV RNA < 6.0 log IU/mL) and *IL28B* TT (rs8099917) were independent factors for SVR.

DISCUSSION

It has been shown that the SNP (rs8099917) in the *IL28B* gene is strongly associated with response to IFN-based therapy for chronic HCV-1b infection, and the SNP (rs1127354) in the *ITPA* gene predicts RBV-induced anemia in the Japanese population^[19,23]. In this study, patients with *ITPA* (rs1127354) genotype CC showed a higher degree of hemoglobin reduction in response to Peg-IFN α + RBV treatment at week 1, 2, 4, 12 and 24 compared

with those with the CA/AA genotype (Figure 1A). The greatest difference in mean hemoglobin reduction was found at week 4. These findings confirmed the reported evidence that *ITPA* deficiency (rs1127354 CA/AA variants) renders protection against the development of RBV-induced hemoglobin decline in Japanese patients infected with HCV-1b^[20-23]. The exact mechanism by which *ITPA* deficiency protects against RBV-induced hemolysis has yet to be resolved. One postulated mechanism for the development of anemia is the accumulation of triphosphorylated RBV in erythrocytes, causing eventual oxidative damage to erythrocyte membranes, and *ITPA* deficiency may confer protection against RBV-induced ATP reduction by substituting for erythrocyte GTP, which is depleted by RBV in the biosynthesis of ATP^[24-26].

Thrombocytopenia, which leads to poor treatment efficacy because of the initial or early dose reduction of Peg-IFN α , is one of the critical adverse events caused by IFN-based antiviral therapy. A previous study has reported that the *ITPA* (rs1127354) CA/AA genotype is independently associated with a greater reduction in platelet count as well as protection against the reduction in hemoglobin, whereas patients with the CC genotype have significantly less reduction in mean platelet count^[27]. We also evaluated whether genetic variants in the *ITPA* gene were associated with IFN-induced thrombocytopenia. In this study, CC genotype showed lesser trend of reduction at week 2 and 4 compared with CA/AA genotype (Figure 1B). The result may support the association of *ITPA* gene SNP (rs1127354) with platelet decline in response to Peg-IFN α + RBV treatment.

Hemoglobin reduction often necessitates dose reduction of RBV and premature withdrawal from therapy, therefore the *ITPA* (rs1127354) genotype CC may be considered as a disadvantageous factor for Peg-IFN α + RBV treatment. However, although *ITPA* polymorphisms are significantly associated with RBV-induced anemia, their effect on therapeutic outcome is unclear. Some studies have shown no association^[14,28-31], and others have reported a possible association with treatment outcomes in chronic hepatitis C patients^[21,22]. In the present study, although there was no significant association between *ITPA* polymorphisms and treatment outcome, there was a trend towards higher SVR rates in patients with *ITPA* CC genotype, which seemed to contradict previous studies^[21,22,28-31]. The different outcome among the institutes may be due to the difference of inclusion and/or exclusion criteria. In this study, the relationship between *IL28B* and *ITPA* variants were additionally analyzed on treatment outcome. When analyzed in the patients available for treatment outcome, all patients were administered > 70% of the scheduled total Peg-IFN α dose, but the incidence of RBV dose reduction (< 60% of the scheduled dose) and withdrawal was significantly higher in patients with the rs1127354 genotype CC. However, the rate of SVR tended to be higher in patients with the CC genotype, especially in a subset of patients with the favorable TT genotype at rs8099917 of *IL28B*, although the difference was not significant between the CC and CA/AA

genotypes (Tables 2 and 3). Independent favorable predictors for SVR identified in multivariate analysis were low viral load (HCV RNA < 6.0 log IU/mL) and TT genotype at rs8099917 of *IL28B*, but not CC genotype at rs1127354 of *ITPA* (Table 5).

There were several limitations to this study. (1) Because of the small sample size which may have contributed to the loss of significance observed or some statistical errors, this study may be ranked at preliminary status; (2) Because of the retrospective nature of the study, enrolled patients may not represent the standard Japanese population infected with HCV; (3) Several other significant SNPs, which have been detected in *ITPA* as well as *IL28B*, may have influenced and distorted the results; and (4) Mutations in other genes and non-genetic factors that may affect response to antiviral therapy against chronic hepatitis C were not determined.

In conclusion, the SVR rates tended to be higher in patients with the CC genotype than the CA/AA genotype, especially in a subset of patients with *IL28B* (rs8099917) TT genotype, despite a higher rate of RBV dose reduction and treatment withdrawal. Multivariate analysis identified *IL28B* SNP (rs8099917) and HCV RNA as independent predictors of SVR. It is plausible that, in a background of *IL28B* (rs8099917) TT genotype, more SVR is achieved in patients with *ITPA* CC variant when full-length (duration of 48 or 72 wk) treatment is accomplished. These findings indicate that *ITPA* (rs1127354) CC genotype is by no means inferior to the CA/AA genotype for viral response to Peg-IFN + RBV combination therapy.

COMMENTS

Background

A single-nucleotide polymorphism (SNP) at rs1127354 of the inosine triphosphatase (*ITPA*) gene is associated with hemoglobin decline during peginterferon (Peg-IFN) + ribavirin (RBV) combination therapy in patients with hepatitis C virus infection. However, the effect of the *ITPA* gene SNP on treatment outcome has not been fully elucidated. Authors analyzed the association between *ITPA* (rs1127354) genotypes and sustained virological response (SVR) rates in Peg-IFN α + RBV treatment.

Research frontiers

ITPA CC genotype was a disadvantageous factor for Peg-IFN α + RBV treatment in relation to completion rates and RBV dose. However, CC genotype was not inferior to CA/AA genotype for SVR rates. When full-length treatment is accomplished, it is plausible that more SVR is achieved in patients with *ITPA* CC variant, especially in a background of Interleukin 28B (*IL28B*) TT genotype.

Innovations and breakthroughs

In patients with *ITPA* CC genotype, hemoglobin decline was significantly greater and the percentage of patients in whom total RBV dose was < 60% of standard and/or treatment was withdrawn was significantly higher compared with CA/AA genotype. However, SVR rates were equivalent between CC and CA/AA genotypes, and within a subset of patients with *IL28B* (rs8099917) TT genotype, SVR rates tended to be higher in patients with *ITPA* CC genotype, although the difference was not significant.

Peer review

The topic is interesting and relevant. The manuscript is well written and concise.

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Outcome of patients with primary sclerosing cholangitis and ulcerative colitis undergoing colectomy

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Abstract

AIM: To study the outcomes of primary sclerosing cholangitis (PSC) patients with ulcerative colitis (UC) undergoing colectomy.

METHODS: We identified 193 patients with PSC and UC undergoing colectomy at the Mayo Clinic (Rochester, MN, United States), between January 1, 1995 and December 31, 2008 using a computerized record system. Eighty-nine patients were excluded due to unclear diag-

nosis, liver transplantation prior to colectomy, age less than 18 years, inadequate follow-up data or known cases of cholangiocarcinoma. We retrospectively reviewed data from patient medical records. Clinical information, date of colectomy, preoperative and follow-up liver tests and pathological findings of the colon were reviewed. The Mayo risk score at baseline was calculated to obtain survival estimates for up to 4 years of follow-up. The primary endpoint was defined by the presence of all-cause mortality and/or liver decompensation requiring liver transplantation. All patients who did not have a clinical note on December 31, 2008 were considered as patients with an incomplete follow-up unless they reached a study endpoint (death or underwent liver transplantation) prior to that date. The study was approved by the Institutional Review Boards of the Mayo Clinic.

RESULTS: Of the 2441 patients with PSC observed in this period, 104 patients (4.3%) had UC and underwent colectomy and were included. The median age was 43.2 years, and 67% were male. The leading indications for colectomy were severe colonic inflammation (49%), the presence of colonic dysplasia during routine surveillance (42%) and bowel perforation (3%). Twenty-six patients were lost to follow-up after a median duration of 3.9 years. The remaining 78 patients included 52 patients (66.7%) who were followed for a median duration of 5.5 years and 26 patients (33.3%) who developed primary endpoints including death ($n = 13$) or underwent liver transplantation ($n = 13$) with a median follow up of 2.6 years. For the secondary endpoint, the liver complications within 1 mo following the colectomy were found in 9 patients (8.6%) and included worsening liver tests ($n = 3$), liver failure requiring liver transplantation ($n = 2$), acute cholangitis ($n = 3$) and right hepatic vein thrombosis with hepatic infarct ($n = 1$). A multivariate logistic analysis demonstrated that only lower platelet count and lower albumin level preoperatively were significantly associated with more primary endpoints (OR = 0.99 and 0.05 respectively).

CONCLUSION: One third of patients with PSC and UC

undergoing colectomy died or underwent liver transplantation within 2.6 years. PSC patients with lower platelet counts and lower albumin levels were significantly more likely to have a poorer outcome.

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Key words: Prognosis; Colectomy; Primary sclerosing cholangitis; Ulcerative colitis; Outcomes

Core tip: To study the outcomes of primary sclerosing cholangitis (PSC) patients with ulcerative colitis (UC) undergoing colectomy. We identified 193 patients with PSC and UC undergoing colectomy at the Mayo Clinic (Rochester, MN, United States), between January 1, 1995 and December 31, 2008. Eighty-nine patients were excluded. Of the 2441 patients with PSC, 104 patients (4.3%) had UC and underwent colectomy and were included. The median age was 43.2 years. One third of patients with PSC and UC undergoing colectomy died or underwent liver transplantation within 2.6 years. PSC patients with lower platelet counts and lower albumin levels were significantly more likely to have a poorer outcome.

Treeprasertsuk S, Björnsson E, Sinakos E, Weeding E, Lindor KD. Outcome of patients with primary sclerosing cholangitis and ulcerative colitis undergoing colectomy. *World J Gastrointest Pharmacol Ther* 2013; 4(3): 61-68 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v4/i3/61.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v4.i3.61>

INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease and is associated with inflammatory bowel disease (IBD) in 60%-80% of patients^[1-3]. Ulcerative colitis (UC) is more commonly prevalent than Crohn's disease (CD) in patients with PSC^[4,5]. The colitis associated with PSC has unique findings and is usually extensive^[4,6]. UC in patients with PSC is associated with an increased risk of colorectal neoplasia compared to patients with UC alone (OR = 4.8)^[7]. The incidence of colorectal neoplasia at 5 years in PSC patients with IBD is significantly higher than in patients with UC alone (33% *vs* 13%, *P* = 0.054; borderline statistical significance by unmatched log rank test)^[4].

A recent study reported that PSC was the third leading cause (15.4%) of abnormal liver tests among 545 patients with underlying IBD undergoing colectomy with ileal pouch-anal anastomosis (IPAA), after a transient elevation of liver tests (49%) and fatty liver (15.4%)^[8]. Another study evaluated the progression of liver disease after proctocolectomy in patients with PSC and UC^[9]. After proctocolectomy with IPAA, they found that 5 of 30 patients (16.7%) underwent liver transplantation at intervals of 1 to 11 years^[9]. Previous studies have shown that

patients with liver cirrhosis can experience worsening of their liver disease after surgery and poor outcomes^[10,11]. Surgery may lead to severe complications such as decompensated liver disease, worsening of a pre-existing decompensation or even death. Very limited information exists on the prognosis of patients with PSC and UC undergoing colectomy^[12]. We aimed to assess the outcomes and predictors of outcomes of PSC patients undergoing colectomy at the Mayo Clinic, Rochester, MN, United States.

MATERIALS AND METHODS

This was a retrospective study using a computerized record system of patients who had been diagnosed with PSC and UC and were undergoing colectomy at the Mayo Clinic, Rochester, MN, United States, between January 1, 1995 and December 31, 2008. PSC was defined as present when all the following criteria were met: (1) chronic cholestatic disease of at least six months' duration; (2) elevation of serum alkaline phosphatase (ALP) levels; (3) retrograde, operative, percutaneous or magnetic resonance cholangiography demonstrating intrahepatic and/or extrahepatic biliary duct obstruction, beading or narrowing consistent with PSC; and (4) exclusion of secondary sclerosing cholangitis or other causes of cholestatic liver diseases^[3].

A diagnosis of PSC was made using the Hospital International Classification of Disease Adaptation (HICDA) codes of 05760310. A diagnosis of IBD was based on the following HICDA codes: cholangitis, sclerosing (05760310); disease, Crohn's, nos (05630110); enteritis, regional, nos (05630111); ileitis (regional)-see also enteritis (05630112); colitis, Crohn's (05630113); disease, Crohn's, recurrent (05630120); enteritis, regional, recurrent (05630121); colitis, ulcerative, chronic-cuc (05631110); colitis, ulcerative, nos (05631120); colitis, thrombo-ulcerative (05631121); colitis, ulcerative, acute (05631130); colitis, granulomatous (05632110); disease, granulomatous, colon (05632111); disease, inflammatory bowel, nos (05639212). HICDA is an adaptation of the International Classification of Diseases (ICD)-8 for hospital morbidity, which was used at Mayo Clinic to maintain continuity of the Medical Index and the Rochester Epidemiology Project for on-going longitudinal studies^[13]. Of the 2441 patients with PSC, we identified 193 PSC patients with IBD (7.9%) who had undergone colectomy and retrospectively reviewed data from their medical records.

We retrospectively reviewed data from the medical records. A detailed history and physical examination was recorded by a health care provider using standardized protocols. Clinical information, date of colectomy, preoperative and follow-up liver tests and pathological findings of the colon were reviewed. The Mayo risk score at baseline was calculated to obtain survival estimates through up to 4 years of follow-up. The Mayo risk score calculations can be accessed from the web site: <http://www.mayoclinic.org/gi-rst/mayomodel3.html>, and the MELD

model/UNOS modification can be accessed from <http://www.mayoclinic.org/meld/mayomodel6.html>.

Inclusion criteria

We included PSC patients who underwent colectomy and had results of preoperative liver tests and at least one post-operatively. Colectomy cases included open or laparoscopic colectomy. All included patients must have had at least one follow-up visit after the colectomy. The liver tests included total bilirubin, direct bilirubin, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and ALP levels in the serum.

Exclusion criteria

Of the 193 patients with PSC and UC undergoing colectomy, we excluded the patients with the followings: underwent liver transplantation prior to colectomy, inadequate follow-up data, CD, age less than 18 years and known cases of cholangiocarcinoma.

Follow-up data

The primary endpoint was defined as the presence of all-cause mortality and/or liver decompensation requiring liver transplantation and it has been measured at 1 mo and at the end of follow-up. All causes of death listed on the death certificates or pathological findings (underlying, intermediate, immediate and other major conditions) were recorded using the ICD-10 revision.

The secondary end point was defined as the presence of liver complications post-operatively occurred within 1 mo which included ascites, variceal bleeding, clinical hepatic encephalopathy or liver failure and required hospitalization. The worsening liver tests were defined as increases in AST, ALT or total bilirubin to at least 2-fold greater than the baseline values. Other information including the length of hospital stay and general post-operative complications were recorded.

All patients who did not have a clinical note on December 31, 2008 (the end of follow up in this study) were considered as patients with incomplete follow up unless they developed an endpoint (death or underwent liver transplantation) prior to that date. The study was approved by the Institutional Review Boards of the Mayo Clinic, and all participants provided permission for their medical information to be used for research.

Statistical analysis

Statistical analyses were performed with SPSS version 15.0 software. Subjects were categorized by the presence or absence of primary endpoints. Continuous variables were presented as the mean $\pm$ SD or median [interquartile range (IQR)] as appropriate. Comparisons between the two groups were performed using independent *t* tests if values were normally distributed or by the Wilcoxon rank sum test if the distribution was not normal. Categorical data were presented as numbers (percentage) and were compared by Fisher's exact test or the χ^2 test where appropriate. All tests were two sided, and the chosen level

of significance was $P < 0.05$. A logistic regression analysis was used to identify factors significantly associated with the presence of primary endpoints in PSC patients with UC undergoing colectomy. Only variables with a *P* value < 0.1 in a univariate analysis were included in the multivariate analysis. We estimated receiver operating characteristic (ROC) curves of related variables for detection of the primary endpoints in patients with PSC to maximize the area under the curve (AUC).

RESULTS

Clinical features at presentation

Of the 2441 patients with PSC, 193 patients with PSC and UC undergoing colectomy were identified. Eighty-nine patients were excluded due to liver transplantation prior to colectomy ($n = 30$), inadequate follow-up data ($n = 35$), CD ($n = 16$), age less than 18 years ($n = 6$) and known cases of cholangiocarcinoma ($n = 2$). The remaining of 104 patients (4.3% of 2441 PSC patients) were included in this study. The median age was 43.2 years, and 67% were male. The demographic and biochemical data of the 104 patients are shown in Table 1. The median (IQR) Mayo risk score was -0.05 (-0.7, 1.1) while the median (IQR) MELD score was 9 (6, 12). The leading indications for colectomy were severe colonic inflammation (49%), colonic dysplasia observed during routine surveillance (42%) and bowel perforation (3%). Most of the preoperative total bilirubin, direct bilirubin and albumin levels were within normal range, while the mean ALP value was two fold greater than normal.

Clinical outcomes

Table 1 summarizes the postoperative clinical outcomes of the 104 patients with a median (IQR) hospital stay of 7 d (6, 11). Of 104 patients with PSC and UC, 26 were lost to follow-up after a median duration of 3.9 years. The remaining 78 patients included 52 patients (66.7%) who continued follow up, with a median duration of 5.5 years, and 26 patients developed primary endpoints including death or underwent liver transplantation (33.3%), with a median follow up of 2.6 years (Figure 1). The causes of death of the 13 patients were liver-related complications: hepatocellular carcinoma, hepatic renal syndrome and/or liver failure ($n = 4$), metastatic cancer to the liver ($n = 5$), acute cholangitis ($n = 1$), amyloidosis ($n = 1$) and unknown causes ($n = 2$). Two patients died at 10 and 20 d following colectomy. For the secondary endpoint, the liver complications within 1 mo following the colectomy were found in 9 patients (8.6%) and included worsening liver tests ($n = 3$), liver failure requiring liver transplantation ($n = 2$), acute cholangitis ($n = 3$) and right hepatic vein thrombosis with hepatic infarct ($n = 1$) (Table 2). General postoperative complications were found in 36 patients (34.6%) within 1 mo. The most common complications were anemia or blood loss requiring blood transfusion ($n = 11$; 10.6%), intra-abdominal abscess requiring drainage ($n = 4$; 3.8%), acute bowel obstruction

Table 1 Baseline characteristics data, laboratory tests and clinical outcomes of 104 patients with primary sclerosing cholangitis and ulcerative colitis who underwent colectomy

Patient characteristics ¹	Total (n = 104)	Patients who continued follow-up or developed primary endpoints (n = 78)
Baseline characteristics data		
Age at colectomy (yr)	43 (30-53)	42 (28-52)
Gender, male	70 (67)	56 (72)
Race, Caucasian	98 (94)	76 (97)
Presence of advanced liver fibrosis at baseline	27 (32)	24 (39)
Mayo risk score at baseline	-0.05 (-0.7, 1.1)	-0.001 (-0.8, 1.4)
BMI (kg/m ²)	25 (22.6, 28.6)	24.7 (22, 27.4)
Previous use of immunosuppressive drugs	38 (36.5)	29 (37)
History of receiving ursodeoxycholic acid	33 (31.7)	26 (33.3)
Diabetes mellitus or impaired glucose tolerance	7 (7)	6 (7.6)
History of current smoking	2 (2)	2 (2.6)
Indication for colectomy		
Severe colonic inflammation	51 (49)	37 (47)
Colonic dysplasia	44 (42)	36 (46)
Bowel perforation	3 (2.8)	3 (3.8)
Other indications	6 (5.7)	2 (2.6)
Laboratory tests at baseline		
ALT (< 40 U/L)	70 (43, 113)	73 (43, 135)
AST (< 40 U/L)	50 (30, 96)	55 (32, 100)
Albumin (g/dL)	3.9 (3.5, 4.2)	3.9 (3.5, 4.2)
Total bilirubin (mg/dL)	0.7 (0.5, 1.5)	0.8 (0.5, 1.9)
Direct bilirubin (mg/dL)	0.2 (0.1, 0.7)	0.3 (0.1, 0.9)
ALP (U/L)	359 (194, 657)	385 (197, 839)
Glucose (mg/dL)	93 (86, 106)	93.5 (86, 107)
Creatinine (mg/dL)	1.0 (0.9, 1.1)	1.0 (0.9, 1.1)
CA 19-9 (normal < 55 U/mL)	17.2 (8.8, 51)	16.8 (8.5, 51)
Platelet ($\times 10^9$ /L)	289 (201, 350)	289 (205, 351)
INR	1 (0.9, 1.1)	1 (0.9, 1.1)
Clinical outcomes at the end of follow-up		
Undergoing ileal pouch-anal anastomosis	78 (75)	58 (74)
Length of hospital stay (d)	7 (6, 11)	8 (5, 12)
New diagnosis of malignancy after colectomy	32 (30.8)	26 (33.4)
Colorectal cancer	19 (18.3)	13 (16.7)
Other malignancy	13 (12.5)	13 (16.7)
Pathological findings		
Colonic inflammation	62 (60)	45 (57.7)
Presence of colonic dysplasia	24 (23)	21 (27)
Colon cancer	19 (18.3)	12 (15.4)
Post-operative general complications within 1 mo	36 (34.6)	34 (43.6)
Post-operative liver complications within 1 mo	9 (8.7) ²	9 (11.5)
Results of follow-up		
All-cause mortality	13 (12.5)	13 (16.7)
Liver transplantation	13 (12.5)	13 (16.7)
Continued follow-up	52 (50)	52 (66.6)
Lost to follow-up	13 (25.0)	-

¹Median (interquartile range; IQR) or n (%); ²Including worsening liver tests (n = 3), liver failure requiring liver transplantation (n = 2), acute cholangitis (n = 3) and right hepatic vein thrombosis with hepatic infarct (n = 1). BMI: Body mass index; ALT: Alanine transaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; CA 19-9: Cancer antigen 19-9.

requiring re-exploration (n = 4; 3.8%), bowel ileus (n = 4;

3.8%), high ileostomy output (n = 3; 2.8%), wound infection or delayed wound healing (n = 3; 2.8%) and other complications (n = 8; 7.7%) including urinary retention (n = 3), fever with unknown causes (n = 2), acute pancreatitis (n = 1), abdominal pain with unknown causes (n = 1) and portal vein thrombosis (n = 1).

By the end of the follow-up of patients with PSC and UC who underwent colectomy, 13 patients developed colorectal cancer (15%) and 13 patients (16.7%) were diagnosed with other malignancies. The primary location of the malignancies were cholangiocarcinoma (n = 6), hematologic malignancy (n = 4), gallbladder cancer (n = 1), hepatocellular carcinoma (n = 1) and intradural extramedullary spinal cord tumor (n = 1). Colonic dysplasia was found in 21 patients (21.2%) including low-grade dysplasia in 16 and high grade dysplasia in 5.

Predictors for primary endpoints (death or undergoing liver transplantation)

Table 3 shows the comparison of clinical characteristics of the 78 PSC patients with UC who underwent colectomy based on the presence of primary endpoints. Table 4 shows the results of 3 models from the multivariate analysis to identify the best-fit model for predictors of primary endpoints. Model 1 was the best-fit model, which found that only a higher platelet count and higher albumin level preoperatively were significantly associated with fewer primary endpoints (OR = 0.99 and 0.05, respectively; *P* < 0.05). Using the ROC curves for the detection of primary endpoints, we found that a preoperative platelet count of 194×10^9 /L was the best cutoff value based on a sensitivity of 46%, a specificity of 88.5%, a positive predictive value (PPV) of 66.7%, and a negative predictive value (NPV) of 76.7% with an AUC of 0.67. The best cutoff value of the preoperative albumin level for the presence of primary endpoints was 3.7 g/dL with a sensitivity of 73%, a specificity of 82%, a PPV of 70%, an NPV of 84%, and an AUC of 0.80.

Figure 2 shows the survival curve of the 104 patients with PSC and UC who underwent colectomy. The smooth line represents median survival estimates calculated from the Mayo risk scores at baseline, and the stepped line corresponds to survival per the Kaplan-Meier method. The two survival curves were found to significantly differ over this time period (*P* = 0.01) which indicated that PSC patients with UC who underwent colectomy died or required liver transplantation more often than those PSC patients with UC who had no colectomy regarding to the same baseline calculated Mayo risk scores.

DISCUSSION

Our study indicates that one third of PSC patients with UC who underwent colectomy died or required liver transplantation within an average interval of 2.6 years. This result was similar to a previous study from the Cleveland Clinic showing that 38% of cirrhotic patients with PSC who underwent colectomy experienced early

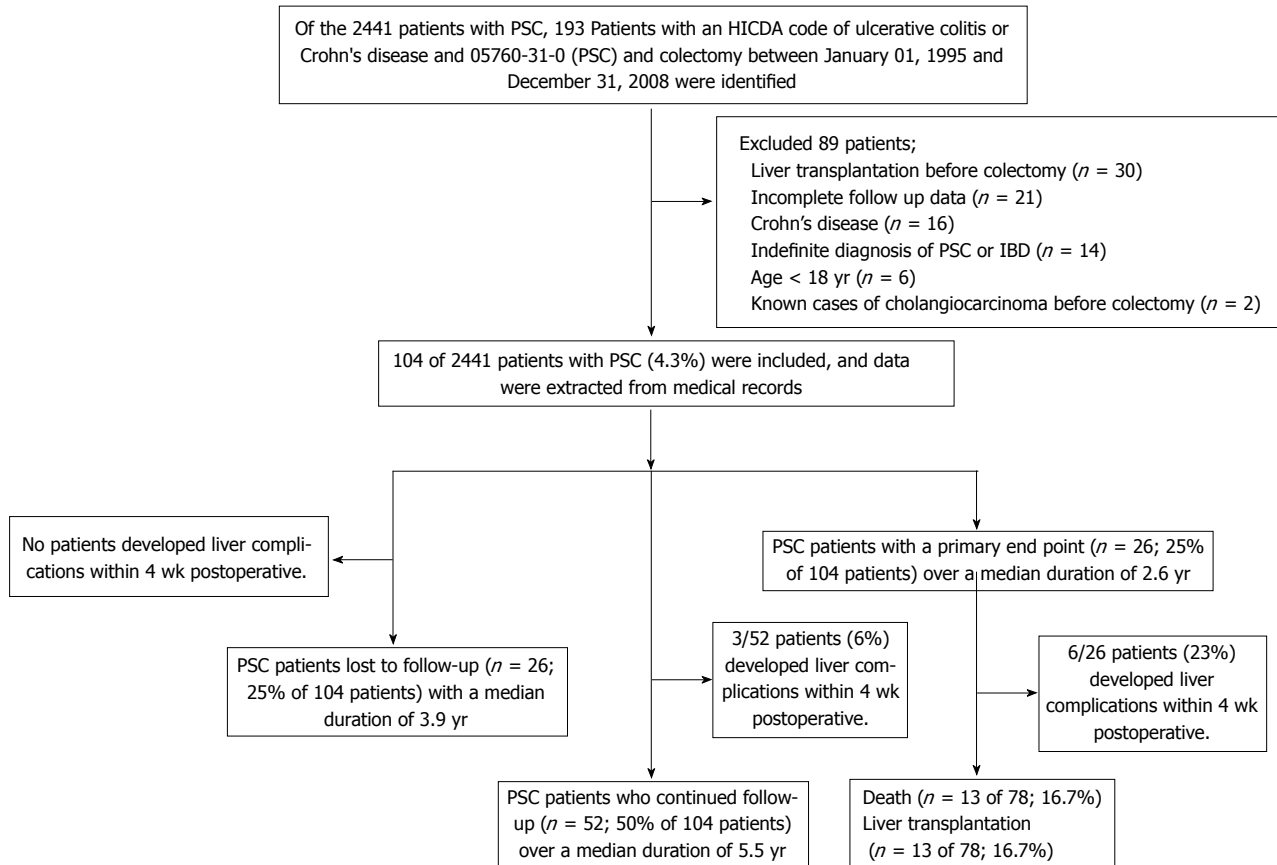


Figure 1 Outcomes of 104 patients with primary sclerosing cholangitis and ulcerative colitis who underwent colectomy. IBD: Inflammatory bowel disease; PSC: Primary sclerosing cholangitis; ICD-10: Hospital international classification of disease adaptation.

Table 2 Nine patients with primary sclerosing cholangitis and ulcerative colitis who underwent colectomy had worsening liver tests postoperatively

Case, Sex	Age at colectomy (yr)	Presence of advanced liver fibrosis	Pathological findings in the colon	Blood loss requiring transfusion	Liver complications	Other complications	Length of stay (d)	Presence of primary endpoints	Duration of follow up (yr)
1, M	28	Yes	Moderate inflammation	No	Worsening liver tests	Abdominal pain, Dehydration	18	No	8.5
2, M	28	Yes	Transverse colon cancer grade 3/4 T3N2	Yes; 2 units	Worsening liver tests	High ileostomy output	15	Death; colon cancer metastasis to liver	1.2
3, F	52	Yes	Mild inflammation	No	Worsening liver tests	Delayed wound healing, Blood loss	8	Death; liver failure	0.3
4, M	32	No	Moderate inflammation	No	Acute cholangitis	None	9	Liver transplant	1.1
5, M	33	Yes	Moderate inflammation	No	Acute cholangitis	None	6	No	8.3
6, F	54	No	Mild inflammation	Yes; 6 units	Liver failure	Severe blood loss, shock	8	Liver transplant; liver failure	0.3
7, F	21	Yes	Necrotized distal ileum with perforation	Yes; 9 units	Liver failure	DIC, Respiratory failure, GI-bleeding	30	Death; liver failure	0.03 (12 d)
8, F	21	No	Moderate inflammation	Yes; 2 units	SMV and hepatic vein thrombosis	Anemia,	15	Death; liver failure	8
9, M	41	No	Moderate inflammation	No	Acute cholangitis	Wound infection	10	No	3.6

M: Male; F: Female; DIC: Disseminated intravascular coagulation; HCC: Hepatocellular carcinoma; SMV: Superior mesenteric vein.

Table 3 A comparison of the clinical characteristics of 78 patients with primary sclerosing cholangitis and ulcerative colitis who underwent colectomy categorized by the presence or absence of primary endpoints

Clinical characteristics ¹	Without primary endpoints (<i>n</i> = 52)	With primary endpoints (<i>n</i> = 26)	<i>P</i> value ²
Gender, %female	11 (21)	11 (42)	0.05 ²
Age at colectomy (yr)	38.7 (27.8, 51.6)	45.8 (29.4, 52)	0.40
Presence of advanced liver fibrosis	12 (23)	12 (46)	0.03 ²
Pre-operative Mayo risk score	-0.1 (-0.9, 0.9)	1.3 (-0.2, 2.2)	0.01 ²
Pre-operative MELD score	7 (6, 9)	14 (11, 18)	< 0.001 ²
History of anemia or blood loss requiring a post-operative blood transfusion within 1 mo	4 (7.7)	7 (27)	0.02 ²
Post-operative liver complications within 1 mo	3 (5.8)	6 (23)	0.02 ²
Length of hospital stay (d)	7 (5, 10)	9 (7, 15)	0.07
Hemoglobin (g/dL)	13.1 (11.9, 14.4)	10.8 (9.8, 13.1)	< 0.001 ²
Platelet count ($\times 10^9$ /L)	296 (247, 357)	244 (126, 337)	0.02 ²
INR	0.9 (0.9, 1.0)	1.1 (1.1, 1.3)	< 0.001 ²
Total bilirubin (mg/dL)	0.7 (0.5, 1.3)	2.3 (0.6, 5.0)	0.001 ²
Direct bilirubin (mg/dL)	0.2 (0.1, 0.4)	0.9 (0.2, 3.5)	0.002 ²
ALP (U/L)	352 (180, 494)	709 (276, 1232)	0.003 ²
AST (U/L)	44 (31, 90)	80 (36, 129)	0.09
Albumin (g/dL)	4.1 (3.4, 4.3)	3.5 (3.3, 3.9)	< 0.001 ²
Duration of follow up from colectomy to the last follow-up (yr)	5.5 (3.8, 8.8)	2.6 (0.8, 5.6)	0.007 ²

¹Median [interquartile range (IQR)] or *n* (%); ²*P* value < 0.05 for primary sclerosing cholangitis patients with or without primary endpoints and those variables with a *P* value < 0.1 in a univariate analysis were included in the multivariate analysis. AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; MELD: Model for End-Stage Liver Disease.

postoperative death compared to 0% of non-cirrhotic patients^[14]. However, the previous study was limited by the small number of included patients with PSC who underwent colectomy (*n* = 24) and the need for a preoperative diagnosis of cirrhosis. The present study builds on previous reports from our center regarding the risk of colectomy in patients with PSC and UC^[15,16].

However, three previous studies reported that proctocolectomy had little effect on the progression of liver disease in patients with PSC and UC and there was no significant difference in the survival of patients undergoing colectomy compared to unoperated patients^[17-20]. A study from England showed that PSC patients who underwent colectomy prior to or concurrent with liver transplantation (*n* = 17) had a mortality rate of 12%, and they concluded that colectomy was a relatively safe procedure and believed that considering colectomy pre-, during, or shortly after liver transplantation in selected patients with risk factors for colorectal cancer would reduce the risk of colorectal cancer^[19]. The low colectomy rate of 4% in our study might reflect the usually quiescent colitis in PSC. The majority of our patients were the large duct PSC which might have an impact on the poorer outcome from liver complications^[9,21,22]. Recently, the outcomes after elective colectomy in patients with cirrhosis were examined and showed that cirrhotic patients undergoing

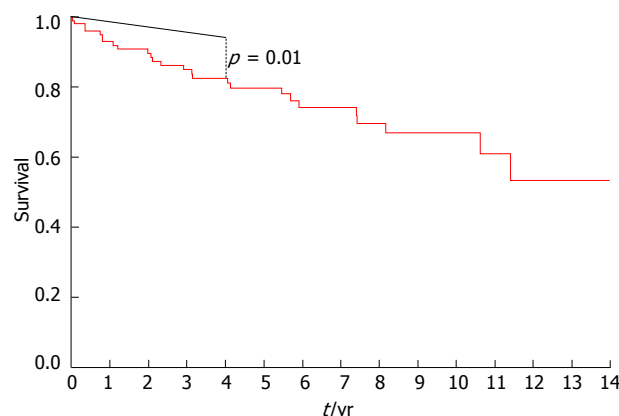


Figure 2 The survival curves of 104 patients with primary sclerosing cholangitis and ulcerative colitis undergoing colectomy. The smooth line represents the median survival estimate calculated from the Mayo risk scores at baseline, and the stepped line corresponds to survival calculated using the Kaplan-Meier method. The two survival curves were found to significantly differ over this time period (*P* = 0.01) which indicated that primary sclerosing cholangitis (PSC) patients with ulcerative colitis who underwent colectomy died or required liver transplantation more often than those PSC patient with ulcerative colitis who had no colectomy regarding to the same baseline calculated Mayo risk scores.

colectomy had a 3.7-fold increased risk of death (HR of 3.7; 95%CI: 2.6-5.2)^[23]. The in-hospital mortality (6%), length of stay (9 d), and total expenses of cirrhotic patients were significantly higher than for those without cirrhosis^[23,24].

Our results showed that a lower platelet count and lower albumin level preoperatively were associated with poorer outcomes. Thus, using simple preoperative blood test screening may provide useful information for monitoring patients pre-operatively. The timing of colectomy is an important issue. Recently, a study from Italy^[20] showed that eight of 16 patients with PSC and UC post-liver transplantation had active colitis despite immunosuppressive medications with a median interval from liver transplantation to colectomy of 6.5 years. Few studies showed that the colitis condition in PSC patients with UC remained inactive or under controlled of at least 60% of cases after orthotopic liver transplantation^[25,26]. Another studied revealed that liver transplantation for PSC independently reduced the need for colectomy (HR = 0.43; 95%CI: 0.25-0.75; *P* = 0.003)^[11]. Additionally, the presence of colon carcinoma and high grade dysplasia were more frequent in the non liver transplantation group and this group of patient had increased inflammation of the colonic mucosa at histology (*P* = 0.011)^[10]. Thus, the patients with severe progressive PSC requiring liver transplantation should proceeded for supportive care of colitis and listed for liver transplantation which might reduced the disease activity of UC and the need for colectomy^[10,11].

About 17% of our PSC patients with UC developed colorectal cancer, which was similar to a previous report from England showing that the cumulative risks of developing colorectal cancer in patients with an intact colon and IBD were 14% and 17% after 5 and 10 years, respec-

Table 4 Multivariate analysis models showing the association between primary sclerosing cholangitis patients with ulcerative colitis who underwent colectomy and the primary endpoints

Multivariate analysis	P value	OR	95%CI
Model 1 ¹			
Platelet ($\times 10^9/L$)	0.030	0.991	0.98-0.999
Albumin (g/dL)	0.006	0.05	0.007-0.44
Model 2 ²			
Pre-operative Mayo risk score	0.390	1.2	0.77-1.97
Hemoglobin (g/dL)	0.010	0.6	0.45-0.91
Model 3 ³			
Pre-operative MELD score	0.090	18.6	0.6-567
Hemoglobin (g/dL)	0.090	0.03	0.001-1.9

¹Model 1, to avoid overestimation of the model, we excluded the Mayo risk score and the Model for End-Stage Liver Disease (MELD) score from model 1; ²Model 2, we included the Mayo risk score in the model and removed the individual variables used for Mayo risk score calculation; ³Model 3, we included the MELD score in the model and removed the individual variables used for MELD score calculation. $P < 0.05$, all variables with $P < 0.1$ in a univariate analysis were included in the multivariate analysis models.

tively^[19]. Recent study showed that the colonic neoplasms that developed in PSC-UC patients were spread throughout the colon on colonoscopy and they were found predominantly on right sided colon^[5]. Thus, surveillance colonoscopy and biopsies should be performed in patients with PSC and UC at 1-year to 2-year intervals^[3].

The main strengths of our study are the inclusion of a large number of PSC patients with PSC and UC and the available clinical data and pathological findings, which were useful for outcome assessment. However, our study is limited by its retrospective nature in a tertiary center, and it contains data derived from multiple physicians from 1995 to 2008, which may have resulted in a selection bias. Additionally, surgeons excluded the colectomy procedure for all patients with poor liver conditions. Second, we included all PSC patients who underwent colectomy and had results from preoperative liver tests and at least one post-operative test, which may explain the small number of patients with liver complications. Thus, further multicenter prospective studies of post-operative liver complications and poor outcomes in patients with PSC and UC undergoing colectomy should be performed to provide clearer guidance for the selection of patients to be referred for a liver transplantation and colectomy rather than colectomy alone.

Unfortunately, we had to exclude a number of patients (10%) who had incomplete data because they were lost to follow-up. Additionally, the Mayo risk score and MELD score could not be calculated annually from our retrospective data therefore the colectomy might changed the progression of the PSC severity which cannot be concluded. Last, we had only a small number of patients with liver complications, and we can therefore not draw a firm conclusion regarding the association between liver complications and poor outcomes.

In conclusion, one third of PSC patients with UC who underwent colectomy died or underwent liver trans-

plantation within an average interval of 2.6 years. PSC patients with advanced liver fibrosis (lower platelet count and lower albumin level) and UC who underwent colectomy were associated with significantly poorer outcomes.

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COMMENTS

Background

The colitis associated with primary sclerosing cholangitis (PSC) has unique findings and is usually extensive. Ulcerative colitis (UC) in patients with PSC is associated with an increased risk of colorectal neoplasia compared to patients with UC alone. Previous studies have shown that patients with liver cirrhosis can experience worsening of their liver disease after surgery and poor outcomes. Surgery may lead to severe complications such as decompensated liver disease, worsening of a pre-existing decompensation or even death. Very limited information exists on the prognosis of patients with PSC and UC undergoing colectomy.

Research frontiers

Authors aimed to assess the outcomes and predictors of outcomes of PSC patients undergoing colectomy at the Mayo Clinic, Rochester, MN, United States.

Innovations and breakthroughs

One third of patients with PSC and UC undergoing colectomy died or underwent liver transplantation within 2.6 years. PSC patients with lower platelet counts and lower albumin levels were significantly more likely to have a poorer outcome.

Applications

PSC patients with UC who underwent colectomy died or required liver transplantation more often than those PSC patients with UC who had no colectomy regarding to the same baseline calculated Mayo risk scores.

Terminology

The primary endpoint was defined as the presence of all-cause mortality and/or liver decompensation requiring liver transplantation and it has been measured at 1 mo and at the end of follow-up. All causes of death listed on the death certificates or pathological findings (underlying, intermediate, immediate and other major conditions) were recorded using the International Classification of Diseases-10 revision. The secondary end point was defined as the presence of liver complications post-operatively occurred within 1 mo which included ascites, variceal bleeding, clinical hepatic encephalopathy or liver failure and required hospitalization. To concisely and accurately describe, define or explain the specific, unique terms that are not familiar to majority of the readers, but are essential for the readers to understand the article.

Peer review

Few studies showed that the colitis condition in PSC patients with UC remained inactive or under controlled of at least 60% of cases after orthotopic liver transplantation. Another studied revealed that liver transplantation for PSC independently reduced the need for colectomy. Additionally, the presence of colon carcinoma and high grade dysplasia were more frequent in the non liver transplantation group and this group of patient had increased inflammation of the colonic mucosa at histology. Thus, the patients with severe progressive PSC requiring liver transplantation should proceeded for supportive care of colitis and listed for liver transplantation which might reduced the disease activity of UC and the need for colectomy.

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Reduced esophageal cancer incidence in statin users, particularly with cyclo-oxygenase inhibition

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Abstract

AIM: To examine the association between statin use and the development of esophageal cancer

METHODS: We performed a systematic review and meta-analysis. Multiple databases (Pubmed, EMBASE, Cochrane Library, Web of Science, Wiley Interscience and Google Scholar) were systematically searched for studies reporting the association of statin use and the development of esophageal cancer. Literature searching and data abstraction were performed independently by two separate researchers. The quality of studies reviewed was evaluated using the Newcastle-Ottawa Quality assessment scale. Meta-analysis on the relationship between statin use and cancer incidence was performed. The effect of the combination of statin plus a cyclo-oxygenase inhibitor was also examined.

RESULTS: Eleven studies met eligibility criteria, 9 high

and 2 medium quality. All were observational studies. Studies examining adenocarcinoma development in Barrett's esophagus included 317 cancers and 1999 controls, population-based studies examining all esophageal cancers included 371203 cancers and 6083150 controls. In the Barrett's population the use of statins (OR = 0.57; 95%CI: 0.43-0.75) and cyclo-oxygenase inhibitors (OR = 0.59; 95%CI: 0.45-0.77) were independently associated with a reduced incidence of adenocarcinoma. Combined use of a statin plus cyclo-oxygenase inhibitor was associated with an even lower adenocarcinoma incidence (OR = 0.26; 95%CI: 0.1-0.68). There was more heterogeneity in the population-based studies but pooled adjusted data showed that statin use was associated with a lower incidence of all combined esophageal cancers (OR = 0.81; 95%CI: 0.75-0.88).

CONCLUSION: Statin use in patients with Barrett's oesophagus is associated with a significantly lower incidence of adenocarcinoma. The chemopreventive actions of statins, especially combined with cyclo-oxygenase inhibitors deserve further exploration.

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Key words: Aspirin; Barrett's oesophagus ; Chemoprevention; Cancer risk; Esophageal carcinoma; Non-steroidal anti-inflammatory drugs; Statins

Core tip: Esophageal cancer remains a major burden upon health. The incidence of esophageal adenocarcinoma has increased dramatically in western countries. Experimental studies have suggested that statins may have useful actions against esophageal cancer cells. This systematic review and meta-analysis of observational studies shows that statin use was associated with a reduced incidence of all esophageal cancers (19% decrease). A more striking reduction in adenocarcinoma incidence in patients with Barrett's esophagus taking statins was seen (43% decrease) and this effect was

enhanced in those also taking cyclo-oxygenase inhibitors (74% decrease). This combination offers promise for chemoprevention and further interventional studies are warranted.

Beales ILP, Hensley A, Loke Y. Reduced esophageal cancer incidence in statin users, particularly with cyclo-oxygenase inhibition. *World J Gastrointest Pharmacol Ther* 2013; 4(3): 69-79 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v4/i3/69.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v4.i3.69>

INTRODUCTION

Esophageal cancer remains an important worldwide problem with high rates of incidence and death as well as considerable morbidity and burdens of treatment^[1,2]. In the developed world, the incidence of esophageal adenocarcinoma (EAC) has increased dramatically over the last 30 years and now outstrips esophageal squamous cell cancer (ESC) in some countries^[3-7]. Although the incidence of squamous cancer appears relatively flat in the developed world it continues to be a major health problem in many places^[2]. Despite improvements in the diagnosis, screening and treatment, the mortality and morbidity of these conditions remains substantial.

Chemoprevention remains one attractive way to reduce the incidence of esophageal cancer. Most of the attention has been devoted to EAC, as this appears to develop in most cases from a pre-malignant phenotype, metaplastic (columnar-lined) esophagus (Barrett's esophagus), providing both a means to study, and intervene in cancer development^[8,9]. At present there are no proven chemotherapeutic agents, although aspirin appears to offer the most attractive combination of risks and benefits, and the results of the large United Kingdom (ASPECT) trial are awaited with interest^[10].

Experimental laboratory studies have suggested that statins, hydroxymethylglutaryl-CoA reductase inhibitors (HMG-CoA), might have useful anti-cancer effects against the progression of Barrett's esophagus and EAC. In our laboratory we have shown that 4 different statins (simvastatin, pravastatin, lovastatin and rosuvastatin) inhibit the proliferation and induce apoptosis in both malignant EAC cell lines (OE33 and Flo-1) and non-malignant QhERT Barrett's cells^[11,12]. These effects appear to be due to inhibition of HMG-CoA reductase, which not only reduces the intermediates which are required for the subsequent formation of cholesterol but also limits the availability for other metabolic intermediates that are required for the prenylation of signalling G-proteins. This prenylation of G-proteins, localises them to the cell membrane where there are key players in pro-proliferative and anti-apoptotic signalling^[13]. We have shown that statins inhibit signalling *via* the ERK and Akt cascades in Barrett's cells, which contribute to the anti-proliferative and pro-apoptotic effects^[11]. Similar effects, albeit with less detailed char-

acterization, have also been reported in other EAC cells (simvastatin in OE19 cells^[14] and simvastatin, and less so atorvastatin, in FLO-1 cells^[15]). Experiments using pharmacological inhibitors and RNA interference have shown that the anti-cancer effect of statins in Barrett's cells seems to be separate from, but additive to, the effects produced by inhibition of the cyclooxygenase (COX)-2/prostaglandin E2 pathway^[11,12]. There is a single laboratory study showing that lovastatin has some modest anti-proliferative and pro-apoptotic effects in TE-8 and SKGT-4 esophageal squamous cancer cell lines^[16].

Although these experimental studies are clearly promising, it is important that these are correlated with clinical outcomes before embarking on either significant change in practice or even an adequately powered randomised trial to further explore these effects.

Although several studies have attempted to explore the association of statin use and esophageal cancer incidence: individually these have often been relatively small and underpowered^[17,18]. To place this in context, a prospective study in patients with Barrett's esophagus would require approximately 4000 subjects followed up 5 for years, assuming a statin use rate of 40% and a cancer incidence of 0.5% per annum, to have 80% power to detect a 50% reduction in cancer incidence. A proportionately larger study would be needed based on the latest and more conservative rates (0.1%-0.3% per annum) of malignant progression in Barrett's esophagus^[19,20]. No individual study has come close to this recruitment. Therefore to further explore the potential cancer-protective effects of statins in esophageal cancer we have performed a systematic review and meta-analysis of published literature examining the association of statin use and esophageal cancer, following the MOOSE guidelines^[21]. Our review of the literature demonstrated two distinct categories of studies: those examining statin use in relation to malignant progression to EAC in Barrett's oesophagus and those examining statin use on a population scale which either combined or did not differentiate between EAC and ESC. We have analysed these separately.

MATERIALS AND METHODS

The Pubmed, EMBASE, Cochrane Library, Web of Science, Wiley Interscience and Google Scholar databases were searched for relevant publications, published in English up to February 1st 2013 using the search terms "esophageal neoplasm," "Barrett's esophagus," "esophageal adenocarcinoma," "statin" and "Hydroxymethylglutaryl-CoA reductase inhibitor." The reference lists of these papers were then hand searched for any additional publications. Randomised controlled studies, case-control studies and prospective cohort studies were eligible for inclusion. Two investigators (Beales ILP, Hensley A) independently reviewed the articles and extracted the data, differences were clarified by discussion and mutual agreement.

Data extraction

The following information was abstracted from the pub-

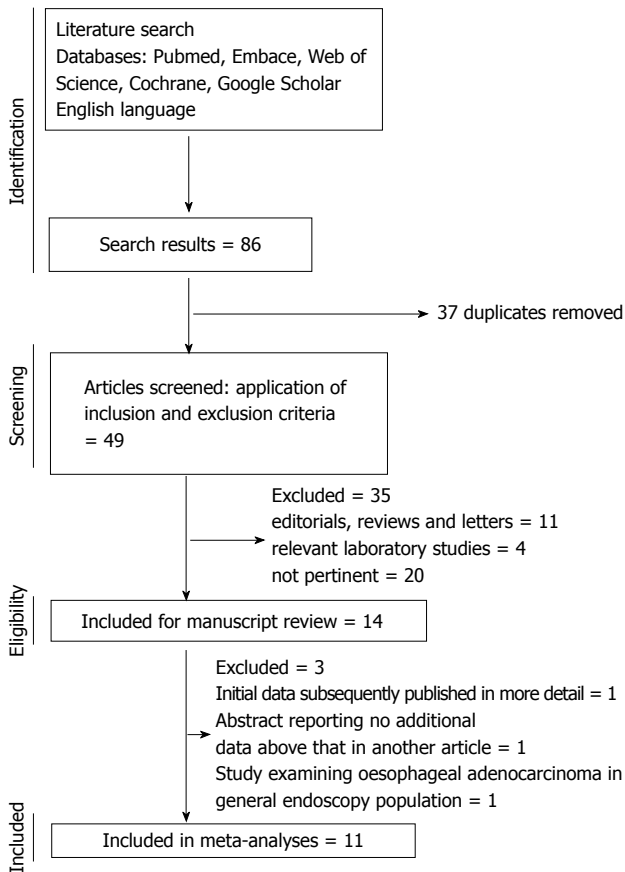


Figure 1 Flow chart showing process of study selection and data extraction.

lications: type of study, numbers of participants, raw data for ever or never use statins and unadjusted and adjusted risk estimates for statins (where available).

Study characteristics

A pre-specified protocol was used to record the following from the eligible studies: authors, journal, participant source, selection criteria, drug exposures of interest, ascertainment of drug exposure and outcome, and confounding factors adjusted for.

Assessment of risk of bias and study quality

We checked the validity of the included studies based on possibility of confounding and potential for misclassification of tumour pathology and/or drug exposure. Risk of bias assessment was focused on the selection of participants, comparability of cases and controls (with any adjustments for confounding), and methods used in ascertaining drug exposure and outcomes. The quality of all studies was assessed using the Newcastle-Ottawa Quality Assessment Scales for cohort and case-control studies using the star rated system as previously described^[22]. In brief, this scores studies in each of 9 categories, with a star rating awarded for high quality in separate areas related to the selection of subjects, comparability of groups, reliability of outcomes and exposures. We regarded 9 stars as high-quality studies, 7-8 stars as medium quality, 5-6 stars as low quality and < 4 stars as very low quality.

Statistical analysis

Review Manager (RevMan) version 5.023 (Nordic Cochrane Center, Copenhagen, Denmark) was used to calculate the pooled risk ratio (based on ORs or hazard ratios from individual studies) using the inverse variance method, random effects model. Statistical heterogeneity was assessed using the Cochrane I^2 statistic, with $I^2 > 25\%$ indicating moderate statistical heterogeneity, and $I^2 > 50\%$ indicating a substantial level of heterogeneity^[23]. A sensitivity analysis was performed by separately omitting one study at a time to assess if the pooled estimate had changed significantly compare to the results of all pooled studies.

RESULTS

The search yielded 146 potentially eligible publications, after exclusion of experimental and animal studies, reviews, editorials and other papers irrelevant to the current study, 14 relevant papers were reviewed and 11 were eligible for inclusion in the meta-analysis. The flow chart of study selection is shown in Figure 1. Of these 11 papers, 10 were published in full^[17,18,24-31] and one only published in abstract form^[32]. Of those excluded from the final analysis, one paper reported initial data^[12] that were subsequently published in full with larger cohorts in 2 subsequent papers and one abstract reported on essentially the same cohort reported in another abstract but with insufficient extra information to be utilised in the meta-analysis (we attempted to clarify the data with the author but received no response)^[33]. One further study was the only one which examined the association between statin use in EAC patients compared to all-comers without cancer^[34], as all other studies examined either EAC in the Barrett's esophagus population or all esophageal cancers in general population, this paper was not analysed in the meta-analysis but the data were extracted for review. Two of the studies included in the meta-analysis involved different methodologies of interrogating the same research database and generated different data sets, hence both were included^[28,31]. The studies reviewed are summarised in Table 1. No randomised studies were identified; 6 case-control studies and 5 cohort studies were included. There was heterogeneity in the methods of presentation of the results between the studies with reference to the adjustment for risk factors; therefore we performed separate meta-analyses on the adjusted and unadjusted ORs. Data on individual statins, dose or duration of exposure were reported too variably to be analysed robustly in the meta-analysis. Overall 9 of the papers were rated as high quality (9 stars out of a possible 9) and 2 of medium quality (7-8 stars out of a possible 9) using the Newcastle-Ottawa scale.

Statin use and esophageal adenocarcinoma

Five separate studies examined the association of statin use with the development of esophageal adenocarcinoma in patients with Barrett's esophagus^[17,18,24-26]. Where high-grade dysplasia was reported as an outcome, this was

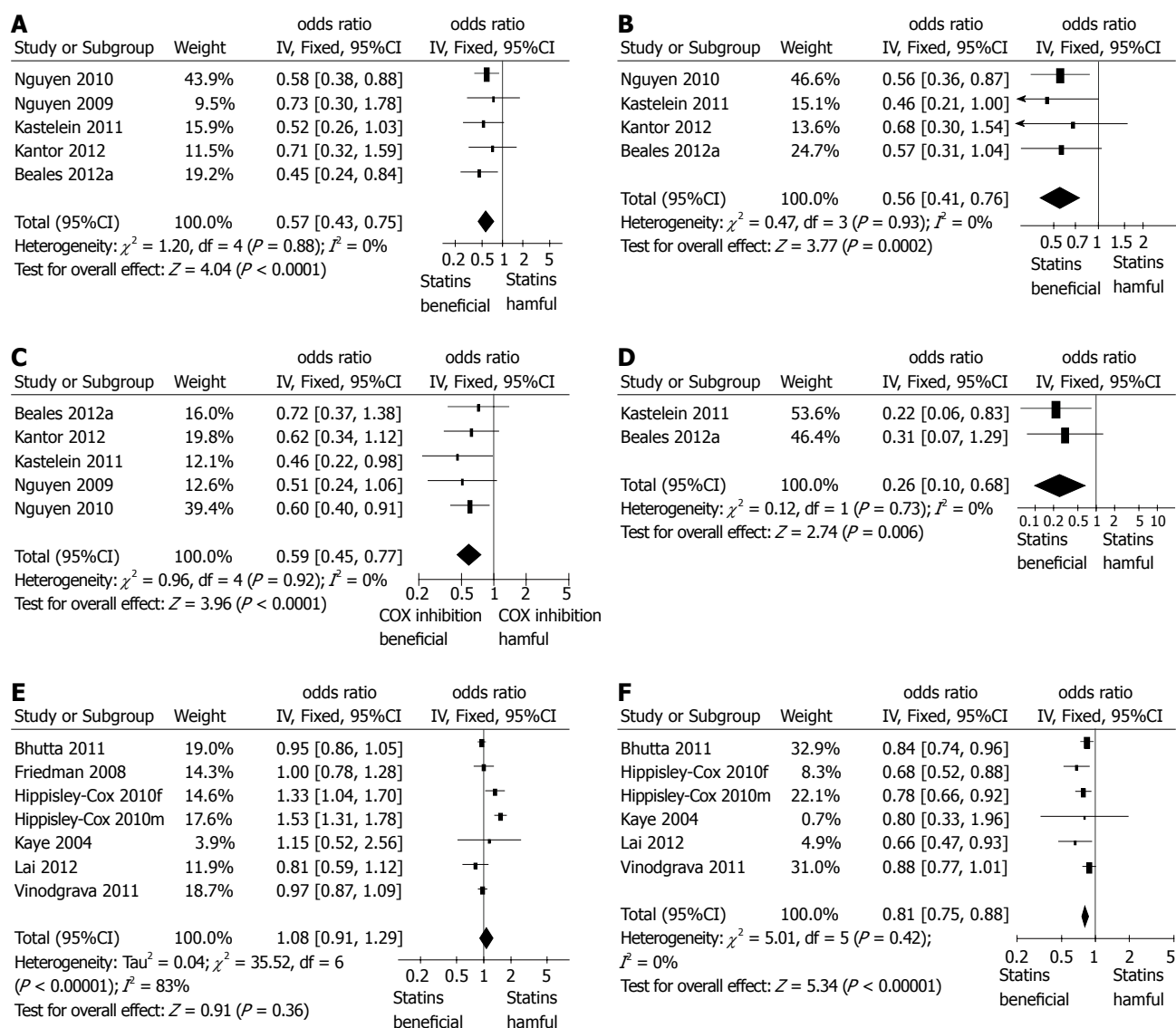


Figure 2 Meta-analysis. A: Meta-analysis of pooled unadjusted odds ratios for the effect of statin use on the development of esophageal adenocarcinoma in patients with Barrett's esophagus; B: Meta-analysis of pooled adjusted odds ratios for the effect of statin use on the development of esophageal adenocarcinoma in patients with Barrett's esophagus; C: Meta-analysis of pooled adjusted odds ratios for the cyclo-oxygenase inhibitor [aspirin, non-steroidal anti-inflammatory drug (NSAID) or coxib] use on the development of esophageal adenocarcinoma in patients with Barrett's esophagus; D: Meta-analysis of pooled adjusted odds ratios for the effect of combined statin and cyclo-oxygenase inhibitor (aspirin, NSAID or coxib) use on the development of esophageal adenocarcinoma in patients with Barrett's esophagus; E: Meta-analysis of pooled unadjusted odds ratios for the effect of statin use on the development of all esophageal carcinomas in population-based studies; F: Meta-analysis of pooled unadjusted odds ratios for the effect of statin use on the development of all esophageal carcinomas in population-based studies.

included with adenocarcinoma for analysis due to the indication for intervention at that stage. Data from a total of 317 adenocarcinomas and 1999 non-cancer Barrett's controls were included in the meta-analysis. In addition, one further study examined the association of statin use in patients with esophageal adenocarcinoma compared to cancer-negative a controls rather than just Barrett's esophagus patients, this study was not included in the meta-analysis^[34].

Meta-analysis of all the 5 studies providing crude, unadjusted ORs showed a significant negative association between statin use and the development of esophageal adenocarcinoma in patients with Barrett's esophagus (OR = 0.57, 95%CI: 0.43-0.75) without any significant heterogeneity in the results (Figure 2A). Very similar results

were seen the meta-analysis using pooled adjusted ORs (combined OR = 0.56; 95%CI: 0.41-0.76), again without heterogeneity (Figure 2B). Sensitivity analysis showed that omitting any single study did not demonstrably alter the results. Interestingly in the additional study examining the association of statins and esophageal adenocarcinoma compared to endoscopy negative controls, not included in the meta-analysis, the unadjusted OR was very similar (adjusted OR = 0.52; 95%CI: 0.27-0.92)^[34].

We also performed a meta-analysis of the relationship between cyclo-oxygenase inhibitor use and esophageal adenocarcinoma development reported in these studies. This showed that the use of aspirin or other cyclo-oxygenase inhibitors was associated with a significantly lower incidence of adenocarcinoma in Barrett patients (OR =

Table 1 The conclusion of the studies reviewed

Study	Setting	Studies	Participants	Ascertainment of statin use for inclusion	Risk estimates for statins (vs no statin) and factors adjusted for	Limitations, notes and quality
Kastelein <i>et al</i> ^[25]	Netherlands, hospital based	Prospective cohort	Cohort of 570 BO, 38 developed EAC or HGD	All statins, statin use during study period, patient interview and questionnaire, pharmacy records	Statin use = 1 mo HR = 0.46 (95%CI: 0.21-0.99); statin use = 5 yr HR = 0.51 (0.18-0.1.47); statin use = 5 yr HR = 0.49 (95%CI: 0.22-0.85); statin plus aspirin HR = 0.22 (95%CI: 0.06-0.85); adjusted for age, sex, length of BO, baseline histology and aspirin use	No adjustment for BMI or smoking; limited categorisation of duration-, and dose-relationship; Newcastle-Ottawa 9 stars
Nguyen <i>et al</i> ^[24]	United States, hospital based, veterans administration	Case-control	116 EAC, 696 BO	All statins, at least 1 filled statin prescription in study period, pharmacy database	At least 1 statin prescription HR = 0.56 (95%CI: 0.36-0.87); statin use < 12 mo HR = 0.63 (95%CI: 0.38-1.06); statin use > 12 mo HR = 0.52 (95%CI: 0.30-0.91); adjusted for race, outpatient encounters, non-cancer comorbidity, use of other medications	97% male, veterans' population; Not adjusted for BMI, alcohol, smoking; no categorisation of dose-relationship; Newcastle-Ottawa 9 stars
Beales <i>et al</i> ^[26]	United Kingdom, hospital based	Case-control	85 EAC, 170 BO	All statins, statin use for > 6 mo prior to cancer diagnosis, questionnaire and clinical and prescribing records	Statin use OR = 0.57 (0.28-0.94); statin and aspirin combined OR = 0.31 (95%CI: 0.04-0.69); adjusted for age, sex, smoking, aspirin, NSAIDs, proton pump inhibitors, BMI, diabetes mellitus, metformin, alcohol; significant negative associations with statin dose and duration.	Cancers were a mix of <i>de novo</i> and screening-detected cancers; Newcastle-Ottawa 8 stars
Beales <i>et al</i> ^[24]	United Kingdom, hospital based	Case-control	112 EAC, 448 cancer negative gastroenterology outpatients	All statins, statin use for > 6 mo prior to cancer diagnosis, questionnaire and clinical and prescribing records	Statin use OR = 0.52 (95%CI: 0.27-0.92); statin and aspirin combined OR 0.27 (95%CI: 0.05-0.67); adjusted for age, sex, smoking, aspirin, NSAIDs, proton pump inhibitors, BMI, alcohol, diabetes mellitus, metformin; United Kingdom, population based	Controls were hospital outpatients; Newcastle-Ottawa 8 stars
Fang <i>et al</i> ^[12]	United Kingdom, hospital based	Case-control	EAC 63, cancer-negative gastroenterology outpatients 252	All statins, statin use for > 6 mo prior to cancer diagnosis, questionnaire and clinical and prescribing records	Unadjusted statin OR = 0.42 (95%CI: 0.19-0.89); unadjusted statin plus aspirin OR = 0.11 (95%CI: 0.01-0.82)	Controls were hospital outpatients. Unadjusted for any risk factors; not included in meta-analysis as more extensive dataset published subsequently; not quality rated
Kantor <i>et al</i> ^[18]	United States	Prospective cohort	BO 411 in cohort, EAC developed in 56	All statins, any statin use during study period, questionnaire	Statin use OR = 0.68 (95%CI: 0.30-1.54); adjusted for sex, age, smoking, NSAIDs	No adjustment for BMI; no data on dose or duration relationship. Included any use of statin, Relatively low incidence of statin use in BO population; Newcastle-Ottawa 9 stars
Nguyen <i>et al</i> ^[17]	United States, hospital based, veterans administration	Retrospective cohort	BO 344 in cohort, EAC or HGD developed in 33	All statins, any statin prescription during the period of study, pharmacy and clinical records	Statin use OR = 0.73 (95%CI: 0.30-1.78), unadjusted	94% male, veterans population. Incomplete adjustment for potential confounding factors; Newcastle-Ottawa 8 stars
Bhutta <i>et al</i> ^[32]	United Kingdom, population based	Case-control	4242 cancers, 17233 controls	All statins, statin prescription for 10 mo in the year preceding diagnosis of cancer; read codes within GPRD	Use of statins OR = 0.84 (95%CI: 0.73-0.95); adjusted for BMI, smoking, aspirin, NSAIDs, proton pump inhibitors, vasodilators	No categorisation of statin dose; related to Hippisley-Cox 2010 but different methodology to interrogate the same research database; Newcastle-Ottawa 9 stars
Vinogradova <i>et al</i> ^[28]	United Kingdom, population based	Case-control	3159 cancers, 13041 controls	All statins, statin use as defined by 2 prescriptions over a 5 year period at least 12 mo prior to cancer diagnosis; read codes within QResearch database	Use of statins OR = 0.88 (95%CI: 0.77-1.01); adjusted for Townsend score, smoking, circulatory disease, diabetes mellitus, rheumatoid arthritis, COX-2 inhibitors	Data for EAC and ESC combined; no individual confirmation of pathology. No categorisation of statin dose; related to Hippisley-Cox 2010 but different methodology to interrogate the same research database; Newcastle-Ottawa 9 stars

Hippisley-Cox <i>et al</i> ^[31]	United Kingdom, population based	Prospective cohort	1809 cancers, 2004692 overall participants	All statins, new users of statins defined by a new statin prescription in the study period; read codes within QResearch database	Men, statin HR = 0.78 (95%CI: 0.66-0.91); women, statin HR 0.68 (95%CI: 0.52-0.88); adjusted for, age, BMI, smoking, townsend score, type 2 diabetes	Data for EAC and ESC combined. No individual confirmation of pathology; no adjustment for aspirin or NSAIDs; no data on duration or long term statin exposure; Newcastle-Ottawa 9 stars
Kaye <i>et al</i> ^[29]	United Kingdom, population based	Case-control	100 cancers, 430 controls	All statins, current use defined as a statin prescription that started within 12 mo of cancer diagnosis; read codes within GPRD	Statin use OR = 0.80 (95%CI: 0.30-1.80); adjusted for smoking, BMI, number of GP visits	Data for EAC and ESC combined. No individual confirmation of pathology; no adjustment for aspirin or NSAIDs; no data on duration or long term statin exposure; Newcastle-Ottawa 9 stars
Friedman <i>et al</i> ^[30]	United States, population based	Retrospective cohort	68 cancers, 4413032 controls	All statin, any statin use prior to cancer diagnosis, Kaiser Permanente Cancer Registry and Pharmacy management systems	Overall unadjusted statin use OR = 1.0 (95%CI: 0.77-1.27); men with > 5 yr statin use OR = 1.70 (95%CI: 1.05-12.75).	Data for EAC and ESC combined; no individual confirmation of pathology; no dose-effect relationship examined; no correction for confounding variables; small number of cancers; Newcastle-Ottawa 9 stars
Lai <i>et al</i> ^[27]	Taiwan, population based	Case-control	549 cancers, 2196 controls.	All statins, statin prescription prior to cancer diagnosis; data from Taiwanese NHI programme	Statin use OR = 0.66 (0.45-0.95); atorvastatin = 12 mo OR = 0.14 (95%CI: 0.04-0.56); adjusted for esophageal diseases, <i>H. pylori</i> infection, alcoholism, smoking, lipid lowering drugs, proton pump inhibitors, H2RA, NSAIDs and aspirin	Data for EAC and ESC combined; no individual confirmation of pathology; no dose-effect relationship examined; Newcastle-Ottawa 9 stars
Bhutta <i>et al</i> ^[31]	United Kingdom, population based	Case-control	Not clearly defined	Not clearly defined; read codes within general practice research database	Statin use OR for EAC 0.61 (95%CI: 0.35-0.94), OR for ESC (95%CI: 0.21-0.80); unclear what adjustments applied	No individual confirmation of pathology; insufficient data for inclusion in meta-analysis appears to be essentially the same cohort as Bhutta 2011; no response from author when asked for further information; not quality rated or included

EAC: Esophageal adenocarcinoma; HGD: High-grade dysplasia; ESC: Esophageal squamous cell cancer; H2RA: Histamine-2 receptor antagonist; NSAIDs: Non-steroidal anti-inflammatory drug; *H. pylori*: Helicobacter pylori; NHI: National Health Insurance; BMI: Body mass index; COX-2: Cyclooxygenase-2.

0.59, 95%CI: 0.45-0.77), again without any heterogeneity (Figure 2C).

Two of the studies specifically reported the association of the combination of cyclo-oxygenase inhibitors (aspirin and/or non-steroidal anti-inflammatory drug, NSAIDs/coxibs) with a statin and esophageal adenocarcinoma in patients with Barrett's esophagus^[25,26]: as shown in Figure 2D this combination was associated with a significantly lower incidence of esophageal adenocarcinoma (OR = 0.26; 95%CI: 0.1-0.68), that seen with either statins or aspirin/NSAIDs alone (Figure 2D).

Only one of the five studies reported data on statin dose: in this one study, higher doses (greater than 40 mg simvastatin or equivalent daily) were associated with a lower incidence of EAC compared to lower doses^[26].

Similarly there were inconsistencies in the reporting of duration of statin use: Kastelein *et al*^[25] reported no difference with either more or less than 5 years use of statin (OR both approximately 0.50), whilst Nguyen *et al* reported that more than one year of statin was associated with a lower incidence of EAC [corrected incidence density ratio (0.52, 95%CI: 0.30-0.91)] than use for less than 12 mo [corrected incidence density ratio (0.63, 95%CI:

0.38-1.06)]^[24]. Beales *et al*^[26] reported that more than 5 years of statin use (OR = 0.41; 95%CI: 0.15-0.85) was associated with lower incidence of EAC than use for less than 2 years (OR = 0.77; 95%CI: 0.29-1.87).

Statin use and all esophageal cancers

A total of 6 studies reported the association of statin use and all cancers of the esophagus^[27-32]. These were all population-based studies utilizing databases, without any individual confirmation of the precise pathology involved. There were no studies specifically examining the relationship between statins and squamous cell cancer. One study reported separate data for men and women and these were included separately in the meta-analysis^[31]. Data from a total of 371203 esophageal cancers and 6083150 controls were included in this meta-analysis. There was considerable and significant heterogeneity in the data for unadjusted OR: overall there was no association of statin use and esophageal cancer (OR = 1.08; 95%CI: 0.91-1.29, $I^2 = 83\%$) (Figure 2E). There was less heterogeneity in the pooled adjusted OR (pooled OR = 0.81; 95%CI: 0.75-0.88, $I^2 = 0\%$) which showed a significant negative association between statin use and the incidence of all

esophageal cancers (Figure 2F). Sensitivity analysis of the pooled adjusted data showed that omission of any one single study did not alter the overall effects.

Again data on dose, duration and individual statins were inconsistently presented and formal meta-analysis of these data is problematical if not impossible. Vinogradova *et al*^[28] reported that the OR for less than 12 mo statin use (OR = 0.90; 95%CI: 0.67-1.20) was similar to that in those using statins for greater than 73 mo (OR = 1.03; 95%CI: 0.07-1.52). Lai *et al*^[27] reported that use atorvastatin but not other statins for greater than 12 mo was associated with a significantly reduced incidence of esophageal cancers, (adjusted OR = 0.14; 95%CI: 0.04-0.56). Sub-groups analysis of the study by Hippisley-Cox and Coupland^[51] showed that there seemed to be a dose-response relationship but only in men: low simvastatin dose (10/20 mg), (adjusted OR = 0.91; 95%CI: 0.73-1.12), compared to high dose (40/80 mg) (adjusted OR = 0.66; 95%CI: 0.48-0.91). Statin dose-relationships were not reported in the other studies.

DISCUSSION

Our meta-analysis has confirmed a significant negative association between the use of statins and a reduced incidence of esophageal adenocarcinoma in patients with Barrett's esophagus. This suggests that statins may have important chemopreventive effects that should now be explored further in interventional studies. The results from all 5 studies are consistent with each other, with no statistical heterogeneity.

Our data have consistency with those previously published: the pooled adjusted OR for cyclo-oxygenase inhibitor use (combined aspirin, NSAIDs and coxibs) in the 5 studies of adenocarcinoma development in Barrett's esophagus is 0.59 (95%CI: 0.45-0.77). This result is consistent with previous studies and meta-analysis^[35-37], although other studies have failed to show a negative association between cyclooxygenase inhibitor use and adenocarcinoma development in Barrett's esophagus^[38]. Within these current studies there were sufficient data to perform a meta-analysis on the combined effects of statin and cyclooxygenase inhibitor usage and this showed that the combination was associated with a greater reduction in adenocarcinoma incidence. These findings are consistent with the laboratory data in Barrett's cancer and non-cancer cell lines, where the anti-proliferative and pro-apoptotic effects of statins are mechanistically both separate from, and additive to, the effects of pharmacological inhibitors of the COX/prostaglandin production pathway^[11,12,14]. Our data strongly support further experimental and interventional studies exploring the combination of aspirin and statin for chemoprevention. Further studies are required to define which of the various families of cyclo-oxygenase inhibitors have the greatest negative association with EAC. The available do not allow differentiation between traditional NSAIDs, coxibs and aspirin.

All the studies included in the meta-analysis were observational in nature, and despite the consistency of results the possibility of bias must still be considered. The pooled adjusted and adjusted ORs both showed that statin use is associated with a lower incidence of adenocarcinoma in Barrett's esophagus, but it is possible that a degree of confounding by uncorrected factors remains. In general the known risk factors that direct the clinical use of statins (risk of circulatory disease, obesity, smoking *etc.*) also increase the risk of adenocarcinoma development, which would tend to diminish the apparent protective effects of statins^[39-41]. It is possible that other factors related to the use of statins within a cohort of Barrett's patients (perhaps some dietary factor) may have led to residual confounding. However the consistency of the results in 5 geographically distinct cohorts suggests that this is not likely to be a significant effect. Singh *et al*^[42] recently published a similar meta-analysis examining statins and esophageal cancers, although with slightly different inclusion criteria and an earlier cut-off point for the literature review. The results are very similar to ours: in that study statin use was associated with a reduced incidence of adenocarcinoma in Barrett's esophagus [pooled unadjusted OR = 0.57 (95%CI: 0.44-0.75), pooled adjusted OR = 0.59 (95%CI: 0.45-0.78)]. Whilst further suitably sized randomized studies are required to fully inform choices over statin and aspirin use as chemopreventive agents in Barrett's esophagus, the currently available data do suggest that these should certainly be prescribed to Barrett's patients with increased risk of circulatory diseases.

Despite the meta-analysis including over 300 cancers and nearly 2000 Barrett's non-cancer controls, there are insufficient data on the dose- and duration-relationships, in the negative association between statin use and adenocarcinoma development. These areas require further investigation. There are also insufficient data on either individual statins or lipophilic versus hydrophilic statins. All 5 studies grouped all statins together and only in one were individual drugs examined. These areas also require important follow up studies. Based on available data, the most plausible mechanism underlying the chemopreventive effect of statins is inhibition of the mevalonate synthetic pathway and subsequent reduction in the availability of functional signalling mediators that promote proliferation and inhibit apoptosis within the Barrett's epithelium^[11,13]. The cell line studies suggest that these effects are mediated by statins at the level of the Barrett's epithelial cells, but the contribution of overall reduction in mevalonate pathway synthetic function (predominantly in the liver) to any esophageal clinical effects remain to be explored. This may have some bearing as lipophilic statins (simvastatin and atorvastatin) are thought to be able to enter all cells by passive diffusion, whereas the hydrophilic statins (pravastatin and rosuvastatin) require the presence of an active transport mechanism^[43]. The latter is expressed by hepatocytes and not usually in other cells (although to our knowledge has not been specifically explored in normal and pathological esophageal epitheli-

um)^[44]. In addition other mechanisms such as altered adipokine secretion or altered inflammatory responses could contribute to the possible protective effects of statins against esophageal adenocarcinoma^[45] and the individual statins or their chemical properties, such as intrinsic antioxidants effects, could be important determinants of these effects^[43].

These current clinical data are important when discussing the mechanistic cell-line studies: some of the latter are open to, (perhaps valid) criticism that the statin concentrations employed *in vitro* (often significantly greater than 1 mmol/L)^[46] are rather higher than those generally seen with *in vivo* therapeutic use (in the nmol/L range)^[47,48]. The correlation of positive clinical and laboratory studies is supportive of a chemopreventive effect of statins against EAC.

The data from the population-based studies examining the incidence of all esophageal cancers in relation to statin use are rather less robust than the more specific Barrett's-adenocarcinoma data. There was considerable heterogeneity in the crude pooled data but the pooled adjusted ORs did show a significantly lower incidence of all esophageal cancers in statin users. In contrast to the Barrett's group studies, all of these population-based studies relied on interrogation of databases and were not specifically designed to examine esophageal cancer incidence (this was one of many outcomes assessed). Data on drug exposure is probably not as complete in this set of studies as aspirin, NSAIDs and statins are all available over the counter in many of the relevant areas and non-prescription use would not have been detected in these prescribing database studies. We feel that this is unlikely to greatly affect the results but does increase the level of uncertainty. The previously mentioned meta-analysis by Singh *et al*^[42] did not separately examine population-based (but non-Barrett's) esophageal cancers, and included overall less subjects (9285 cases and 1132969 total patients) than our current study. However the pooled results for all studies examining statin use and esophageal cancer incidence was similar to ours [pooled unadjusted OR = 0.74 (95%CI: 0.62-0.90), pooled adjusted OR = 0.72 (95%CI: 0.60-0.86)], considering the that the Singh *et al*^[42] results are affected by the inclusion of the Barrett's adenocarcinoma studies, where statins seem to be associated with greater protection against cancer compared to the true population-based studies.

The major difficulty in interpreting the population-based studies is that the cancer diagnoses would have included a mixture of esophageal adenocarcinoma, gastroesophageal junction adenocarcinoma and esophageal squamous cell cancer. These have different risk factors and pathology and it is not clear whether the negative association with statin use reported reflects a similar effect against all possible esophageal cancer types or whether there is a more obvious negative association with adenocarcinoma, as suggested by the Barrett's-cancer data and a less obvious, or indeed no, association with a reduced incidence of squamous cell cancer. This area needs fur-

ther clarification. Again there were insufficient data, and what were available were too inconsistently reported to draw any conclusions regarding the dose and duration relationships between statin use and esophageal cancer incidence or whether individual statins or statin classes had different effects.

In addition to the single study showing some modest effects with lovastatin in esophageal squamous cell lines^[16], anti-cancer effects of statins have been also demonstrated *in vitro* against non-esophageal squamous cancer cell lines, such as lung, skin or head and neck cancers^[49-52]. However although some clinical studies have suggested a non-statistically significant trend to improved outcomes in statin-treated squamous cell cancer patients^[50], other large studies have failed to show any benefit. Further studies are clearly required to examine the associations (if any) between statin use and esophageal squamous cell cancer^[28,53]. Similarly, further studies are required to examine whether statin use has any association with the incidence of Barrett's metaplasia, all the studies in our meta-analysis examined adenocarcinoma (or high-grade dysplasia) development in Barrett's mucosa.

The potential cancer chemopreventive effects of statins continue to attract widespread attention: statins have been reported to be associated with reduced overall cancer-related mortality^[54] but data on the clinical effects of statins on the incidence or prognosis of cancers various different sites have often been inconclusive and require that different cancers are addressed separately (reviewed by Boudreau *et al*^[55]).

Our meta-analysis has shown that statin use is consistently associated with a reduced incidence of adenocarcinoma in populations of patients with Barrett's esophagus. The combination of a cyclo-oxygenase inhibitor and statin is associated with a greater reduction in the incidence of adenocarcinoma. In population based studies of all esophageal cancers statin use was also associated with a reduced cancer incidence. The chemopreventive actions of statins, especially in combination with aspirin/NSAIDs deserves further exploration in interventional trials.

COMMENTS

Background

Cancers of the esophagus are common causes of mortality and morbidity worldwide. The incidence of esophageal adenocarcinoma is increasing in the Western world and although it is accepted that most cases of esophageal adenocarcinoma arise from metaplastic Barrett's esophagus, there are, as yet, no proven chemopreventive interventions. Laboratory-based experimental cell-line studies have shown that statins have potentially useful anti-cancer effects against esophageal cancer and that in some model systems, at least, these effects can be enhanced by combining with a cyclo-oxygenase inhibitor. At present there are only limited data on the clinical correlations of these observations.

Research frontiers

It is not clear if the clinical use of statins is associated with a reduced incidence of esophageal cancers and equally the effects of combined use of statins and cyclo-oxygenase on the development of esophageal cancer are unclear. Several of the laboratory cell-line studies have used relatively high concentrations of statins to show anti-cancer effects, probably higher than seen in usual clinical therapeutic use, and hence it is important to determine the relationship between

statin use and esophageal cancer incidence with usual clinical use of the drugs.

Innovations and breakthroughs

This is the largest systematic review and meta-analysis in this area and has included over 300 cases of Barrett's-related adenocarcinoma, 1999 non-cancer Barrett's controls. In addition the population-based studies included over 370000 total cases of esophageal cancer and almost 6 million controls. The results show that statin use in patients Barrett's esophagus was associated with a 43% reduction in the incidence of adenocarcinoma. Inhibition of cyclo-oxygenase (COX) with aspirin, non-aspirin non-steroidal anti-inflammatory agents or selective COX-2 inhibitors was independently associated with a reduced adenocarcinoma incidence in Barrett's esophagus (41% decrease). The combination of a statin plus a cyclo-oxygenase inhibitor was associated with a greater reduction in adenocarcinoma incidence than either alone (74% reduction). The data from the population-based studies are more heterogeneous, containing a mixture of esophageal cancer types but again statin use was associated with a reduced incidence of cancer development (19% reduction).

Applications

These data from observational studies suggests that statins may have useful chemopreventive effects against esophageal cancer; particularly against the development of adenocarcinoma in Barrett's esophagus when used in combination with a cyclo-oxygenase inhibitor. Further interventional studies are warranted. As patients with Barrett's esophagus are at increased risk of circulatory diseases, statins should not be withheld from such patients where otherwise indicated.

Terminology

Statins are inhibitors of the enzyme hydroxymethylglutaryl-CoA reductase. This is the rate limiting step on cholesterol biosynthesis. These drugs are widely used to treat and prevent circulatory diseases. Intermediates of the cholesterol synthetic pathway are also essential in other cell signalling pathways which are important in controlling many functions including cell proliferation and survival.

Peer review

Chemoprevention for esophageal cancers, especially in the context of Barrett's esophagus, is an area of active interest around the world. This systematic review and meta-analysis examined the association with statin use and the incidence of esophageal cancers. The study results show a consistent and significant negative between statin use and the development of esophageal adenocarcinoma. It also showed that the combined use of statins with aspirin or other cyclooxygenase inhibitors was associated with even lower incidence of adenocarcinoma development in patients with Barrett's esophagus. The results would further stimulate research and interest in combined chemoprevention. The findings are topical and relevant to clinical practice.

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Diarrhoea in a patient with metastatic melanoma: Ipilimumab ileocolitis treated with infliximab

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gest that infusional therapy of infliximab is effective in ipilimumab induced ileocolitis.

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Key words: Melanoma; Ipilimumab; Colitis; Infliximab; Cytotoxic T-lymphocyte associated antigen-4

Core tip: This paper presents a case of ipilimumab induced ileocolitis which was successfully treated with infliximab, an anti-tumor necrosis factor monoclonal antibody, after corticosteroid therapy failure. Although formal trials are lacking, recently published series suggest that infusional therapy of infliximab is effective in ipilimumab induced ileocolitis.

Slangen RME, van den Eertwegh AJM, van Bodegraven AA, de Boer NKH. Diarrhoea in a patient with metastatic melanoma: Ipilimumab ileocolitis treated with infliximab. *World J Gastrointest Pharmacol Ther* 2013; 4(3): 80-82 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v4/i3/80.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v4.i3.80>

Abstract

Administration of ipilimumab, a cytotoxic T-lymphocyte associated antigen-4-blocking monoclonal antibody, leads to enhancement of the anti-tumor T-cell response and as a result shows a significant survival benefit in metastatic melanoma patients. Therefore patients are currently receiving this promising therapy as a second-line strategy. Unfortunately, by activation of the T-cell immune response, ipilimumab therapy may lead to an unwanted induction of different autoimmune phenomena. Diarrhoea and colitis occur in up to one third of patients. Here we present a case of ipilimumab induced ileocolitis which was successfully treated with infliximab, an anti-tumor necrosis factor monoclonal antibody, after corticosteroid therapy failure. Although formal trials are lacking, recently published series sug-

INTRODUCTION

Ipilimumab administration has shown a survival benefit in metastatic melanoma patients, therefore more patients are likely to receive this therapy as a second-line treatment. Unfortunately, ipilimumab therapy may lead to an unwanted induction of autoimmune phenomena. Here we present a case of ipilimumab induced ileocolitis successfully treated with infliximab after corticosteroid therapy failure.

CASE REPORT

A 53-year-old man with a medical history of metastatic melanoma (metastasized to lungs, lymph nodes and peri-

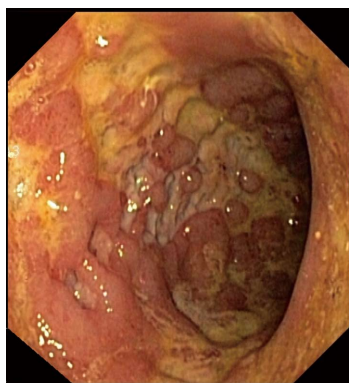


Figure 1 Deep ulcerations in the colon (endoscopic image).

cardium), was presented at our endoscopy ward because of highly frequent, non-bloody diarrhoea without fever. His medication consisted of morphinomimetics and haloperidol. Four weeks earlier, he started with ipilimumab (3 mg/kg body weight), a fully humanized IgG antibody against the Cytotoxic T-lymphocyte associated Antigen-4 (CTLA-4), of which he had received two administrations. His diarrhoeal complaints had started one week after the second infusion. Routine stool cultures, including *Clostridium difficile*, were negative. A colonoscopy was performed, which showed a patchy colitis with deep, confluent Crohn-like ulcerations (Figure 1). Histopathological examination demonstrated a severe cryptitis with a few abscesses. No granulomas or architectural changes were seen (Figure 2). Furthermore, cytomegalovirus infection was excluded. A computed tomography-scan was performed, showing diffuse thickening of the wall of the entire colon and terminal ileum. A diagnosis of ileocolitis associated with anti-CTLA-4 treatment was made. Our patient was treated with prednisolon (1 mg/kg) for 10 d without beneficial clinical effect. For that reason, intravenous infliximab therapy was initiated (a chimeric IgG antibody against tumour necrosis factor- α) in a dosage of 5 mg/kg body weight (at week 0 and 2)^[1,2], after two administrations his diarrhoeal complaints resolved completely.

DISCUSSION

Two recent studies demonstrated that ipilimumab therapy improves survival of patients with metastatic melanoma^[1,2]. Unfortunately, blocking of CTLA-4 by ipilimumab^[3], may lead to an induction of a variety of autoimmune phenomena. This may comprise inflammation of the gastrointestinal tract, leading to diarrhoea and colitis being reported in up to 31% of patients^[1].

As ipilimumab administration has shown a survival benefit in metastatic melanoma patients^[1,2], more patients are likely to receive this therapy as a second-line treatment. Moreover, trials of ipilimumab are ongoing in metastatic non-small cell lung cancer^[4] and in castration-resistant metastatic prostate cancer patients^[5]. Therefore, it is to be expected that ipilimumab induced colitis will be encountered more often.

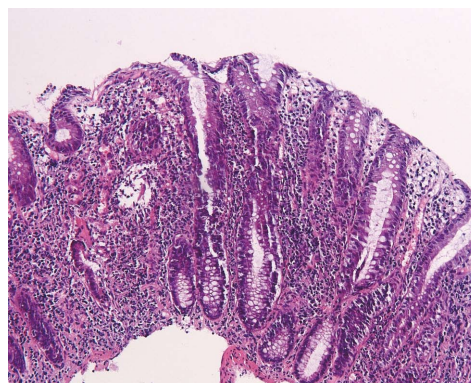


Figure 2 Histopathology of colon biopsies (hematoxylin and eosin staining, $\times 10$).

So far, by clinical judgement, corticosteroids are most often prescribed as a first-line treatment for ipilimumab induced colitis. In prednisone-refractory cases, infliximab has shown to be an effective second line treatment^[6-9]. The beneficial administration of infliximab in these patients is underlined by our case.

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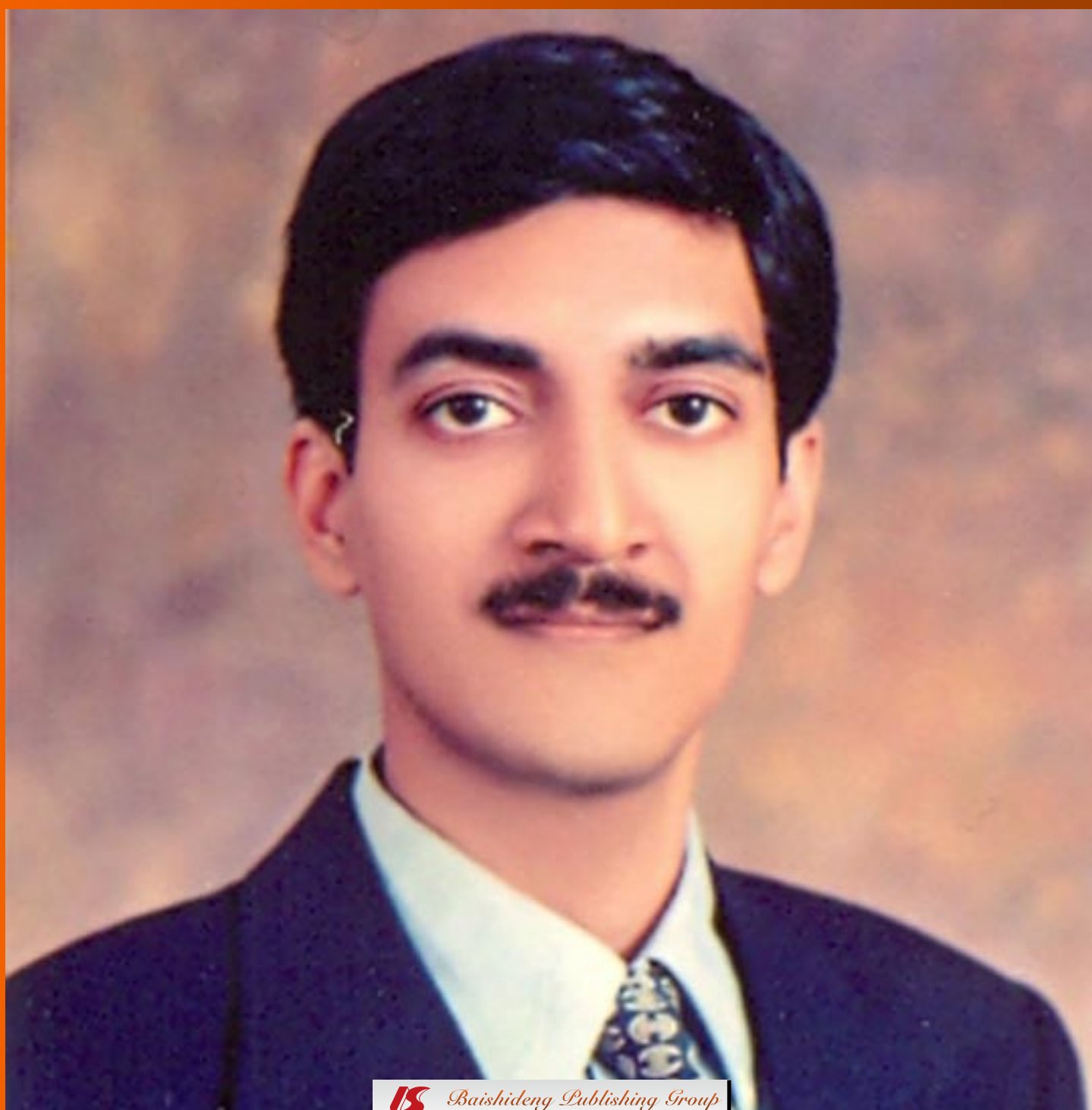
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Barriers to colorectal cancer screening in the developing world: The view from Pakistan

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Abstract

Colorectal cancer screening has become a defining concern of current gastroenterological practice in many Western nations. This same focus does not exist in many developing countries, including Pakistan. There is a need to develop a model for the developing world. Here are several areas that need to be pursued: (1) epidemiological research; (2) physician and public education; (3) training of gastroenterologists, especially female ones; (4) less expensive and more culturally acceptable screening options (fecal occult blood testing); and (5) cost-effectiveness analyses. Gastroenterologists in developing countries need to step up to educate people and promote, where possible and in keeping with local conditions, the prevention and early diagnosis of colorectal cancer.

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Key words: Colon cancer; Cancer screening; Pakistan; Cancer

Core tip: Gastroenterologists in developing countries need to step up to educate people and promote, where possible and in keeping with local conditions, the prevention and early diagnosis of colorectal cancer.

Ahmed F. Barriers to colorectal cancer screening in the develop-

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INTRODUCTION

Colorectal cancer screening has become a defining concern of current gastroenterological practice in many Western nations. This same focus does not exist in many developing countries, including Pakistan.

LACK OF EPIDEMIOLOGICAL DATA

A basic prerequisite for any screening program is knowledge of the incidence and prevalence of the disease in question. In the absence of this information any screening process is unjustifiable. Until as recently as last year, there was no useful incidence data on colorectal cancer in Pakistan. Recently a study has shown that Pakistan falls into a low incidence region/category for colorectal cancer^[1]. The crude incidence rate is 3.2% in both males and females. Most significantly, however, the incidence appears to be rising, particularly in males. This study also suggested that given an aging population, a strong tradition of consanguineous marriages, and a high prevalence of colorectal cancer risk factors, including a trend towards a more "westernized" dietary intake, this low incidence may, in fact, be an artifact. This data may also be an underestimation of colorectal cancer in Pakistan because the registry is voluntary and some cases may have gone unreported.

FINANCIAL LIMITATIONS

Implementation of Western models of large scale colonoscopic screening programs would place an insurmountable burden on already struggling health care systems in many developing countries. As a reflection of the state of healthcare in Pakistan, data on life expectancy

Table 1 Life expectancy, healthcare expenditure per capita in Pakistan compared with other countries

Country	Life expectancy (M/F, yr)	Expenditure on health per capita (\$, 2011)
Pakistan	66/68	69
India	64/67	141
Bangladesh	69/70	67
Sri Lanka	71/78	191
Singapore	80/85	2787
United Kingdom	79/82	3322
United States	76/81	8608

Available from: URL: <http://www.who.int/countries/>, accessed on May 16, 2013.

and healthcare expenditure per capita are given in Table 1. In Pakistan there is no health insurance system and the burden of any investigation rests solely with the patient. Given that the average annual income in Pakistan is \$650, the cost of different screening options is of paramount consideration. A colonoscopy costs \$100 here and fecal occult blood testing costs \$1.30. Regardless, it is still cheaper to diagnose colorectal cancer early than treat advanced malignancies.

LACK OF RESOURCES

Even if money to support a large scale colorectal cancer screening process were to be suddenly available, many trained gastroenterologists would be required which are already in short supply in many developing countries. In Pakistan, a country of 180 million people, there are limited number of gastroenterologists and endoscopy units and these are mostly concentrated in urban areas leaving the majority of the population without any access to gastroenterologic facilities.

LACK OF PHYSICIAN AND PUBLIC AWARENESS

There is a great lack of awareness about many malignancies, including colorectal cancer, in Pakistan. Even amongst physicians, there is a lack of awareness about the symptoms of colorectal cancer. For example, many physicians do not know that the presence of blood in the stool, especially in someone older than 50, needs to be investigated further and can't simply be attributed to hemorrhoids and ignored. Risk factors for colorectal cancer need to be highlighted, in particular the genetic aspects of colorectal cancer risk. First degree relatives of patients with colon cancer are rarely told that they are at increased risk for developing this malignancy and, therefore, need to be screened appropriately. Beyond this, the concept of screening asymptomatic persons, at average risk for colorectal cancer needs to be introduced and promoted here.

CULTURAL BARRIERS

There are many cultural barriers that exist in Pakistan

and would impede the implementation of a colon cancer screening program. Patients are wary of talking about even the possibility of cancer, there is widespread fear of endoscopic procedures due to concerns about potential complications and rumors of excruciating procedure-induced pain, and there is a widespread misconception that biopsying a malignant lesion invariably leads to spread of cancer. Finally, with Pakistan being a conservative Muslim country, female patients here are reluctant to have colonoscopy exams performed by male doctors and in this country of 180 million people, there are only a handful of female gastroenterologists.

The Asia Pacific consensus recommendations for colorectal cancer have focused primarily on data from East and Southeast Asia and have overlooked the Indian Subcontinent (Pakistan, India, Bangladesh) which together comprise more than one billion people^[2]. A prospective multinational colonoscopy screening study found that the prevalence of advanced colorectal neoplasms in asymptomatic Asians is comparable to that in the West^[3]. Again, the Indian Subcontinent was under-represented. Finally, cost-effective analyses conducted in other parts of the world are not necessarily directly applicable to our setting.

For all the reasons mentioned above, the implementation of more well-established cancer screening protocols (for breast cancer, cervical cancer, prostate cancer) have also not yet occurred in Pakistan. Is colon cancer screening a luxury of developed nations, unaffordable in the developing world? There are possible solutions to these obstacles. There is a need to develop a model for the developing world. Here are several areas that need to be pursued: (1) epidemiological research; (2) physician and public education; (3) training of gastroenterologists, especially female ones; (4) less expensive and more culturally acceptable screening options (fecal occult blood testing); and (5) cost-effectiveness analyses.

In a country beset by terrorism, militancy, and political uncertainty, it is easy to lose sight of issues relating to cancer screening. The initiation and implementation of any large-scale cancer screening program requires careful thought. Before starting a colon cancer screening program in Pakistan, efforts must be made to increase physician and public awareness regarding colon cancer, in particular, and the philosophy behind cancer screening, in general. Gastroenterologists in Pakistan and other developing countries need to step up to educate people and promote, where possible and in keeping with local conditions, the prevention and early diagnosis of colorectal cancer.

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Eosinophils and mast cells as therapeutic targets in pediatric functional dyspepsia

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prove the rate of symptom resolution in pediatric FD.

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Key words: Eosinophils; Mast cells; Functional dyspepsia; Abdominal pain; Stress

Core tip: Current evidence implicates gastric mast cells and duodenal eosinophils in the pathophysiology of functional dyspepsia and as mediators between psychologic and physiologic factors. Increased antral mast cell density is associated with anxiety, electromechanical dysfunction, and the postprandial distress syndrome (PDS) subtype of functional dyspepsia. Likewise, increased duodenal eosinophil density is associated with anxiety and the PDS subtype, however, effects on electromechanical function are more indirect. More importantly, mast cells and eosinophils appear to be therapeutic targets offering newer options for treating functional dyspepsia.

Abstract

There is an increasing appreciation for the importance of inflammation as a pathophysiologic entity that contributes to functional gastrointestinal disorders including functional dyspepsia (FD). Importantly, inflammation may serve as a mediator between psychologic and physiologic functions. This manuscript reviews the literature implicating two inflammatory cell types, mast cells and eosinophils, in the generation of dyspeptic symptoms and explores their potential as targets for the treatment of FD. There are a number of inciting events which may initiate an inflammatory response, and the subsequent recruitment and activation of mast cells and eosinophils. These include internal triggers such as stress and anxiety, as well as external triggers such as microbes and allergens. Previous studies suggest that there may be efficacy in utilizing medications directed at mast cells and eosinophils. Evidence exists to suggest that combining "anti-inflammatory" medications with other treatments targeting stress can im-

Friesen CA, Schurman JV, Colombo JM, Abdel-Rahman SM. Eosinophils and mast cells as therapeutic targets in pediatric functional dyspepsia. *World J Gastrointest Pharmacol Ther* 2013; 4(4): 86-96 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v4/i4/86.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v4.i4.86>

INTRODUCTION

A majority of children with chronic abdominal pain presenting to pediatric gastroenterology practices fulfill criteria for a functional gastrointestinal disorder (FGID), with the two most common being functional dyspepsia (FD) and irritable bowel syndrome (IBS)^[1-4]. Prevalence estimates for FD are 3.5%-27.0% in children/adolescents and 20%-30% in adults, highlighting the pervasive nature of this disorder^[5,6]. FD is defined as persistent or recur-

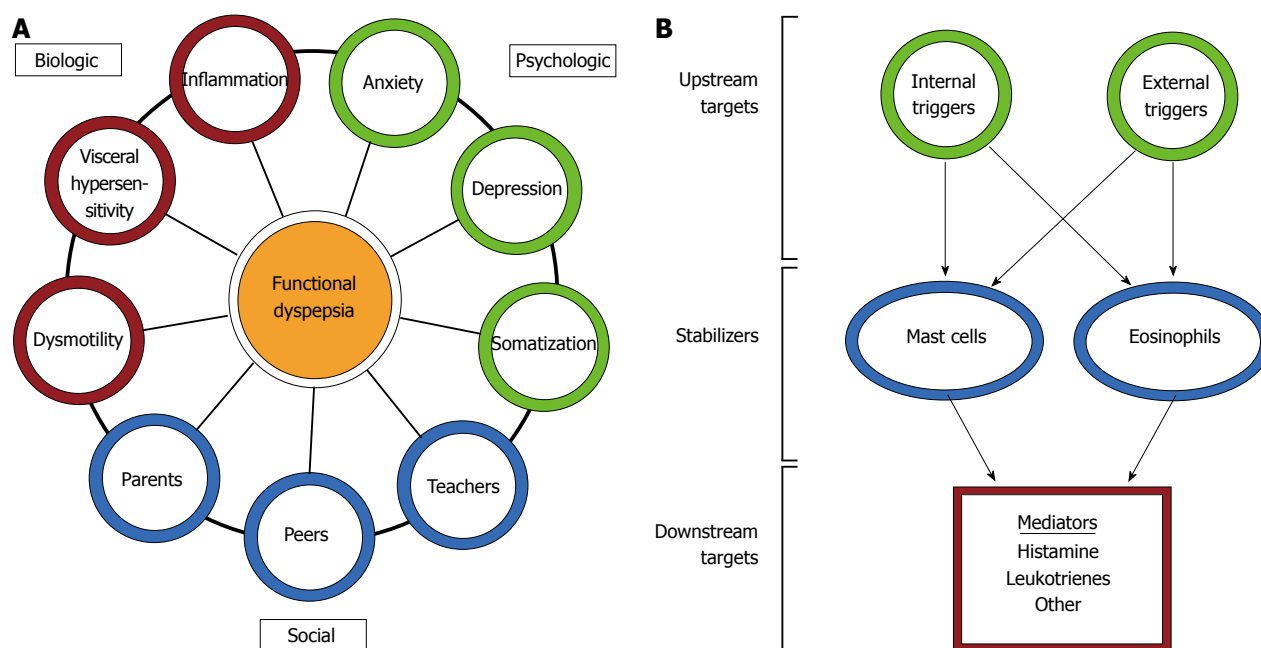


Figure 1 Overview of symptom contributors and points of intervention. A: Overview of symptom contributors in the biopsychosocial model of abdominal pain; B: Overview of the points of intervention in treatments targeting mast cells and eosinophils in functional dyspepsia.

rent pain or discomfort centered in the upper abdomen (above the umbilicus) that is unrelated to a change in stool frequency or form and not exclusively relieved by defecation. A diagnosis of FD is accompanied by the lack of evidence for an inflammatory, anatomic, metabolic, or neoplastic process that explains the patient's symptoms; however, mild, chronic inflammatory changes on mucosal biopsies do not preclude the diagnosis^[5,6].

In adults, there are two recognized FD subtypes based on studies utilizing factor analysis, postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS). PDS is defined as bothersome postprandial fullness occurring after ordinary sized meals and/or early satiation that prevents finishing a regular meal. EPS is defined as intermittent pain or burning of at least moderate severity localized to the epigastrium. The Rome pediatric subcommittee did not adopt the adult subtypes because of a lack data to support their existence in children and adolescents. However, there is now some data to suggest that these adult subtypes may have meaningful associations with mucosal inflammation and psychosocial functioning in children with FD^[7]. For example, pediatric dyspepsia is associated with lower quality of life, increased functional disability, and increased likelihood of meeting criteria for an anxiety disorder, however, the association with anxiety appears to predominate in patients experiencing symptoms consistent with PDS^[7,8]. A similar relationship between PDS and anxiety has been described in adults^[9].

FGIDs, including FD, are probably best understood through a biopsychosocial model. This model suggests that interactions between biological/physiological factors (*e.g.*, inflammation, mechanical disturbances, hypersensitivity), psychological factors (*e.g.*, anxiety, depression, somatization), and social factors (*e.g.*, interactions with

parents, teachers, or peers) collectively contribute to the symptoms of FD (Figure 1). The biological factors most often implicated in FD include motility disturbances, such as delayed gastric emptying, gastric electrical disturbances, and impaired accommodation, and visceral hypersensitivity to distension, acid, and/or lipids^[10-12]. Delayed gastric emptying and gastric electrical disturbances have been demonstrated in a substantial proportion of children with FD^[13,14]. Similarly, water load volume, as an indicator of visceral sensitivity, differs between children with FD and healthy controls^[15,16]. Consequently, electromechanical disturbances and visceral hypersensitivity represent frequent targets for therapeutic intervention in FD.

Recently, there is an increasing appreciation for the importance of inflammation as a pathophysiologic entity that contributes to FGIDs including FD. Importantly, inflammation may serve as a mediator between psychologic and physiologic functions. This manuscript reviews the literature implicating two inflammatory cell types, mast cells and eosinophils, in the generation of dyspeptic symptoms and explores their potential as targets for the treatment of FD.

MAST CELLS

In the context of FGIDs, mast cells have been studied primarily in adults with IBS where their numbers are generally elevated in the ileum and colon^[17,18]. In addition, adult IBS has been associated with an increase in the density of degranulating mast cells and mast cells in close proximity to nerves which correlate with abdominal pain severity and frequency^[19]. Increased mucosal mast cell density has also been demonstrated in the gastric corpus and antrum of adults with FD^[20,21]. Increased mast cell

density is generally isolated to the stomach of adults with FD, while increased mast cell density in the duodenum is generally associated with IBS^[21,22].

Due to a lack of normal control data, it is unclear whether gastric mast cells are elevated in pediatric FD, however, antral mast cells do appear to be actively degranulating in children with FD. The mean reported degranulation index is 67% with more than 80% of patients demonstrating degranulation indices of greater than 50%^[23]. Mast cells in the proximal stomach have been shown to degranulate with balloon distension of this region, demonstrating possible hypersensitivity in adults with FD^[24]. Although equivalent data are not available for children, mast cell density is positively correlated with slower gastric emptying and pre-prandial dysrhythmia in children with FD^[23]. Of note, the presence of pre-prandial dysrhythmia appears to be associated with increased post-prandial pain^[13].

EOSINOPHILS

Ethical considerations preclude tissue sampling for evaluation of eosinophil density in otherwise healthy children. Existing pediatric data provide suggestive evidence of eosinophil densities that may be abnormal in the absence of established “norms”. In a pediatric autopsy study (for which presence of gastrointestinal symptoms could not be evaluated), eosinophil density was $< 10/\text{hpf}$ in the antrum and $\leq 20/\text{hpf}$ in the duodenum in 100% and 82% of samples evaluated, respectively^[25]. Review of biopsies from 682 presumably symptomatic children referred for endoscopy found eosinophil density was $\leq 10/\text{hpf}$ in the antrum and $\leq 20/\text{hpf}$ in the duodenum of 90% and 93% of children, respectively^[26]. Maximum eosinophil density was 8/hpf in the antrum and 26/hpf in the duodenum. Thus, eosinophil density cut points of 10/hpf in the antrum and 20/hpf in the duodenum seem reasonable, but may need to be considered in tandem with measures of activation.

Eosinophil biologic activity results from mediator release with most mediators active in a concentration-dependent fashion. Thus, eosinophil effects are not just dependent on cell density, but on the extent of degranulation. However, these events may not be tightly correlated^[27]. In a previous study involving 20 children with a diagnosis of FD, an eosinophil density $> 20/\text{hpf}$ was present in only 15%, but moderate to extensive degranulation was demonstrated by electron microscopy in 95% of those evaluated^[28].

Location of eosinophils also may be important to consider. Dyspeptic adults have demonstrated increased eosinophil density in the duodenum as compared with controls; however, the quantity of antral eosinophils did not differ between groups^[29,30]. Duodenal biopsies from adult dyspeptics also revealed more extensive degranulation, with enhanced extracellular major basic protein (MBP)^[30]. This is consistent with observations of degranulation and MBP release in pediatric patients with FD^[28].

In adults, an increased eosinophil density and a higher prevalence of duodenal eosinophilia has been specifically associated with the PDS subtype of FD^[30]. Eosinophilia within the upper gastrointestinal tract has been evaluated in children with unspecified (by Rome criteria) abdominal pain, as well as children with FD, providing some pediatric information regarding the association between eosinophil location and patient symptoms. In a study of 1191 children with unspecified chronic abdominal pain, eosinophilia was identified in the antrum or duodenum in 11.4%^[31]. Another study found gastric eosinophilia in 19% and duodenal eosinophilia in 32% of children with unspecified chronic abdominal pain^[32]. In contrast, in children specifically fulfilling FD criteria, duodenal eosinophilia has been demonstrated in 79% of patients, which closely mirrors adult findings^[33].

INTERACTIONS BETWEEN MAST CELLS AND EOSINOPHILS

Mast cells and eosinophils exert their biologic functions almost exclusively by the release of mediators after activation. The effects of specific mediators depend, to some extent, on the local biochemical milieu of the involved tissue^[34]. As a consequence of this paracrine activity, mast cells and eosinophils interact highly with each other. In addition, mast cells and eosinophils demonstrate self-sustaining autocrine activity. For example, both eosinophils and mast cells produce interleukin (IL)-5 which augments mast cell cytokine production and is critical for the growth, chemotaxis, and activation of eosinophils^[35,36]. Mast cells and eosinophils both produce eotaxin which, in conjunction with mast cell-produced histamine, serves as a chemoattractant for eosinophils^[35,37]. Mast cells and eosinophils both also produce and express receptors for leukotrienes and tumor necrosis factor- α (TNF- α) which effect chemotaxis, survival, and activation of these two cell types^[35,37]. Given the countless mediators that these cells produce, it is likely that activation of either cell will result in alteration of function of the other.

SPECIFIC CONDITIONS ASSOCIATED WITH MUCOSAL INFLAMMATION

There are a number of triggers or inciting events which may initiate an inflammatory response, and the subsequent recruitment and activation of mast cells and eosinophils, in the gastrointestinal tract. These include internal triggers such as stress and anxiety, as well as external triggers such as microbes and allergens.

Internal triggers

Anxiety and stress are the most highly implicated internal triggers in the development and/or maintenance of FGIDs, including FD. Children with FGIDs tend to have more concurrent symptoms of anxiety and depression than do their peers^[38]. Approximately 50% of children

with FD demonstrate elevated anxiety scores either in isolation or as part of more global psychosocial dysfunction^[39]. Further, mucosal eosinophil density, as well as antral mast cell density, correlates with anxiety scores in children with FD^[7,40].

Thus, the role of inflammation in the biopsychosocial model is probably best illustrated by examining the stress response. Corticotropin releasing hormone (CRH), produced by the hypothalamus (as well as immune cells including lymphocytes and mast cells) is a major mediator of the stress response in the hypothalamic-pituitary-adrenal (HPA) axis and, subsequently, within the brain-gut axis. The stress response results in physiologic effects which appear relevant to FGIDs including inflammation, altered gastric accommodation, gastric dysmotility, and visceral hypersensitivity. CRH also has CNS effects which may alter central processing of nociceptive messages including anxiogenic effects. Of note, the relationship between the CNS and gastrointestinal pathophysiology is bidirectional. In a rodent model, gastric irritation in the neonatal period induces a long lasting increase in depression- and anxiety-like behaviors, as well as an increased sensitivity of the HPA axis to stress^[41]. CRH stress systems may be activated by afferent nerves from inflamed sites or *via* cytokines including TNF- α , IL-1, IL-6 and IL-12^[42].

CRH receptors are widely expressed within the gastrointestinal tract and immune cells. Mast cells express both CRH1 and CRH2 receptor subtypes at their surface. Most of the inflammatory cell actions, including those on mast cells, occur *via* CRH-R2 receptors^[43]. Once mast cells are activated, they release mediators which recruit and activate eosinophils, with both cell types interacting in a bi-directional fashion with T helper cells (Th). There also may be a direct effect for CRH on eosinophils. In a rodent model, psychologic stress results in eosinophilic expression of CRH^[44]. CRH is not expressed by eosinophils in the intestines of the mice except under psychologic stress and decreases after the stress is removed^[44].

Once activated by CRH, mast cells may release pro-inflammatory cytokines^[45]. Adults have demonstrated selective luminal release of tryptase and histamine from jejunal mast cells under cold stress at a magnitude similar to that induced by antigen exposure in food allergic patients^[46]. Once released, mast cell and eosinophil mediators can stimulate afferent nerves signaling pain, can sensitize afferent nerves resulting in visceral hypersensitivity, and can alter electromechanical function. Histamine can stimulate afferent sensory nerves *via* H2 receptors^[47]. CRH has been shown to activate gastrointestinal mast cells with resultant mediator sensitization of afferent sensory enteric nerves^[48-50]. Low grade inflammation may lead to visceral sensitivity and motility disturbances; the key appears to be a shift from a TH1 to a TH2 response, with eosinophils and mast cells as the key effector cells^[51]. Stress has been shown to shift the relative proportion and trafficking of T helper lymphocytes towards a Th2 or "allergic" phenotype^[42]. This shift is driven by central and peripheral CRH, catecholamines, and histamine *via*

H2 receptors. The Th2 phenotype is associated with release of IL-4, IL-10, and IL-13 which stimulate growth and activation of mast cells and eosinophils^[42].

External triggers

A number of external triggers have been identified, such as microbes and allergens, which may result in eosinophil and/or mast cell recruitment and activation. The immune system may be activated by an acute infection and continue to generate symptoms after the infection resolves, resulting in so-called post-infectious FD (PI-FD). *Helicobacter pylori* (*H. pylori*) colonization represents a unique situation where symptoms may result from chronic infection and, in many patients, persist after eradication.

FD has been reported at a higher prevalence following both bacterial and parasitic infections^[52]. It seems likely that FD may also be induced by viral gastroenteritis in a manner similar to that of IBS. In a study of 88 children with a previous positive bacterial stool culture, FD was present in 24% and IBS in 87%^[53]. Fifty-six percent of the patients reported the onset of abdominal pain after the acute infection. Another study identified 82 adults with persistent abdominal symptoms following *Giardia* infection, with FD in 24.3% and IBS in 80.5%^[54]. Over half of these patients reported exacerbation due to specific foods and, consistent with the biopsychosocial model, nearly half reported exacerbations with physical or mental stress^[54].

PI-FD appears to represent an impaired ability to terminate the inflammatory response after elimination of the offending pathogen. It may also be associated with neuroplastic changes in visceral and central afferent pathways^[55]. Duodenal eosinophilia has been described in PI-FD and gastric mast cells are significantly increased in PI-FD as compared to healthy controls^[51,56]. PI-FD is associated with increased gastric release of histamine and 5-hydroxytryptamine as well as increased number of mast cells in close proximity to nerve fibers as compared to healthy controls or non-PI-FD^[57].

The role of *H. pylori* in FD remains incompletely defined. Multiple studies have demonstrated a moderate reduction in FD symptoms with eradication of this organism while others have shown no clinical benefit^[57-60]. A Cochrane review concluded that eradication was significantly better than placebo^[61]. However, a large number of patients continue to experience symptoms following eradication. These may be patients in whom *H. pylori* had no pathologic role or may represent patients with PI-FD and prolonged submucosal inflammation^[62].

H. pylori colonization in children is associated with a mixed inflammatory infiltrate including eosinophils which decrease with eradication^[63]. *H. pylori* colonization also may be associated with increased antral mast cell density, though this appears to be *H. pylori* strain specific^[64]. In the setting of *H. pylori*-associated nodular gastritis, eosinophils may be of particular significance. Patients with nodular gastritis have a higher incidence of dyspeptic symptoms which resolve with eradication therapy^[62].

Nodularity is associated with an increased density of eosinophils^[65]. Even in the absence of nodularity, *H. pylori* colonization is associated with increased antral eosinophils, as well as increased gastric fluid eosinophil cationic protein indicating that the eosinophils are actively degranulating^[26,63,66]. These findings would suggest a possible pathophysiologic role for eosinophils in contributing to symptoms in patients with *H. pylori* colonization prior to and following eradication.

The role of allergies in the development of FD has not been well studied; however, their potential to contribute given the observed increases in and activation of mast cells and eosinophils in FD is certainly plausible. FGIDs occur more commonly in children with a history of cow's milk allergy as infants^[67]. In these children, mucosal application of cow's milk is associated with increased eosinophils and mast cells and rapid degranulation within 10 min of application^[68]. In addition, cow's milk exposure is associated with increased mast cells in close proximity to nerves^[68]. A history of allergy is associated with increased duodenal eosinophil density in adults with FD^[30]. Whether food allergy accounts for a substantial portion of children with FD is not clear. We previously found no significant increase in immunoreactivity to common food allergens in FD children with duodenal eosinophilia, though it is possible that the reactions were localized to the mucosa and thus missed in our assessment^[69]. It is also possible that environmental allergens may play a role in FD. Antigen exposure in adults with birch pollen allergy results not only in an increase in symptoms of FD but also an increase in mucosal MBP+ eosinophils and IgE-bearing cells in the majority of patients^[70].

"ANTI-INFLAMMATORY" THERAPY

Treatments with the potential to impact symptoms related to inflammatory cells would primarily act by three mechanisms: (1) controlling upstream factors which recruit or activate inflammatory cells; (2) controlling the release of mediators from inflammatory cells; and (3) antagonizing the downstream effects of mediators once released from inflammatory cells (Figure 1).

Treatment directed at upstream factors

Treatments directed at upstream factors would include those which interfere with activation of mast cells or eosinophils by internal triggers (*e.g.*, CRH antagonists, selective serotonin reuptake inhibitors (SSRI) anti-depressants, anti-anxiety treatments) or external triggers (*e.g.*, corticosteroids, anti-TNF- α , anti-IL-5 and anti-IgE).

The biopsychosocial model and CRH physiology would suggest a potential role for antagonizing CRH, or controlling its secretion by modulating anxiety and the stress response either through the use of SSRIs, anti-anxiety medications, or relaxation techniques. Though there are no previous controlled studies evaluating CRH-antagonists or SSRIs in pediatric FD, some evidence exists for the role of relaxation *via* biofeedback-assisted relaxation

training (BART). Biofeedback is a technique whereby individuals are trained to relieve physical or emotional symptoms using signals from their bodies that are displayed visually or aurally. Biofeedback can be paired with relaxation training to yield BART. BART paired with fiber supplementation has been shown to be superior to fiber alone in children with non-specific abdominal pain^[71]. The effect of BART directly on inflammation has not been studied. However, BART has been studied as an adjunctive treatment to medications directed at inflammation in children with FD in association with duodenal eosinophilia^[72]. Children receiving medication plus BART demonstrated better outcomes with regard to pain intensity, duration of pain episodes, and global clinical improvement as compared to children receiving medications alone^[72].

Corticosteroids represent another group of agents which may be used in the setting of mucosal eosinophilia to block upstream activation and upstream effects, although they have not been studied directly in patients with FD. Prednisone has long been considered the mainstay in the treatment of eosinophilic gastroenteritis though there are no placebo-controlled studies evaluating efficacy. The less than favorable side effect profile represents a significant draw back in considering its use as a long term agent. Budesonide may represent a safer alternative. Budesonide is a synthetic corticosteroid with high topical activity, substantial first pass elimination and relatively low systemic bioavailability. Among the commercially available preparations is an oral enteric-coated capsule formulated to optimize delivery to the ileum and colon^[73]. The delivery pattern would suggest that budesonide may be less effective for proximal small bowel disease. However, the budesonide granules dissolve at an alkaline pH normally present in the proximal small bowel. Although acid suppression with omeperazole does not affect absorption, acid suppression in combination with delayed gastric emptying, as might be expected with mucosal inflammation, has not been evaluated^[73]. The literature regarding budesonide and eosinophilic gastroenteritis consists of case reports where budesonide therapy has been reported to be effective against eosinophilia in the duodenum and jejunum^[74-76].

TNF- α represents another theoretical "upstream" treatment target for FD. CysLTs induce TNF- α production which has been demonstrated to recruit and prolong survival of eosinophils, as well promote a TH2 response depending on other chemokines present in the micro-environment^[77-79]. In a variety of allergic mouse models, anti-TNF antibodies have been shown to decrease eosinophilic infiltration and local Th2 cytokine transcription and secretion^[80-82]. Pre-treatment serum TNF- α concentrations correlate negatively with the clinical response to montelukast in pediatric FD in association with duodenal eosinophilia indicating that mediation by TNF- α may represent an alternative pathway for symptom generation in these patients. Although there are no controlled studies, anti-TNF- α antibody has been reported to be

effective in a series of children with resistant eosinophil disease including patients with FD^[83].

Eosinophils and/or mast cells exhibit a number of cell surface markers which also serve as potential therapeutic targets in blocking upstream activation. These have been well reviewed elsewhere^[84]. However, there are two of these, IL-5 and IgE, which have been targeted in humans with gastrointestinal eosinophilia and, thus, warrant specific mention.

IL-5 serves to stimulate the expression of eosinophils. In general, most clinical studies evaluating anti-IL-5 antibodies have demonstrated decreases in eosinophil density but little clinical benefit^[85]. There are limited reports on the use of anti-IL-5 in patients with gastrointestinal eosinophilia and none specifically in patients with FD. In a small pilot study of adults with eosinophilic gastroenteritis, a single dose of anti-IL-5 resulted in a 50%-70% decrease in mucosal eosinophil density in 3 of the 4 patients but with minimal symptom improvement^[86]. The effect of anti-IL-5 on duodenal eosinophil density was assessed in 11 adult patients treated for eosinophilic esophagitis^[87]. While esophageal density decreased significantly, there was no significant effect on duodenal eosinophil density. This may simply indicate that the normal physiologic duodenal eosinophil population is unaffected.

Anti-IgE antibody has also been evaluated in a small study of adults with eosinophilic gastroenteritis but not specifically in patients with FD. In an uncontrolled, open-label study of 9 patients, anti-IgE resulted in a non-statistically significant reduction in eosinophil density in the antrum (69%) and duodenum (59%)^[88]. Symptoms significantly improved but improvement had no direct relation to the decrease in mucosal eosinophil density.

Treatment directed at controlling mediator release

Mast cell stabilizers, including cromolyn and ketotifen, represent an attractive potential therapy given data implicating mast cells in the generation of dyspeptic symptoms as previously discussed. These agents inhibit the release of mast cell mediators and, consequently, their pathophysiologic effects.

There have been no adult studies on the use of mast cell stabilizers in patients with FD. Benefit has been demonstrated in adults with IBS where it is suggested that the response may be related to blocking allergic or immunologic reactions to foods^[89-91]. In an open-label observational study of oral cromolyn in children with FD in association duodenal eosinophilia, resolution of pain was demonstrated in 89% of patients who had previously failed to respond to H2 and combined H1/H2 antagonism^[92].

Ketotifen, which antagonizes the H1-receptor, in addition to stabilizing mast cells has been shown to significantly decrease pain in adults with IBS and to increase the threshold for discomfort in patients with visceral hypersensitivity though this effect could not be correlated with pain improvement^[93]. Whether the observed response to this drug is related to H1-receptor antagonism or mast cell stabilization is unclear.

Treatment directed at downstream mediators

In general, treatments directed at antagonizing the downstream effects of mediators released by mast cells and/or eosinophils are associated with a more rapid onset of action and fewer side effects. Therefore, they should probably be viewed as first line agents in treatment directed at mast cells and eosinophils in FD. It should be noted that the two most common downstream targets, histamine and leukotrienes, also have pro-inflammatory effects that may result in further upstream activation. Further, the simple experience of symptoms may cause physical and/or emotional stress that promotes upstream activation through the pathway previously described. Thus, addressing these downstream treatment targets may have direct effect on reducing symptoms in the short-term, while also indirectly serving to reduce activation of inflammatory cells in the long-term.

Acid reduction remains the most common treatment prescribed empirically by pediatric gastroenterologists for children with dyspepsia^[1]. While there are numerous adult studies to support this practice, pediatric studies are limited. In adults, H2 antagonism has been shown to improve at least some symptoms associated with FD (abdominal pain, indigestion, belching, and gastroesophageal reflux symptoms) and appear to be superior to prokinetic medications and short term use of anxiolytics^[94-97]. In adults with dyspepsia, proton pump inhibitors (PPIs) are superior to placebo in symptom reduction although this appears limited to patients with ulcer-like or reflux-like dyspepsia^[98-101]. Whether PPIs are superior to H2 antagonism is not completely clear. Omeperazole was found to have a modest increase in efficacy as compared to ranitidine at 4 wk (51% *vs* 36%) but there was no benefit at 6 mo^[97].

In children with abdominal pain, famotidine was superior to placebo in global improvement with clear benefit in those with dyspepsia^[102]. In a large pediatric study, omeperazole was shown to have a very modest advantage in the relief of all symptoms as compared to either famotidine or ranitidine but there was no significant difference between the three with regard to resolution of abdominal pain, epigastric pain, nausea or vomiting^[103].

Given the response to PPIs, it would appear that at least some of the clinical improvement from H2 antagonism or PPIs is related directly to acid suppression. A significant portion of responders may derive benefit from treatment of overlap GER or possibly from peptic gastritis or duodenitis, however, the benefit may also be due to limiting acid exposure in patients with acid hypersensitivity. With H2 antagonism, the benefit may also be unrelated to acid reduction as histamine has direct gastric myogenic actions, modulates afferent enteric nerve excitability, and acts as an immunomodulating agent^[104-108]. H2 receptors affect not only acid secretion but influence neurotransmission and immune responses^[47].

There may also be additional benefit from H1 antagonism. Combining an H1 antagonist with an H2 antagonist has been reported to relieve symptoms in 50% of

children with FD in association with duodenal eosinophilia and in 79% of adults with FD in association with increased antral mast cell density who had previously failed to respond to acid reduction therapy^[92,109]. H1 receptors have direct effects on smooth muscle contraction and visceral sensitivity^[47]. Some benefit from H1 antagonism may also be due to an anxiolytic effect. Immune modulators, such as sucralfate sodium, may also indirectly inhibit H1 receptor expression by suppressing IL-4 and IL-5 production from TH2 cells^[110]. Shirai *et al.*^[111], reported successful treatment with sucralfate sodium in an adult with eosinophilic gastroenteritis. It has not been specifically used in patients with FD.

H4 antagonists are currently in development and may represent a treatment option in the future. H4 receptors are abundant in the small intestine, largely on hematopoietic cells including eosinophils and mast cells, as well as endocrine cells^[112,113]. H4 receptor activation results in eosinophil and mast cell chemotaxis (but not degranulation) as well as T cell cytokine production^[112]. Current H1 antagonists do not inhibit H4 receptors but they do share common ligands^[112].

CysLTs also are a potential downstream therapeutic target. The pattern of eosinophil degranulation in pediatric FD is consistent with the release of major basic protein, which is known to enhance the synthesis of cysLT. CysLT, in turn, stimulates smooth muscle contraction and recruitment of eosinophils^[114]. CysLTs have been shown to alter mast cell function *via* induction of IL-5 and TNF- α production in primed mast cells, an effect blocked by cysLT inhibition^[115]. Leukotrienes have the potential to increase intestinal sensory nerve sensitivity during inflammation as LT receptors are expressed on spinal nerve terminals and cysLTs have been shown to increase excitability of enteric neurons and to have a pro-contactile effect on the esophagus, stomach, small intestine, colon, and gallbladder^[116-123].

In a double-blind, placebo-controlled, cross-over trial of children with FD in association with duodenal eosinophilia, montelukast, a cysLT receptor antagonist, was found to be superior to placebo with regard to relief of pain^[124]. The response rate was 84% in patients with eosinophil density between 20 and 29/hpf as compared to a 42% response rate with placebo. This high response rate was confirmed in a second study which also determined that the short term positive clinical response was unrelated to a decrease in eosinophil density or activation^[33]. This would suggest that the effect may be mediated through an enteric nerve effect on motility or sensitivity though that remains to be demonstrated. Other leukotriene antagonists (*e.g.*, pranlukast, zafirlukast) have not been evaluated in FD or eosinophilic gastroenteritis.

CONCLUSION

Current evidence implicates gastric mast cells and duodenal eosinophils in the pathophysiology of FD and as mediators between psychologic and physiologic factors.

Increased antral mast cell density is associated with anxiety, electromechanical dysfunction, and the PDS subtype of FD. Likewise, increased duodenal eosinophil density is associated with anxiety and the PDS subtype, however, effects on electromechanical function are more indirect.

While empirical data is limited, previous studies suggest that there may be efficacy in utilizing medications directed at mast cells and eosinophils. Most current data regarding treatment response consists of case series utilizing H1/H2 antagonists, mast cell stabilizers, and anti-TNF- α , as well as a controlled trial demonstrating clinical efficacy for the use of montelukast. Evidence exists to suggest that combining “anti-inflammatory” medications with other treatments targeting stress can improve the rate of symptom resolution in pediatric FD.

FUTURE DIRECTIONS

There remains a need for placebo-controlled trials of the various medications and other treatments targeting mast cells and eosinophils which have been suggested to have efficacy, either alone or in combination. Likewise, there is a need to better define the upstream and downstream mediators for both mast cells and eosinophils as potential therapeutic targets for future drug development or as potential targets for agents currently available, such as lipoxygenase inhibitors, prostaglandin synthetase inhibitors, or newer drugs targeting eosinophil adhesion or Siglec-8^[125,126].

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Metabolomics as a diagnostic tool in gastroenterology

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Abstract

Metabolomics has increasingly been applied in addition to other "omic" approaches in the study of the pathophysiology of different gastrointestinal diseases. Metabolites represent molecular readouts of the cell status reflecting a physiological phenotype. In addition, changes in metabolite concentrations induced by exogenous factors such as environmental and dietary factors which do not affect the genome, are taken into account. Metabolic reactions initiated by the host or gut microbiota can lead to "marker" metabolites present in different biological fluids that allow differentiation between health and disease. Several lines of evidence implicated the involvement of intestinal microbiota in the pathogenesis of inflammatory bowel disease (IBD). Also in irritable bowel syndrome (IBS), a role of an abnormal microbiota composition, so-called dysbiosis, is supported by experimental data. These compositional alterations could play a role in the aetiology of both diseases by altering the metabolic activities of the gut bacteria. Several studies have applied a metabolomic approach to identify these metabolite signatures. However, before translating a potential metabolite biomarker into clinical use, additional validation studies are required. This review summarizes contributions

that metabolomics has made in IBD and IBS and presents potential future directions within the field.

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Key words: Metabolomics; Microbiota; Inflammatory bowel diseases; Irritable bowel syndrome

Core tip: Metabolic profiling is a powerful exploratory tool for understanding interactions between nutrients, the intestinal metabolism and the microbiota composition in health and disease and, to gain more insight in metabolic pathways. Metabolomics may advance our understanding, diagnosis and treatment of inflammatory bowel disease and irritable bowel syndrome. Metabolic reactions initiated by the host or gut microbiota can lead to "marker" metabolites present in different biological fluids that allow differentiation between health and disease. Disease-related mechanisms may be uncovered and verified, and candidate diagnostic biomarkers in biological samples are characterized.

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INTRODUCTION

Different "Omic" approaches are currently applied to identify novel diagnostic targets and disease specific markers, and to characterize the link between gut microbiota or host metabolism and functional alterations in the pathophysiology of different diseases. Genomics, transcriptomics and proteomics provide extensive information regarding the genotype but convey limited information about the phenotype (Figure 1). Gene expression and protein data mainly indicate the potential for specific

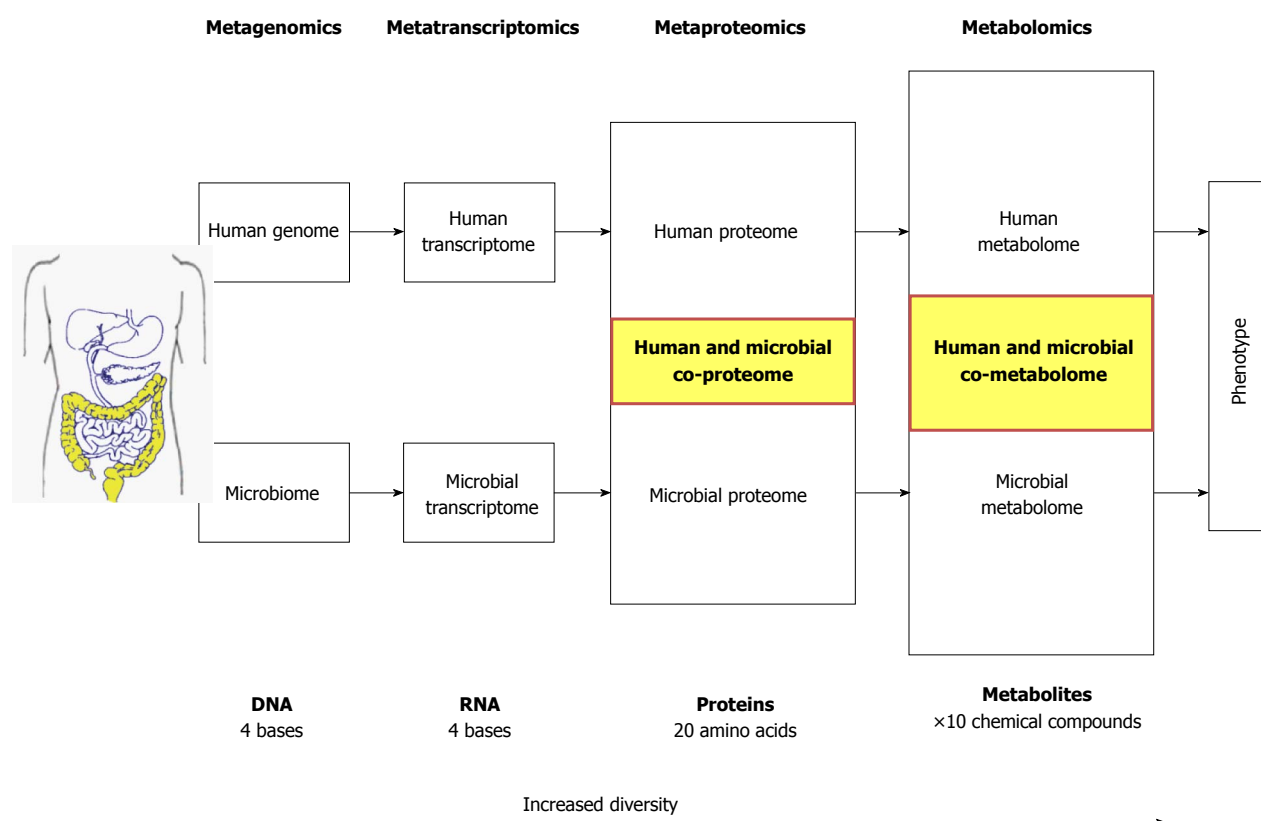


Figure 1 Overview of different “Omic” approaches (metagenomics, metatranscriptomics, metaproteomics and metabolomics) determining an individual’s “Genotype to phenotype”.

metabolic functions and do not always reflect the effective physiological processes as several downstream regulatory mechanisms are involved. As compared to other “omics”, metabolic profiling or metabolomics, integrates the effects of gene regulation, post-transcriptional regulation and pathway interactions. This downstream synthesis of diverse signals ultimately makes metabolites direct molecular readouts of cell status that reflect a meaningful physiological phenotype (Figure 1)^[1,2]. In addition, changes in metabolite concentrations are also induced by exogenous factors such as environmental and dietary factors which do not affect the genome. Metabolomics is defined as “the non-biased identification and quantification of all metabolites in a biological system”^[3]. For the quantitative analysis of metabolites in response to disease, Nicholson and colleagues introduced the term metabonomics or “the quantitative measurement of the multiparametric metabolic responses of a living system to pathophysiological stimuli or genetic modification”^[4]. In practice, within human disease research, both terms are used indifferently.

Metabolic profiling is a powerful exploratory tool for understanding interactions between nutrients, the intestinal metabolism and the microbiota composition in health and disease and, to gain more insight in metabolic pathways. Metabolomic studies allow evaluation of metabolites by a top-down approach bypassing the need for an *a priori* hypothesis. Generally, metabolomic analysis has in view two major opportunities. First, untargeted analysis

of a large number of metabolites enhances the chance to discover metabolites that are associated with the disease and might serve as biomarkers. In this respect, biomarker models are designed to discriminate with optimal sensitivity/specificity between groups, but do not presume biological understanding as an absolute prerequisite for biomarker development. However, understanding of the biological pathways can certainly support an assay^[5]. Second, the profile of metabolites affected by the disease may provide new insights into the pathogenesis and eventually reveal new therapeutic targets.

Until now, genomic and proteomic methodologies have often been applied to uncover gastrointestinal related pathophysiological processes^[6-11]. However, currently, metabolomics technologies are increasingly used for discovery of gastrointestinal disease signatures and have been applied for the screening of different pathological conditions that are linked with a metabolic imbalance. This review focuses on the contribution of metabolic profiling in advancing research in the field of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).

THE MICROBIOTA AND ITS METABOLIC ACTIVITY

The microbiota residing in the human gastrointestinal tract, especially the large intestine, is recognized as one of the most metabolically active organs of the human

body. This microbial ecosystem is extremely complex and dynamic with high densities of living bacteria consisting of approximately 500-1000 different species^[12,13]. In healthy adults, 80% of the identified fecal microbiota can be classified into three dominant phyla: *Firmicutes*, *Bacteroidetes* and *Actinobacteria*, but there is substantial variation in the species composition between individuals^[14]. A total of about 10^{14} bacterial cells are present in the adult intestine, which is ten times the number of cells in the human body^[15]. This microbiome outnumbers the host's genetic potential by two orders of magnitude^[16] and provides a diverse range of biochemical and metabolic activities to complement the host's physiology. The presence and metabolic activities of a specific bacterial community play an important role in maintaining the host's overall health and well-being, and has been shown to respond to metabolic challenges and dietary factors. This complex microbial system varies with the host's age, diet and health status^[17].

Through the process of fermentation, colonic bacteria produce a wide range of compounds that may influence the physiological processes in the colon. The human microbiota is characterized by a significant degree of functional redundancy, meaning that different bacteria can perform similar functions and metabolize the same substrate, thereby producing similar metabolites^[18]. Therefore, not only the composition but also the functional capacity of the intestinal microbiota is highly important regarding the clinical end points. Nevertheless, metabolic insights remain limited due to the inaccessibility of the intestinal habitat and the complexity of the microbiota composition^[12]. A number of factors, such as nutrient availability, physicochemical nutrient properties, colonic transit time, and age of the host, influence the composition and the metabolic activity of the colonic microbiota. Nutrient availability is believed to be the most important regulator of bacterial metabolism. Especially the ratio of available carbohydrate to nitrogen determines the degree of saccharolytic *vs* proteolytic fermentation^[19]. Colonic fermentation of carbohydrates results in the generation of short-chain fatty acids (SCFA) which are generally assumed to be beneficial for the host^[20]. Protein fermentation gives rise to a variety of metabolites such as phenolic compounds, branched-chain fatty acids, S-containing compounds, amines and ammonia^[21,22].

HOST AND INTESTINAL MICROBIOTA CO-METABOLOME

The intestinal microbiota produces a number of compounds during the metabolism of nutrients and xenobiotics (compounds of non-host origin that enter the gut with the diet or are produced by the microbiota). Some of these metabolites are excreted in feces whereas others are absorbed through the colonic mucosa and enter the systemic circulation where they can be further modified by human metabolism. For instance, p-cresol is a bacte-

rial fermentation product produced in the colon from tyrosine that is effectively absorbed. It is conjugated in the colon mucosa or liver to p-cresol sulfate or p-cresol glucuronide which improves the water solubility and facilitates its urinary excretion^[23]. These metabolites are called human commensal co-metabolites. Also the opposite occurs. A number of metabolites that are derived from host metabolism are returned to the gut *via* biliary excretion where they can be further metabolized by the microbiota. For instance, bile acids that have escaped absorption in the terminal ileum can be deconjugated and converted to secondary bile acids by microbial metabolism^[24].

These host-microbiota metabolic interactions complicate the interpretation of metabolite profiles. In addition, this co-metabolism explains that the outcome of metabolome analyses clearly depends on the biomatrix chosen. The contribution of the microbial metabolism is more likely reflected in the fecal metabolome than in urinary, serum or breath profiles. Urinary profiles contain human and human-microbial co-metabolites whereas serum profiles seem less influenced by bacterial metabolism.

METABOLOMICS-BASED METHODS

Analytical strategies

Fiehn *et al.*^[1] defined metabolomic analysis as "a comprehensive and quantitative analysis of the metabolome" with the metabolome defined as the whole of metabolites produced by an organism. However, due to the chemical diversity and different physicochemical properties of the metabolites and the large dynamic range of metabolite concentrations in different biological samples, it is virtually impossible to measure the complete metabolome. By selecting a specific analytical platform and a biofluid in which metabolites will be measured, the metabolome will be reduced to those specific conditions. Serum, plasma, urine, feces and tissue are the most studied biological matrices^[25]. An overview of the different steps involved in the analytical process is shown in Figure 2.

Multiple analytical techniques have been used for the analysis of the metabolome. Gas chromatography (GC), liquid chromatography (LC) and high/ultra performance liquid chromatography (H/UPLC) coupled to mass spectrometry (MS), and nuclear magnetic resonance spectroscopy (NMR) enable detection, identification and quantification of metabolites^[26,27]. Other analytical options consist of Fourier transform infrared spectroscopy (FTIS) or capillary electrophoresis (CE) coupled to MS^[28,29].

The applicability of these analytical techniques differs. GC-MS provides an extraordinary resolution, permitting the separation of structurally similar compounds. However, this technique requires the compounds to be volatile and thermally stable. A chemical derivatization step can be applied prior to the chromatographic separation to render polar metabolites more volatile. Purge-

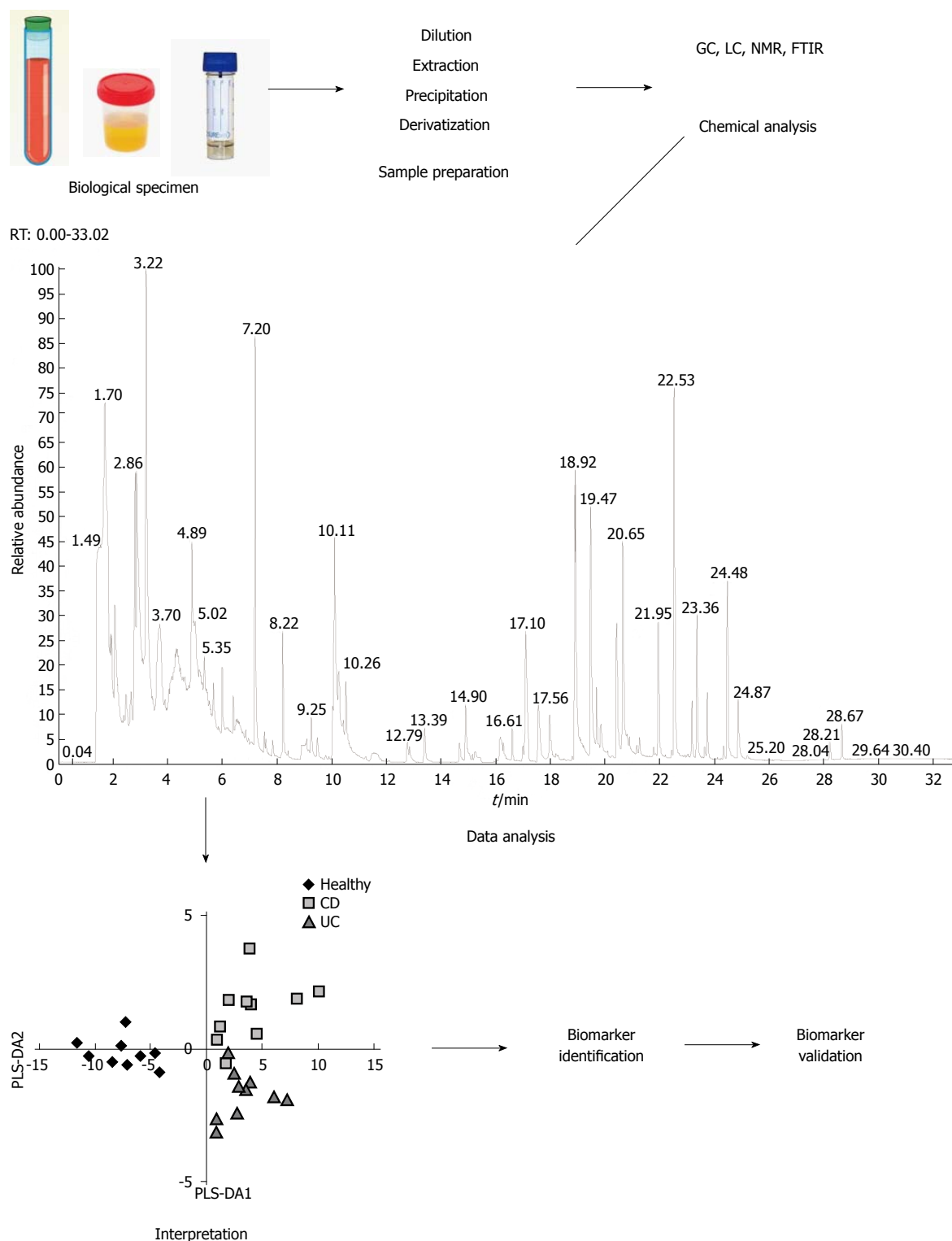


Figure 2 Overview of the different steps involved in the analytical process of metabolomics. GC: Gas chromatography; LC: liquid chromatography; NMR: Nuclear magnetic resonance; FTIR: Fourier Transform infrared spectroscopy; PLS-DA: Partial least squares discriminant analysis; CD: Crohn's disease; UC: Ulcerative colitis.

and-trap and solid phase micro-extraction are sample preparation techniques often used in combination with GC. For metabolites that are not volatile and which cannot be derivatized, LC-MS is applied. LC-MS can detect a relatively broad spectrum of (polar and non-polar) metabolites with ample selectivity and sensitivity^[50]. CE-MS

is a rather new technique in metabolomics that is more sensitive and detects a wider spectrum of (polar) compounds than LC-MS^[31,32]. ¹H-NMR is a non-destructive technique that does not require prior separation of the compounds in the biofluid. It provides detailed information on molecular structure and requires only minimal

sample preparation, but has a lower sensitivity than the MS based techniques^[33]. Often, a combination of different techniques is applied as none of the individual methods will cover the full metabolome^[27]. Several on-line databases for identifying metabolites from experimental NMR and/or MS data are available, as summarized in Table 1. These databases contain chemical, spectral, clinical and molecular data about metabolites found in different human biofluids.

Depending on the technique, detection limits vary: detection limits for NMR and FTIS (mM sensitivity) are much higher as compared to GC-MS (< mM sensitivity) and LC-MS (nM sensitivity). As a consequence, MS-based techniques are preferably applied for quantification of specific metabolites.

Data handling

The analysis and interpretation of complex metabolomics data is facilitated by the application of chemometric and bio-statistical tools. Commonly used tests in metabolomic studies include principal component analysis (PCA) and partial-least squares discriminant analysis (PLS-DA). PCA is an unsupervised classification method, since the variation in the data is analyzed without a priori designation of samples into their classes. In contrast, PLS-DA is considered a supervised classification method, as the samples are designated into their classes for comparison.

In metabolomics, typically the number of variables or metabolites largely exceeds the number of samples measured. This can lead to the discovery of a number of variables that randomly, *i.e.*, by chance, correlate to the outcome variable and in this way give the impression of a good predictive ability. However, if such a set of variables is chosen and the model is applied to new samples, the predictive ability might be very poor. This is known as over-fitting or fitting to the noise and can be avoided by careful cross-validation of the model. Cross-validation implicates that the data set is split in a training set and a test set. The biomarker model is discovered using the training set and the performance of the model is evaluated using the test set. In case of a relatively low sample number (< 100), multiple rounds of cross validation are performed using different partitions of the data in training set/test set and the performance results are averaged^[5].

METABOLIC SIGNATURE AND GASTRO-INTESTINAL PHENOTYPES

Dysbiosis in gastrointestinal disorders

In gastrointestinal diseases such as IBD and IBS, there is an emerging consensus hypothesis that a dysbiosis of the microbiota is involved in initiating the disease or maintaining it. Several studies identified a disproportion of the predominant bacteria in fecal samples of IBD patients^[18,42] and IBS patients^[43]. For example, a reduction in the abundance and diversity of Firmicutes is fre-

quently associated with IBD and IBS. At present, studies comparing the metabolic activity of the microbiota of IBD and IBS patients as compared to normal individuals are emerging thereby investigating whether eventual differences could be related to the pathogenesis of the disease^[44,45] or whether they could be used as a classification tool in clinical diagnosis.

Metabolomics in IBD

Inflammatory bowel diseases comprise Crohn's disease and ulcerative colitis as the two major phenotypes. Although both phenotypes share similar pathophysiological and clinical features, they require different therapeutic management and display different prognosis. Both manifestations are influenced by genetic predispositions as well as microbial and environmental factors. At present, the diagnosis of IBD mainly relies on clinical, endoscopic, radiologic and histologic examination which implicates that diagnosis is only possible at a relatively advanced stage of the disease. Less invasive methods such as analysis of biomarkers from urine, serum, or feces, however, would be of significant advantage and useful for primary diagnosis, surveillance, and early detection of relapses.

Several biomarkers or sets of biomarkers have been tested in clinical trials including acute phase proteins such as C-reactive protein, fecal markers (lactoferrin, calprotectin, and PMN-elastase) and serological markers (antibodies against luminal antigens and anti-glycan antibodies)^[46]. Recently, the exploration of metabolomics in IBD rose from the need to improve diagnosis and to allow better stratification of patients into IBD subtypes.

Several studies have applied a metabolomic approach to discriminate IBD patients from healthy controls, to discriminate CD from UC and patients with active disease from patients in remission. An overview of the studies in humans is presented in Table 2.

The growing acceptance of the involvement of the gut microbiota in the chronic mucosal immune activation underlying the pathogenesis of IBD has led to an interest in the use of fecal extracts or fecal samples as biofluids to apply metabolite profiling.

Marchesi *et al*^[47] was the first to differentiate IBD patients from healthy controls based on ¹H-NMR analysis of fecal water extracts and to discriminate CD patients from UC patients. Fecal water extracts from IBD patients were characterized by a depletion of bacterial degradation products such as SCFA, dimethylamine and trimethylamine suggesting a disruption of the normal bacterial ecology, called dysbiosis, either as the cause or consequence of the disease. In a study in identical twins including healthy twins and twins with inactive CD, either concordant or discordant, fecal extracts were analyzed using ICR-FT/MS with ultrahigh mass resolution. Healthy subjects could nicely be discriminated from CD patients. In addition, it was possible to separate patients with predominantly ileal involvement from patients with predominantly colonic involvement of the disease. Inter-

Table 1 Overview of web-based databases for metabolite identification

Database	URL or web address	Extra information
Human Metabolome Database (HMDB)	http://www.hmdb.ca/	Wishart <i>et al.</i> ^[34]
Madison Metabolomics Consortium (MMC) Database	http://mmcd.nmrfa.wisc.edu/	Cui <i>et al.</i> ^[35]
Biological Magnetic Resonance Data Bank (BMRB)	http://www.bmrwisc.edu/	Ulrich <i>et al.</i> ^[36]
Golm Metabolome Database	http://csbdb.mpimp-golm.mpg.de/csbdb/gmd/gmd.html	Kopka <i>et al.</i> ^[37]
BiGG (a knowledgebase of Biochemically, Genetically and Genomically structured genome-scale metabolic network reconstructions)	http://bigg.ucsd.edu/	Schellenberger <i>et al.</i> ^[38]
SetupX and BinBase	http://fiehnlab.ucdavis.edu/	Skogerson <i>et al.</i> ^[39]
MassBank	http://www.massbank.jp/	Horai <i>et al.</i> ^[40]
METLIN	http://metlin.scripps.edu/	Smith <i>et al.</i> ^[41]

estingly, the separation of the groups was higher for the metabolite profiles than for the microbial community profiles, analyzed on the same samples with T-RFLP fingerprints generated using general bacterial and *Bacteroides* group-specific primers^[48]. The higher discrimination of the metabolite data was attributed to a direct link of the metabolites to function.

In another study, ¹H-NMR profiling of fecal extract allowed to discriminate patients with UC from healthy controls. Elevated levels of taurine and cadaverine in UC patients were the major discriminative findings. Samples were also analyzed for microbiota composition using DGGE. Canonical correlation analysis between NMR and DGGE data sets, based on PC scores accounting for 90% of the original variance, revealed a good correlation ($r = 0.85$, $P < 0.002$) between gut microbiota profile and metabolite composition suggesting a direct link between both parameters^[49].

A recent study analyzed metabolite profiles in feces of chronic gastrointestinal disorders using GC-MS with headspace sample preparation. Samples of CD patients showed significant higher levels of ester and alcohol derivatives of SCFA and indole as compared to healthy controls, UC and IBS patients. Following treatment, the metabolite profile was altered to more closely resemble that of healthy volunteers^[50]. As many microbial metabolites are absorbed and excreted in urine, either as such or after further metabolism by human enzymes, metabolite profiles of urine samples may also reflect the impact of the microbiota composition^[51]. Several studies were able to discriminate IBD patients from healthy controls based on metabolite profiling of urine samples^[52-54]. In all these studies, hippurate levels were lower in IBD patients as compared to controls suggesting hippurate as a biomarker of IBD. Hippurate or N-benzoylglycine is a mammalian-microbial co-metabolite that originates from bacterial fermentation of dietary aromatic compounds (polyphenols, purines or aromatic amino acids) to benzoic acid which is further conjugated to glycine in the liver^[55]. Remarkably, urinary metabolite profiling allowed to differentiate between UC and CD in only one study^[51], whereas two other studies failed to do so^[53,54]. This discrepancy may highlight the fact that IBD is a multifactorial disease with a high variety in phenotypes and severity^[56]. Indeed, the notion that IBD is actually a syndrome comprising several disease subtypes, is gaining

more and more acceptance^[46].

Metabolite profiles in serum or plasma or in colonic mucosal biopsies rather reflect changes in the host's metabolism and provide less information on the impact of the gut microbiota composition and/or activity. As compared to urinary and fecal profiles, metabolite profiles in serum or plasma may be less affected by environmental factors and are subject to less inter-individual variation^[57].

Results from studies that analyzed serum/plasma or colonic mucosa cells indicate that both CD and UC have an impact on the amino acid metabolism^[53,58-63]. Several amino acids occurred in lower levels in colonic mucosal cells from IBD patients as compared to controls. As higher amino acid levels were found in fecal extracts^[47,64], this may be the result of malabsorption due to inflammation. An alternative explanation is that inflammatory conditions induce large energy requirements to repair the damaged mucosa leading to enhanced protein catabolism. Specifically, the role of decreased levels of glutamine in the pathogenesis of IBD has been studied. Besides butyrate, glutamine is an important energy source for the colonocytes and accounts for about 30% of their energy needs. In a mouse model of DSS-induced colitis, similar reductions in glutamine levels in serum and colonic tissue were observed and supplementation with glutamine attenuated the DSS-induced colitis^[65].

In a recent study, Hisamatsu *et al.*^[66] calculated an AminoIndex based on multivariate analysis of amino acid profiles in serum of IBD patients which allowed to distinguish between CD and UC and also reflected disease activity.

Zhang *et al.*^[67] specifically recruited patients with active UC and short disease duration (2.7 years) and evaluated the efficacy of ¹H-NMR metabolic profiling of serum for early stage diagnosis of UC. Although active UC patients could be discriminated from healthy controls in a multivariate OPLS-DA model, the number of altered metabolites in this study was rather limited.

Metabolomics in IBS

Irritable bowel syndrome is a multifactorial functional disorder of the gastrointestinal tract that affects about 10%-15% of the adult population. IBS patients have symptoms of pain and bowel dysfunction. A subset of patients that developed IBS after an infection, so-called

Table 2 Overview of the studies that applied metabolomics to discriminate inflammatory bowel diseases and/or irritable bowel syndrome patients from controls

Reference	Analytical platform	Biofluid	Samples	Observations
Marchesi <i>et al</i> ^[47]	¹ HNMR	Faecal extracts	CD (<i>n</i> = 10), UC (<i>n</i> = 10), HC (<i>n</i> = 13)	Depletion of SCFA and methylamine and trimethylamine in CD patients Higher amounts of amino acids in UC and CD compared to healthy controls
Jansson <i>et al</i> ^[64]	ICR-FT/MS	Faecal water	10 twin pairs with CD, 7 healthy twin pairs	Discrimination based on disease location (ileal or colonic CD) significant differences in the types and number of metabolites within specific pathways, including tyrosine and phenyl-alanine metabolism and bile acid and fatty acid biosynthesis
Le Gall <i>et al</i> ^[49]	¹ HNMR	Faecal water	UC (<i>n</i> = 13; 31 samples), IBS (<i>n</i> = 10; 21 samples), HC (<i>n</i> = 22; 72 samples)	Discrimination between UC and HC; no classification of IBS Increased taurine and cadaverine in UC
Walton <i>et al</i> ^[50]	GC-MS	Faeces	UC (<i>n</i> = 20), CD (<i>n</i> = 22), IBS (<i>n</i> = 26), HC (<i>n</i> = 19)	Increased concentrations of ester and alcohol derivatives of short-chain fatty acids and indole in CD After treatment, metabolite patterns are more similar to those of HC
Williams <i>et al</i> ^[52]	¹ HNMR	Urine	CD (<i>n</i> = 86), UC (<i>n</i> = 60), HC (<i>n</i> = 60)	Discrimination between CD, UC and HC Significantly different metabolites include hippurate, p-cresol sulfate and formate
Schicho <i>et al</i> ^[53]	¹ HNMR	Urine, serum, plasma	CD (<i>n</i> = 20), UC (<i>n</i> = 20), HC (<i>n</i> = 40)	Clustering independent of diet and medication IBD patients could be discriminated from HC, differences between CD and UC less pronounced
Stephens <i>et al</i> ^[54]	¹ HNMR	Urine	CD (<i>n</i> = 30), UC (<i>n</i> = 30), HC (<i>n</i> = 60)	Discriminating metabolites include amino acids, creatine, creatinine, metabolites of urea cycle, monosaccharides, hippurate (urine) Metabolites for distinguishing IBD from HC: TCA cycle intermediates, amino acids metabolites derived from gut microflora (methanol, formate, hippurate, acetate, and methylamine); as well as the other metabolites trigonelline, creatine, urea, and taurine
Ooi <i>et al</i> ^[56]	GC-MS	Colonic biopsies, serum	Colonic biopsies: UC (<i>n</i> = 22), se- rum: UC (<i>n</i> = 13), CD (<i>n</i> = 21), HC (<i>n</i> = 17)	Reduced levels of amino acids resulting in reduced levels of TCA cycle related downstream molecules in colonic tissue of UC Serum amino acid profiling enabled discrimination between UC and CD
Bjerrum <i>et al</i> ^[60]	¹ HNMR	Colonic biopsies, colonocytes, lymphocytes, urine	Active UC (<i>n</i> = 35), quiescent UC (<i>n</i> = 33), HC (<i>n</i> = 25)	No discrimination between active UC, inactive UC and HC based on urine or lymphocyte profiles Inactive UC could not be differentiated from HC Active UC characterized by higher antioxidants and amino acids and lower levels of lipid, myo-inositol, betaine and glycerophosphoglycine
Bezabeh <i>et al</i> ^[63]	¹ HNMR	Colonic biopsies	UC (<i>n</i> = 26; 45 samples), CD (<i>n</i> = 21; 31 samples), controls (38 non-inflamed IBD, 25 cancer patients)	Accurate classification of UC vs CD Some non-inflamed tissues from IBD had abnormal NMR-spectra
Balasubramanian <i>et al</i> ^[61]	¹ HNMR	Colonic biopsies	Active UC (<i>n</i> = 20), Inactive UC (<i>n</i> = 11), Active CD (<i>n</i> = 20), Inactive CD (<i>n</i> = 6), HC (<i>n</i> = 26)	Higher α -glucose and lower amino acids, membrane components, lactate and succinate in active UC and CD compared to HC Lower lactate, glycerophosphorylcholine and myo-inositol in inactive UC and lower lactate in inactive CD compared to HC Lower formate in active UC vs active CD
Sharma <i>et al</i> ^[62]	¹ HNMR	Colonic biopsies (inflamed and non-inflamed)	UC (<i>n</i> = 12), CD (<i>n</i> = 9), controls (<i>n</i> = 25)	No differentiation between inflamed and non-inflamed samples Lower levels of amino acids, membrane components, lactate and formate in IBD vs controls and higher levels of glucose
Hisamatsu <i>et al</i> ^[66]	AA analyzer	plasma	CD (<i>n</i> = 165), UC (<i>n</i> = 222), HC (<i>n</i> = 210)	Multivariate indexes established from plasma aminograms distinguish CD or UC from HC Other indexes distinguish active UC and CD from each remission patients and correlate with disease activity indices
Zhang <i>et al</i> ^[67]	¹ HNMR	Serum	Active UC (<i>n</i> = 20), HC (<i>n</i> = 19)	Active UC displayed increased 3-hydroxybutyrate, β -glucose, α -glucose and phenylalanine and decreased lipid compared to healthy controls
Ponnusamy <i>et al</i> ^[71]	GC-MS	Faeces	IBS (<i>n</i> = 11) vs non-IBS (<i>n</i> = 8)	Elevated levels of amino acids and phenolic compounds that were highly correlated with abundance of lactobacilli and Clostridium

Ohman <i>et al</i> ^[69]	GC-MS	Faeces	IBS-D (<i>n</i> = 30), CD Significantly more esters in IBS-D, association of aldehydes with IBD (<i>n</i> = 62), UC (<i>n</i> = Accurate separation of IBS-D from active CD, UC and HC 48), HC (<i>n</i> = 109)
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SCFA: Short-chain fatty acids; IBD: Inflammatory bowel diseases; IBS: Irritable bowel syndrome; TCA: Tricarboxylic acid; HNMR: Nuclear magnetic resonance; ICR-FT/MS: Fourier transform ion cyclotron resonance mass spectrometry; GC-MS: Gas chromatography-mass spectrometry.

post-infectious IBS, may have microscopic inflammation but with normal mucosal appearance on endoscopy. Recently, a new IBS entity, IBD-IBS, has been described: these patients have pain and diarrhea similar to IBS in association with minimal or no evident intestinal inflammation^[68]. The exact etiology has not been identified. Yet, environmental, psycho-social, physiological and genetic factors are believed to play a role. Also the role of an abnormal microbiota composition is supported by clinical and experimental data^[69]. Disruption of the balance between the host and the intestinal microbiota results in changes in the mucosal immune system that range from overt inflammation, as seen in Crohn's disease, to low-grade inflammation without tissue injury, as seen in a subset of IBS patients^[70].

Surprisingly, very little work has been conducted on metabolite profiling of IBS patients. ¹H-NMR metabolite profiling of fecal extracts allowed to separate IBS patients from healthy controls with moderate success (sensitivity = 57%, specificity = 76%)^[49]. Two other studies applied GC/MS to analyze fecal samples although with different sample preparation. Ahmed *et al*^[71] analyzed the volatile organic compounds (VOC) in the headspace of the fecal samples, *i.e.*, compounds with a vapour pressure that is sufficiently high to enable them to move from the solid or liquid phase into the gaseous phase. Those VOC comprise hydrocarbons, alcohols, aldehydes, ketones, esters and organic acids^[72]. In contrast, the volatility of polar compounds in the fecal samples was increased by trimethylsilyl derivatization in the study by Ponnusamy *et al*^[73]. Although both studies were able to discriminate IBS patients from healthy controls, the metabolites responsible for discrimination were different which is inherent to the different sample preparation. Ahmed *et al*^[71] found increased abundance of esters in diarrhea predominant IBS-patients compared with healthy controls. Of the 28 VOCs positively associated with IBS, 22 belonged to the class of esters. Ponnusamy *et al*^[73] highlighted higher levels of specific amino acids (alanine and pyroglutamate) and phenolic compounds (hydroxyphenyl acetate and hydroxyphenyl propionate) and associated these metabolic changes to alterations of specific gut microbial populations including lactobacilli and *Clostridium*.

Translating metabolites in gastrointestinal biomarkers

Metabolites are promising biomarkers because they can be easily measured from non-invasive breath, urine, feces or blood samples. Several studies have identified such metabolite signatures that should allow classification of samples as healthy or diseased. To translate these models into the clinic, additional validation studies are required. First of all, experiments to validate the biomarker model

need to be performed. This level of validation includes (1) lab repeatability studies where the same samples are analyzed in the same laboratory by the same observer; (2) lab replication studies, where independent samples are analyzed in the same lab by the same observer; (3) inter-lab repeatability studies, where the original samples are analyzed in a different laboratory by a different observer; and (4) inter-lab replication studies, where independent samples are analyzed in a different laboratory.

Secondly, most studies mentioned above have compared metabolite profiles obtained from patients with a distinct diagnosis of either IBD or IBS to healthy subjects. To ensure clinical utility, it might be necessary to include additional control groups including patients with other gastrointestinal or inflammatory disorders like patients with infectious GI disease or neoplastic disease.

Thirdly, the influence of potential confounders on the performance of the models needs to be established. Confounding factors might be related to the subject (gender, age, disease location, comorbidities) or might be of environmental origin (diet, diurnal variation, medication, smoking).

CONCLUSION

Metabolomics may advance our understanding, diagnosis and treatment of inflammatory bowel disease and irritable bowel syndrome. Using this approach, disease-related mechanisms may be uncovered and verified, and candidate diagnostic biomarkers in biological samples are characterized. Before usage as clinical diagnostics, metabolites must be verified and validated in large clinical trials. To translate metabolomics data into a more profound biological understanding of the disease, more knowledge on the relevance of a decrease or increase in certain metabolites is warranted. Metabolomic profiling mainly detects associations between profiles and specific phenotypes which may not always be meaningful. In addition, it often remains unknown whether changes in metabolites are the cause or a consequence of the disease. Integration of other "Omics" with metabolomic may enable a further understanding of gastrointestinal related pathophysiological processes.

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Non-dietary forms of treatment for adult celiac disease

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Abstract

At present, treatment for celiac disease includes a strict gluten-free diet. Compliance, however, is difficult and gluten-free food products are costly, and, sometimes very inconvenient. A number of potential alternative measures have been proposed to either replace or supplement gluten-free diet therapy. In the past, non-dietary forms of treatment were used (*e.g.*, corticosteroids) by some clinicians, often to supplement a gluten-free diet in patients that appeared to be poorly responsive to a gluten-free diet. Some of new and novel non-dietary measures have already advanced to a clinical trial phase. There are still some difficulties even if initial studies suggest a particularly exciting and novel form of non-dietary treatment. In particular, precise monitoring of the response to these agents will become critical. Symptom or laboratory improvement may be important, but it will be critical to ensure that ongoing inflammatory change and mucosal injury are not present. Therapeutic trials will be made more difficult because there is already an effective treatment regimen.

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Key words: Adult celiac disease; Gluten-free diet; Non-dietary treatment of celiac disease; Tight junction inhibition; Protease; Immunotherapy; Vaccination

Core tip: Non-dietary forms of treatment for adult celiac disease are currently being evaluated and some have reached clinical trials. Some novel approaches being investigated include hydrolysis of gliadin peptides, inhibition of intestinal permeability, blockade of T lymphocytes and transglutaminase 2/human leukocyte antigen-DQ2 functions as well as induction of immune tolerance. Future evaluations will need to define effects on specific endpoints and ensure an improvement in symptoms, laboratory test results and, most important, mucosal inflammatory changes. Therapeutic trials with novel agents will be difficult from an ethical perspective as the current form of management with a gluten-free diet already provides an excellent result for most compliant patients with celiac disease. Finally, effects on other known superimposed diseases will need close evaluation (*i.e.*, lymphoproliferative and other malignancies).

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INTRODUCTION AND DIAGNOSIS

Celiac disease is a small bowel disorder that appears to respond clinically and histopathologically to a strict gluten-free diet. Indeed, the only universally accepted form of effective therapy for celiac disease is a gluten-free diet for life after the diagnosis has been accurately established.

Diagnosis involves demonstration of the following, ideally in a sequential fashion: (1) classical histopathological features of celiac disease shown in biopsies from the proximal small bowel; and (2) a response to a gluten-free diet^[1]. A very recent review and update on the prevalence, diagnosis, pathogenesis and treatment of celiac disease has appeared^[2]. Some, but not all clinicians, particularly those evaluating the pediatric age group, believe that serological testing (especially with tissue transglutaminase

antibodies) coupled with definition of human leukocyte antigen (HLA)-DQ2 and HLA-DQ8, rather than biopsy may be sufficient for diagnosis^[3,4].

Most patients present with diarrhea and weight loss. However, in recent years, more and more patients are now being detected with limited or no intestinal symptoms. In part, this reflects a greater appreciation by physicians for a widening spectrum of extra-intestinal changes associated with celiac disease and increased performance of screening using widely available serological markers (e.g., antibodies to tissue transglutaminase, or tTG). In addition, however, some recent studies have also suggested that there may be a very real increase in celiac disease even over the past decade or so, possibly related to some, as yet, unrecognized environmental factor^[5,6]. Typical biopsy changes include “flattening” of the villi with extension of the crypt epithelial cell compartment, increased numbers of plasma cells and lymphocytes in the lamina propria region, and increased numbers of intraepithelial lymphocytes. Although typical, these changes are not, in themselves, diagnostic as several disorders may mimic the changes of celiac disease^[7]. Only celiac disease responds to a gluten-free diet, although some symptoms, incorrectly attributed to celiac disease, may also respond to removal of gluten from the diet.

GLUTEN-FREE DIET AND COMPLIANCE

It is well known that life-long compliance to a gluten-free diet is difficult and expensive. In reality, a major problem underlying this form of prescribed diet therapy in celiac disease is complete removal of gluten since this substance is ubiquitous and present in many foods^[8]. Even foods that some authorities consider as safe, such as oats, may be contaminated with other grains that contain the injurious peptide sequences. The Food and Drug Administration in the United States has arbitrarily established a limit of < 20 ppm gluten (i.e., about 10 ppm gliadin) to be established as a “gluten-free” food. Total daily consumption of gluten also appears to be critical and some experts have estimated a threshold for some individuals with celiac disease to be lower than 50 mg daily^[9]. Even with these numerical considerations though, some patients with celiac disease may be even more sensitive, after only single ingestion of minute amounts of gluten. Even small amounts may provoke increased circulating levels of tissue transglutaminase antibodies and induce inflammatory changes in small bowel biopsies.

In recent years, a number of alternative dietary (e.g., genetically-modified gluten) and non-dietary approaches have been considered^[10-12]. Some are further detailed here including those already studied in some clinical trials as well as some that have not yet been evaluated. These might potentially serve, at least in part, in the future horizon for treatment of celiac disease. It is unlikely that any of these will be designated for independent treatment alone since the gluten-free diet, in spite of being difficult, costly and, often inconvenient, remains a highly effective

management approach.

GLIADIN PEPTIDE HYDROLYSIS

Some plants and micro-organisms express endoproteolytic enzyme activities that can hydrolyze the proline-containing gluten in foods to amino acids and smaller length oligopeptides that might permit later hydrolysis by human intestinal brush border enzymes. The prolyl-endopeptidases (PEP) are a family of enzymes with the ability to cleave internal proline residues in a proline-containing peptide^[13]. Even though PEP activity is expressed in the human small intestine, a gliadin peptide (i.e., 33-mer) that appears to be highly immunogenic is poorly hydrolyzed by human PEP^[14]. Other species, including some bacteria and fungi, express PEP activities and may, in theory, be very effective.

Aspergillus niger PEP can hydrolyze a number of gliadin peptides and its activity has been shown to inhibit the gliadin-induced immunologic response by gluten-specific T-cells^[15]. In a gastrointestinal model system, most hydrolysis appeared to occur in the stomach compartment with little activity required in the small intestine^[16]. Alternative PEPs from other microbial species (*Flavobacterium meningosepticum*, *Sphingomonas capsulata*, *Myxococcus Xanthus*) can hydrolyze gliadin peptides *in vivo* in the rat^[17,18], and pre-treatment of gluten with PEP appeared to reduce malabsorption of fat or carbohydrate in patients with celiac disease^[19].

Use of enzymes that involve other mechanisms could provide different treatment approaches. For example, specific proteases cleave storage proteins during germination of different grains and, as a result, may increase the rate of gluten degradation. A barley proteinase that hydrolyzes wheat gluten in rats has been reported to potentially provide protection against ingested gluten in gluten-sensitive rhesus monkeys^[20,21].

Additional studies have also suggested that different hydrolytic enzyme activities may be used in combination to improve efficiencies. For example, ALV003 consisting of PEP from *Sphingomonas capsulata* and a barley protease may prevent the T-cell response in patients with known celiac disease^[19]. In early clinical studies, orally-administered ALV003 was well tolerated without significant adverse effects^[22]. Phase 2 trials are in process and, have appeared in abstract form, suggesting possible benefit.

Alternative approaches to hydrolyze toxic gluten peptides have also employed enzyme mixtures isolated from germinating *Triticale*, including wheat, rye and barley. *In vitro* studies using intestinal epithelial cells and organ cultures of intestinal biopsies from untreated patients with celiac disease have demonstrated a reduction in markers of epithelial cell injury^[23].

Another suggested alternative to facilitate gluten degradation includes use of whole cultured bacteria. Normally, a complex microbial population is present in the intestinal lumen. A number of studies from different groups^[24,25] have described substantial quantitative and qualitative dif-

ferences in the intestinal microbiome of patients with celiac disease. More specifically, *bifidobacteria*, among several bacterial species, are reportedly abnormal in patients with celiac disease. *In vitro* cell culture studies as well as studies in animals have demonstrated reduced gluten toxicity and results of clinical trials are anticipated^[26,27].

Sequestration of gluten by polymeric binders acting in the intestinal lumen of patients with celiac disease could be a further alternative approach. Gluten may complex with linear co-polymers of hydroxyethylmethacrylate and sodium-4-styrene sulfonate to reduce toxic changes of gliadin induced in intestinal epithelial cells^[28]. In addition, this agent also reduced gliadin-induced alterations in barrier function and the numbers of immunoreactive cells, including intra-epithelial lymphocytes, in mice^[29]. Human effects of polymeric binders are not known, but the apparent limitation in side effects, low cost and potential for improved compliance compared to gluten-free diets is attractive.

INHIBITION OF INTESTINAL PERMEABILITY

The small intestinal mucosa in celiac disease is “leaky” with increased permeability. One of the proteins that contributes to permeability is zonulin. Larazotide acetate (*i.e.*, AT-1001) is a synthetic peptide derived from zonula occludens toxin of *Vibrio cholera*^[30]. It has been hypothesized to inhibit zonulin receptor binding to reduce the gliadin-induced increases in intestinal permeability. A phase 1 evaluation in treated celiac patients suggested that the medication was well tolerated, reduced intestinal permeability, decreased pro-inflammatory cytokine production and symptoms in celiacs after gluten exposure^[31]. A phase 2 evaluation showed a reduction in symptoms and autoantibodies. Added studies are needed^[32].

T-CELL LYMPHOCYTE BLOCKADE AND INHIBITION

Another broad category of agents being explored include agents that function to block key lymphocyte effects on the small intestinal mucosa. Specific antagonists as well as monoclonal antibodies that affect specific lymphokines are being explored^[33,34].

For example, gluten effector T-cells may be directed, at least in part, to the small intestinal mucosa by chemokine 25 and its receptor CC chemokine receptor 9. Blockade of this interaction by a selective antagonist has been hypothesized as a potential clinical approach in celiac disease.

Another suggested approach involves development of monoclonal antibodies, including anti-CD3, anti-CD20, anti-interleukin (IL)-10 anti-IL-15 antibodies^[33,34]. For example, reversal of mucosal damage in the small intestine of mice with overexpression of IL-15 could provide an

avenue for further evaluation.

TG2 AND HLA-DQ2 BLOCKADE

Several approaches may emerge for blockade of the adaptive immune response in celiac patients. One involves blockade of TG2 effects. TG2 enhances the binding of gliadin peptides to HLA-DQ2 and enhances T-cell activation in the small intestinal mucosa^[35]. Inhibition of *in vitro* TG2 activity inhibits gliadin-specific T-cell clones from celiacs. Similar inhibition occurs in the gliadin-induced proliferations of some, but not all (*e.g.*, CD8-positive lymphocytes) lamina propria lymphocytes and epithelial cells. Although TG2 is found in other tissues, TG2 inhibitors could theoretically provide a potential avenue for future therapy.

Another area of focus has been related to development of HLA-DQ2 blocking agents using gluten peptide analogues. These include both cyclic and dimeric gluten peptide analogues as well as gluten peptides with azido-proline residues substituted for proline. By changing the gliadin T-cell stimulatory sequence, conversion to an agonist or antagonist may result^[36].

IMMUNE TOLERANCE INDUCTION

In celiac disease, antigen-based therapy specific for a specific peptide sequence in gliadin might be an important future avenue of treatment. A peptide vaccine could promote tolerance by altering the effects of some immune-mediated cells involved in celiac disease pathogenesis. To date, however, definition of the precise antigen involved may not be sufficiently precise, to permit development of an effective vaccine for all celiac patients. A clinical phase 1 trial with Nexvax 2 peptide vaccine-containing a mixture of immunotoxic gliadins has been initiated^[37].

CONCLUSION

A number of avenues of treatment for celiac disease have been proposed as alternatives to a strict gluten-free diet. Some of these appear to be already advanced at the level of the bench in the laboratory, and even at the bedside in some clinical trials. At this time, there are still difficult issues that need to be addressed. First, the end-point of any treatment regimen will require detailed evaluation. The gold standard is mucosal biopsy, but other forms of non-invasive evaluation require assessment to precisely define, not only the degree of responsiveness to a specific treatment regimen, but also the quality of the treatment response. For example, improved symptoms or improved laboratory parameters may signal an improved state, but if there is ongoing inflammatory change and mucosal injury, the treatment may not be a real advance in management and may still carry the long-term risks of only partially-treated celiac disease. Second, therapeutic trials will be difficult and, by necessity from an ethical

perspective, still require that patients with celiac disease be treated in both a treatment arm and the “placebo” arm with a known effective therapy, *i.e.*, gluten-free diet. At best, in spite of the burdens imposed on the celiac patient at present, the goal of these potentially new forms of therapy in celiac disease may predictably be to supplement the gluten-free diet in long-term management of celiac disease. Finally, the long-term effects of these therapies may not be immediately evident and require many years to define. In celiac disease, there appears to be an increased risk for some malignant diseases, including lympho-proliferative diseases, such as T-cell lymphoma^[38-40]. It is conceivable that some of these novel non-dietary forms of therapy may actually alter this background risk, especially over an extended period.

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Validation of methods to assess potential biomarkers in pediatric patients with esophageal eosinophilia

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Abstract

AIM: To validate methods for determining mast cell density, extracellular major basic protein content, and presence of fibrosis in esophageal eosinophilia.

METHODS: Twenty specimens with > 20 eosinophils/high-power field (hpf) classified as high eosinophil density (HE) and 20 specimens with < 5 eosinophils/hpf classified as low esophageal density (LE) were identified. All 40 specimens underwent immunohistochemical staining and trichrome staining. Mast cell density, extracellular major basic protein (MBP) density, and presence of subepithelial fibrosis were assessed in a standardized manner. All specimens were evaluated by two separate observers and by a single observer on two separate occasions to evaluate reproducibility of the methods.

RESULTS: A strong inter-observer correlation was noted for both peak and mean mast cell counts ($r = 0.725$, $P < 0.0001$ and $r = 0.823$, $P < 0.0001$). A strong intra-observer correlation also was noted for both peak and mean mast cell counts ($r = 0.752$, $P < 0.0001$ and $r = 0.878$, $P < 0.0001$). A very strong inter-observer correlation was noted for both peak ($\tau = 0.867$, $P < 0.0001$) and mean extracellular MBP densities ($r = 0.925$, $P < 0.0001$). A very strong intra-observer correlation was noted for both peak ($\tau = 0.875$; $P < 0.0001$) and mean extracellular MBP densities ($r = 0.956$, $P < 0.0001$). Excellent inter-rater reliability was found for fibrosis ($\kappa = 0.887$). Mast cell and MBP densities, as well as presence of fibrosis, were significantly increased in HE vs LE. The HE group had significantly higher intraepithelial mast cell peak (29.35 ± 21.61 vs 12.45 ± 8.26 , $P = 0.002$) and mean (19.84 ± 15.81 vs 6.35 ± 4.5 , $P = 0.001$) densities than the LE group. The HE group had significantly higher peak extracellular MBP (2.35 ± 0.67 vs 0.45 ± 0.61 , $P < 0.001$) and mean extracellular MBP (1.95 ± 0.76 vs 0.20 ± 0.29 , $P < 0.0001$) densities than the LE group. Seventy-three percent of patients with HE (11/15) had fibrosis, whereas only 10% of patients with LE (1/10) had fibrosis ($P < 0.01$). MBP performed the best in predicting classification of HE vs LE, with mean MBP demonstrating 100% sensitivity and 95% specificity at the optimal cut point.

CONCLUSION: This study provides methodology and proof-of-concept for future evaluation of these biomarkers for differentiating esophageal eosinophilic diseases such as reflux esophagitis and eosinophilic esophagitis.

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Key words: Eosinophilia; Immunohistochemical staining; Tryptase; Major basic protein; Subepithelial fibrosis

Core tip: Esophageal mucosal eosinophilia challenges many clinicians and researchers. Biomarkers have been

proposed to help clarify and better characterize eosinophil driven disease. This study provides validation of methods used previously to differentiate low eosinophil density from high eosinophil density. Eosinophilic major basic protein appears to be the best predictor for classification of eosinophilia at both extremes. This lays the ground work for future studies to examine varying degrees of eosinophilia using biomarkers.

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INTRODUCTION

It is well known that the presence of eosinophils in esophageal mucosa denotes pathology; however, the basis for eosinophilic infiltration is not always clear and has been a topic of numerous studies in both children and adults^[1]. Mucosal eosinophils are increased in both reflux esophagitis (RE) and eosinophilic esophagitis (EoE) as well as Candidal esophagitis, viral esophagitis and Crohn's esophagitis to name a few. The two most common causes of increased eosinophils without other disease features are reflux esophagitis and eosinophilic esophagitis. Determining the density of eosinophils (*i.e.*, cells per high-power field, hpf) present on biopsy specimens is one factor considered in distinguishing between these two diagnoses^[1-6]. Most studies suggest that a significantly higher number of eosinophils are found in patients with EoE; however, the appropriate number needed to make a diagnosis of EoE is unclear^[1]. This is problematic as the two forms of esophagitis have markedly different treatments and prognosis. Thus, finding additional biomarkers to aid in diagnosis is warranted.

Examination of the pathway through which eosinophils exert their influence in EoE may shed light on potential biomarkers. First, eosinophils, which are normally absent in the esophagus, may infiltrate the esophageal mucosa and become activated. Once activated, eosinophils release their granule proteins causing inflammation and tissue damage. Granule proteins, such as major basic protein (MBP), eosinophilic cationic protein, and eosinophilic peroxidase, have cytotoxic effects on esophageal epithelium^[7-9]. Their pro-inflammatory properties continue the cycle of inflammation. Eosinophils elaborate fibrogenic growth factors and induce fibrogenesis through secretion of granule proteins such as MBP. MBP also triggers degranulation of mast cells which, in turn, release inflammatory mediators such as cytokines and histamine^[7,8].

Mucosal mast cells may have a role as a biomarker in that they are increased in pediatric patients with EoE as compared to RE, or gastroesophageal reflux disease (GERD), which is often used interchangeably with RE^[9].

Mast cells are present at higher densities in GERD patients exhibiting > 7 eosinophils/hpf^[10]. Moreover, no intraepithelial esophageal mast cells are present in controls^[10]. Further, mast cell density has been documented to decrease with treatment of EoE^[11].

Extracellular MBP also shows promise as a biomarker for EoE. Chehade *et al*^[12] classified eosinophil degranulation as either absent/mild or extensive based on the pattern of extracellular MBP. Extensive degranulation was more prevalent in EoE patients and the extent of degranulation was unrelated to eosinophil density. Mueller *et al*^[5] studied EoE in adults, evaluating MBP by a semi-quantitative method, and found degranulation in 72%.

Fibrosis also may be a potential biomarker, as indicated by the increased frequency of esophageal stricture in EoE^[1]. In children, esophageal subepithelial fibrosis has been demonstrated in 89% of EoE patients as compared to 37.5% of GERD patients^[13]. Although fibrosis was found in a significant portion of the GERD patients, the pattern was clearly different than with EoE, as the fibrosis was associated with lymphoid tissue in GERD. Chehade *et al*^[12] documented fibrosis in 57% of EoE pediatric patients and in no patients with GERD. Moreover, the presence of fibrosis in the EoE group was associated with a higher proportion of patients demonstrating extensive eosinophil degranulation^[12].

Evaluation of mast cell density, extracellular MBP, and/or fibrosis may be useful in making a diagnosis of EoE, particularly in patients with eosinophil counts placing in the mid-range between typical reflux-associated esophagitis and EoE on biopsy. While mast cell density, extracellular MBP, and fibrosis all appear to be potential markers, the methods for histologic evaluation of these have varied and, further, reproducibility and reliability of these markers in differentiating RE and EoE has not yet been established.

The current study was performed in two steps. In part one, the aim was to establish reproducible methods for determining mast cell density, extracellular MBP content, and presence of fibrosis, respectively. In part two, the primary aim was to determine whether the reproducible markers validated in the first step would reliably differentiate between patients with high esophageal density and patients with low esophageal density. A secondary aim was to explore relationships between eosinophil density and the potential biomarkers.

MATERIALS AND METHODS

The study was approved by the institutional review board at Children's Mercy Hospital, Kansas City, Missouri.

Selection

Archived distal esophageal specimens from patients previously undergoing endoscopy by a pediatric gastroenterologist during a one year period were identified from the pathology database at Children's Mercy Hospital. No medical chart review was performed for this methodology study. Specimens with esophageal eosinophilia were reviewed. Twenty cases classified as low esophageal den-

sity (LE; 1-4 eosinophils/hpf) and twenty cases classified as high esophageal density (HE; > 20 eosinophils/hpf) were chosen. All biopsy specimens, which were previously fixed in formalin, embedded in paraffin, sliced (5- μ m sections), and stained with haematoxylin and eosin, were independently evaluated by a pediatric gastroenterologist who confirmed the number of eosinophils/hpf prior to inclusion of the specimen in this study. The entire specimen was scanned to identify the subjective area of greatest eosinophil density. Eosinophils were counted in five consecutive hpfs. Peak and mean (the average of the 5 hpfs) eosinophil counts were recorded.

Sample processing

Mast cells: The archival tissue blocks of esophageal mucosa were evaluated by immunohistochemical (IHC) staining using the labeled streptavidin-biotin (LSAB) method. The paraffin-embedded tissue specimens were de-paraffinized using a xylene-substitute followed by 100% ethanol then rehydrated in aqueous buffer. No antigen retrieval was performed on tissue for anti-tryptase staining. The primary antibody fixation occurred using mouse monoclonal anti-human mast-cell tryptase (Clone AA1) diluted to 1:1000. Tissues were incubated with anti-tryptase for 1 h at room temperature (20 °C-25 °C). After washing in aqueous buffer, a secondary antibody using biotin-anti immunoglobulin G (IgG) was incubated for 20 min at room temperature. The specimens again were washed in aqueous buffer. Specimens were incubated with streptavidin-horseradish peroxidase at room temperature for 20 min, washed in aqueous buffer, followed by the addition of diaminobenzidine (DAB) hydrogen peroxide solution for 5 min at room temperature. Specimens were washed in running tap water for 5 min. All specimens were counterstained with haematoxylin followed by bluing solution and then mounted for microscopic exam.

Major basic protein: The archival tissue blocks of esophageal mucosa were evaluated by IHC staining using the LSAB method. The paraffin-embedded tissue specimens were de-paraffinized using a xylene-substitute followed by 100% ethanol then rehydrated in aqueous buffer. Tissue was digested with 0.5% pepsin for 30 min at 37 °C for antigen retrieval. Peroxidase activity was inactivated using 3% hydrogen peroxide solution for 5 min at room temperature. To reduce background staining, normal goat serum block (5% goat serum in distilled water) and avidin/biotin blocking systems were used. The primary antibody fixation occurred using mouse monoclonal anti-human e-MBP (Clone BMK13) diluted to 1:30. Tissues were incubated with anti-MBP for 24 h at 4 °C. After washing in aqueous buffer, a secondary antibody using biotin-anti IgG was incubated for 20 min at room temperature. The specimens were washed in aqueous buffer. Specimens were incubated with streptavidin-horseradish peroxidase at room temperature for 20 min, washed in aqueous buffer, followed by the addition of DAB hydrogen peroxide solution for 5 min at room tem-

perature. Specimens were washed in running tap water for 5 min. All specimens were counterstained with haematoxylin followed by bluing solution and then mounted for microscopic exam.

Fibrosis: The archival tissue blocks of esophageal mucosa were subjected to trichrome staining using Gomori's one-step method. The paraffin-embedded tissue specimens were de-paraffinized using xylenes and then rehydrated in distilled water. Slides were pretreated with hot Bouin's solution for 1 h. After washing well in running water to remove all yellow color, slides were placed in Gill's Haematoxylin for 5 min. Slides were washed in tap water then stained with Gomori's trichrome for 10 min. They were rinsed briefly in 1% acetic acid solution then rinsed quickly in distilled water. The tissue specimens were dehydrated in 100% ethyl alcohol and then mounted for microscopic exam.

Sample evaluation

Mast cell density: Mast cell density was assessed by subjectively identifying the area of greatest involvement after scanning the entire specimen and then counting tryptase-positive cells in 5 consecutive hpfs (Figure 1A). Mast cell enumeration was performed by 2 blinded observers and by one of the observers (Observer 1) on 2 separate occasions separated by at least one week to establish inter-rater and intra-rater reliability for the method. Peak and mean mast cell densities were recorded.

Major basic protein: Specimens initially were subjectively evaluated in a blinded fashion by a pathologist and two observers to determine a stratification strategy for extracellular MBP. MBP has been previously been evaluated by semi-quantitative methods with classification into either 2 or 4 categories^[5,12]. A decision was made to employ a 4-point scale as follows: 0, none; 1, mild (< 5% involvement of MBP granules); 2, moderate (5%-25% involvement of MBP granules); and 3, severe (> 25% involvement of MBP granules) (Figure 1B-E). We elected to evaluate with 4 categories because our pre-decision specimen evaluation seemed to indicate that this was a feasible solution and because 4 categories gave us the possibility of a more robust method if reproducible. Extracellular MBP density was assessed by subjectively identifying the area of greatest involvement after scanning the entire specimen and then rating MBP density on the 4 point scale in 5 consecutive hpfs. MBP evaluation was performed by the same 2 blinded observers and by one of the observers (Observer 1) on 2 separate occasions separated by at least one week to establish inter-rater and intra-rater reliability for the method. Peak and mean densities of extracellular MBP were recorded.

Fibrosis: Subepithelial fibrosis was assessed by the trichrome stain and rated as normal (no fibrosis seen) or abnormal (increased collagen deposition) (Figure 1F). The entire specimen was scanned to determine the presence of collagen deposition. Specimens were evaluated

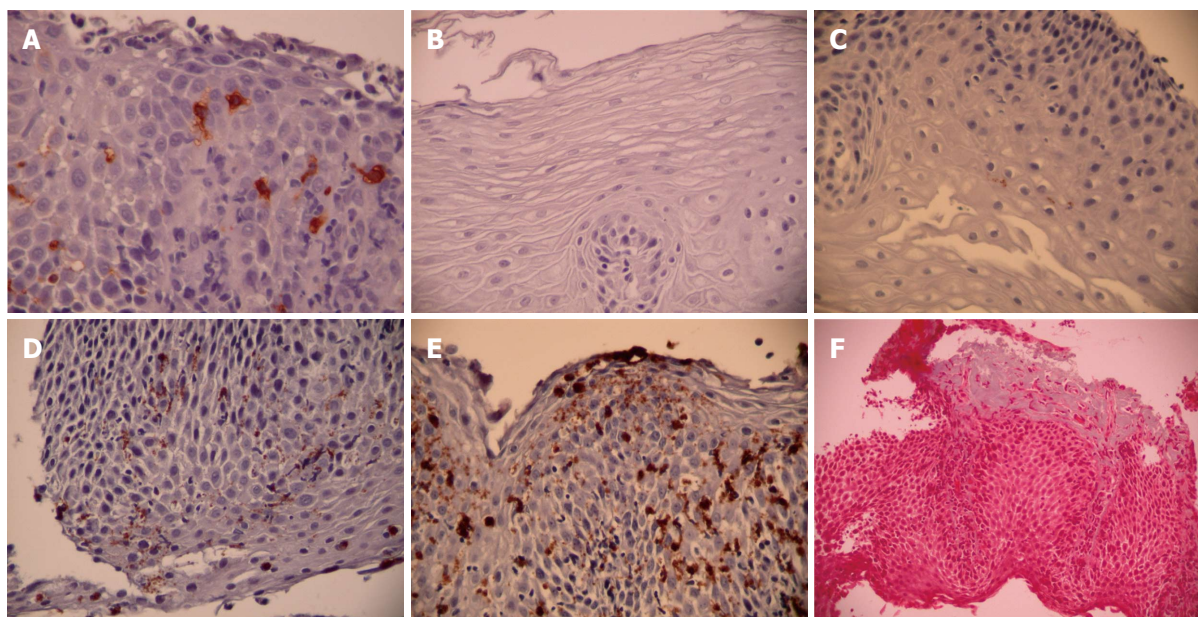


Figure 1 Sample evaluation. A: Tryptase staining for mast cells; B: Grade 0 major basic protein (MBP) involvement (none); C: Grade 1 MBP involvement (< 5%); D: Grade 2 MBP involvement (5%-25%); E: Grade 3 MBP involvement (> 25%); F: Trichrome staining for fibrosis.

by the same 2 blinded observers and by one of the observers (Observer 1) on 2 separate occasions separated by at least one week to establish inter-rater and intra-rater reliability for the method.

Statistical analysis

Measures of agreement were calculated to establish reliability between the two observers for each of the three markers. Measures of agreement also were calculated for mast cell density and extracellular MBP to establish reproducibility on the two separate evaluations by Observer 1; no reproducibility evaluation was conducted for fibrosis given the simple dichotomous nature of this variable. Pearson's correlation was employed for mast cell density and mean extracellular MBP, while Kendall's tau was used for peak extracellular MBP and kappa was used for fibrosis.

Once reproducible methods were obtained, differences in mast cell density, extracellular MBP, and presence of subepithelial fibrosis were compared between HE and LE by a combination of Student's *t* test and χ^2 . Receiver operating characteristic (ROC) curves were used to determine the sensitivity and specificity of different mast cell and extracellular MBP densities, respectively, in predicting classification group membership (*i.e.*, HE *vs* LE) based on eosinophil counts completed at the time of biopsy. Correlations between eosinophil, mast cell, and extracellular MBP densities were evaluated by Pearson's correlation. Statistical analysis was performed with SPSS version 16.0. A *P* value of 0.05 was considered significant.

RESULTS

The HE group had significantly more eosinophils/hpf (peak: 96.45 ± 45.6 ; mean: 63.07 ± 27.99) than the LE group (peak: 2.10 ± 1.07 ; mean: 0.86 ± 0.61 , $P < 0.0001$). Peak eosinophil density ranged from 39-201/hpf in the

HE patients and from 1-4/hpf in the LE group. In all cases, the original classification was confirmed and served as the gold standard for group assignment (HE *vs* LE).

Step 1: Reliability and reproducibility

Mast cell density: A strong inter-observer correlation was noted for both peak and mean mast cell counts ($r = 0.725$, $P < 0.0001$ and $r = 0.823$, $P < 0.0001$). A strong intraobserver correlation also was noted for both peak and mean mast cell counts ($r = 0.752$, $P < 0.0001$ and $r = 0.878$, $P < 0.0001$).

Major basic protein: A very strong inter-observer correlation was noted for both peak ($\tau = 0.867$, $P < 0.0001$) and mean extracellular MBP densities ($r = 0.925$, $P < 0.0001$). A very strong intra-observer correlation was noted for both peak ($\tau = 0.875$, $P < 0.0001$) and mean extracellular MBP densities ($r = 0.956$, $P < 0.0001$).

Fibrosis: Excellent inter-rater reliability was found for fibrosis ($\kappa = 0.887$).

Step 2: Biomarker comparison between HE and LE

Mast cell density: The HE group had significantly higher intraepithelial mast cell peak (29.35 ± 21.61 *vs* 12.45 ± 8.26 , $P = 0.002$) and mean (19.84 ± 15.81 *vs* 6.35 ± 4.5 , $P = 0.001$) densities than the LE group (Figure 2A). Peak mast cell density ranged from 3-89 in the HE group and from 4-32 in the LE group. Mean mast cell density ranged from 1.4-65.0 in the HE group and from 2.0-17.8 in the LE group. ROC curve analysis indicated that both mean (AUC = 0.839, $P < 0.0001$) and peak (AUC = 0.795, $P < 0.001$) mast cell density differentiate between HE and LE, but no specific cut point could be identified with adequate sensitivity and specificity. The best performer in accurately classifying HE was a cut-off 11.2 mast cells/hpf (*i.e.*, aver-

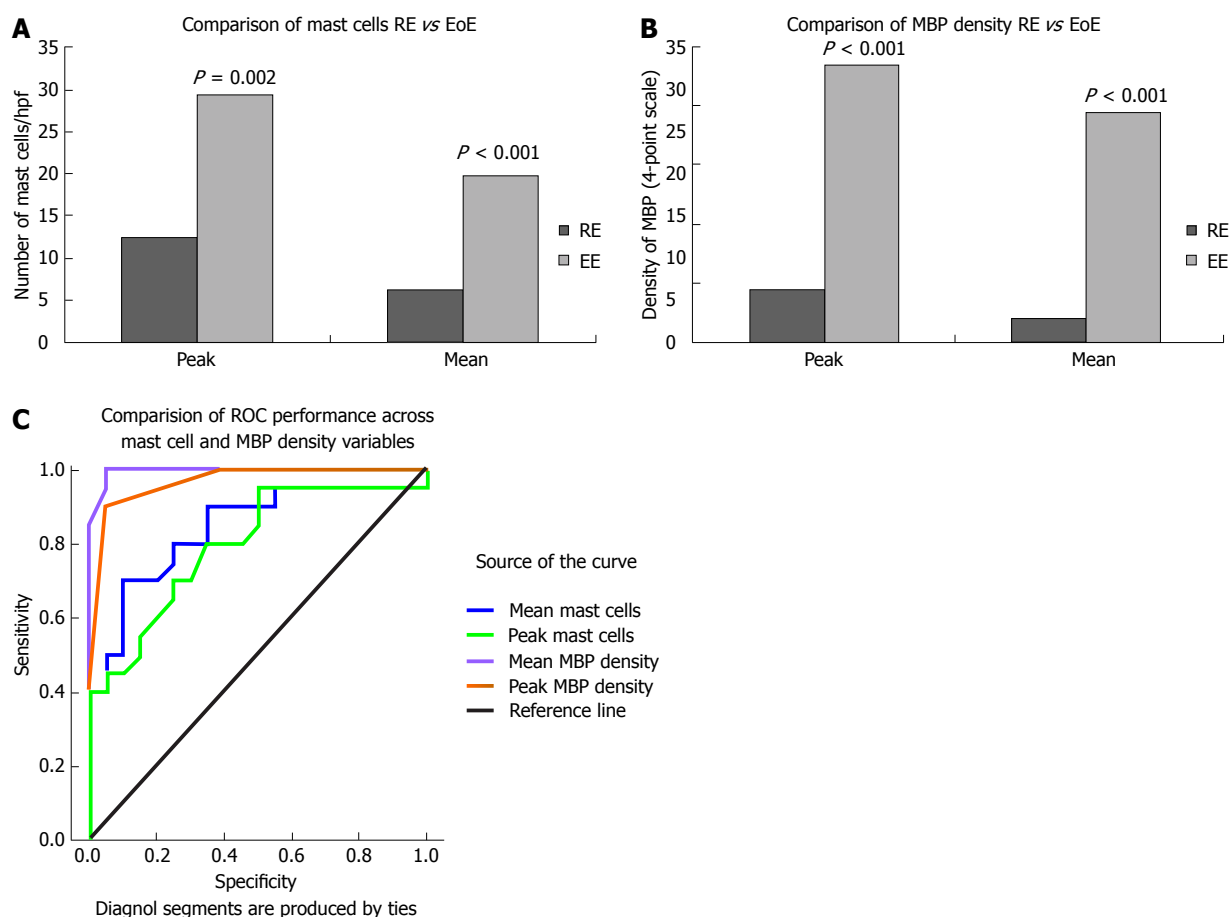


Figure 2 Biomarker comparison between high eosinophil and low esophageal density. A: Comparison of mast cells reflux esophagitis (RE) vs eosinophilic esophagitis (EoE); B: Comparison of major basic protein (MBP) density RE vs EoE; C: Comparison of receiver operating characteristic (ROC) performance across mast cell and MBP density variables.

age across 5 consecutive hpfs in the area deemed to have greatest involvement after visual scan), which detected a true positive classification for HE 70% of the time and a false positive classification for HE 10% of the time.

Major basic protein: The HE group had significantly higher peak extracellular MBP (2.35 ± 0.67 vs 0.45 ± 0.61 , $P < 0.001$) and mean extracellular MBP (1.95 ± 0.76 vs 0.20 ± 0.29 , $P < 0.0001$) densities than the LE group (Figure 2B). Ninety percent of patients with HE (18/20) had moderate to severe peak staining with extracellular MBP, whereas 95% of patients with LE (19/20) had none to mild peak staining for extracellular MBP. ROC curve analysis indicated that both mean (AUC = 0.995, $P < 0.0001$) and peak (AUC = 0.966, $P < 0.0001$) extracellular MBP density differentiate between HE and LE. The best performer in accurately classifying HE was a cut-off of 0.7 for mean extracellular MBP (*i.e.*, average across 5 consecutive hpfs in the area deemed to have greatest involvement after visual scan), which detected a true positive classification for HE 100% of the time and a false positive classification for HE only 5% of the time (Figure 2C).

Fibrosis: Fifteen specimens were excluded because of the lack of lamina propria. Seventy-three percent of patients with HE (11/15) had fibrosis, whereas only 10%

of patients with LE (1/10) had fibrosis ($P < 0.01$).

Relationships between markers in HE patients:

Mean eosinophil density was correlated with both peak ($r = 0.495$, $P = 0.03$) and mean ($r = 0.610$, $P = 0.004$) extracellular MBP density, while peak eosinophil density was correlated with mean ($r = 0.539$, $P = 0.01$), but not peak, extracellular MBP density. There was no correlation between eosinophil density and mast cell density or between mast cell density and extracellular MBP density, respectively. There was no relationship between the presence of fibrosis and densities of eosinophils, mast cells, or extracellular MBP, respectively.

DISCUSSION

When attempting to establish clinically meaningful biomarkers, the process includes several challenging steps. Establishing reproducible and valid methods for measuring or identifying the markers, demonstrating that the markers differ, noting adequate sensitivity and specificity of the markers, and demonstrating that the marker prospectively predicts treatment response, disease progression, and/or prognosis are all necessary components. The current study was undertaken to evaluate the first of these steps for three potential biomarkers supported by

previous studies as differing in patients with HE *vs* LE, namely: mast cell density, extracellular MBP density, and the presence of fibrosis. The esophageal specimens in this study were chosen at extremes (LE < 5 eosinophils/hpf and HE > 20 eosinophils/hpf) in an attempt to validate each of the biomarkers individually. A future direction would apply this validated methodology to varying degrees of eosinophilia and diagnostic dilemmas.

The methodology of immunohistochemical staining to differentiate extreme degrees of eosinophilia is valid based on our study. Applying this method to future studies with less extreme degrees of eosinophilia is warranted. If this method can accurately distinguish varying degrees of eosinophilia on initial biopsies, then follow-up biopsies which are often necessary to clarify diagnoses can be eliminated. For example, distinguishing reflux esophagitis from eosinophilic esophagitis has important implications for the patient, as the treatment and natural history vary greatly for each disorder. Currently, an initial biopsy with determination of the number of eosinophils is performed on patients with clinical symptoms of dysphagia, vomiting, acid reflux, and/or abdominal pain. If a patient is not taking proton pump inhibitor, a trial of high dose proton pump inhibitor is usually followed by a second endoscopy with biopsy to clarify or confirm the diagnosis and hence distinguish between severe RE or GERD and EoE.

Basal cell hyperplasia, papillary elongation, and eosinophilic infiltration are all non-specific findings of esophagitis, even though they tend to be more prominent in patients with EoE^[5,6,13-15]. The actual number of eosinophils per hpf is the only current histologic parameter used, in part, to establish the diagnosis of EoE. This might be simple enough for the most mild cases of RE and the most severe cases of EoE; however, clear cut-offs are not established and in the subtle, indeterminate cases, it is clearly not enough. Identifying biomarkers capable of differentiating EoE and RE has the potential to decrease the diagnostic burden of additional tests, the costs of evaluation, and sequelae associated with treatment delay in EoE.

We also sought to provide proof-of-concept for the second step by determining whether the markers differed between HE and LE. We were able to demonstrate excellent intra- and inter-observer reproducibility for the methodologies employed, with reliabilities above 0.80 for all methods except peak mast cell density counts, which fell only slightly below this threshold (0.73-0.75).

After demonstrating reproducibility, we undertook the next step of evaluating whether the particular markers could predict group assignment for HE *vs* LE. Mast cell density, density of extracellular MBP, and presence of fibrosis were all significantly greater in the HE group when compared to the LE group. These findings are consistent with previous studies evaluating histological differences between RE and EoE in both children and adults^[5,10,12,16,17]. However, no particular cut point could be identified for mast cell density which demonstrated both good sensitivity and specificity. The presence of

fibrosis appeared to be more useful in differentiating between EoE and RE. Fibrosis was present in 73% of HE patients and 10% of LE patients. However, the biggest challenge with using fibrosis as a marker is having biopsies deep enough to be evaluable. We had to exclude 38% of our specimens because we did not have adequate lamina propria. This difficulty has been previously reported^[2]. In the current study, quantifying extracellular MBP appeared to be the most promising method for differentiating HE and LE (and potentially EoE from RE), with mean MBP density, in particular, yielding excellent sensitivity and specificity in the diagnostic classification of HE.

Extracellular MBP is a marker of eosinophil activation and degranulation. Previous electron microscopic studies of esophageal eosinophils in esophagitis have demonstrated eosinophil activation indicated by inversion of core-to-matrix densities and lucency of core protein^[18]. This core lucency corresponds to the release of MBP. From a practical standpoint, the evaluation of extracellular MBP can be performed on the routine biopsies obtained during endoscopy but does require immunohistochemical staining, as MBP or extracellular granules are not sufficiently detected by routine staining^[5]. The cost and feasibility of IHC staining for extracellular MBP granules appear reasonable; however, this would need to be confirmed with future prospective studies. Although there was moderate correlation between eosinophil density and MBP density, eosinophil enumeration alone does not predict MBP density; thus, while related, these two measures appear non-redundant and may add unique information helpful in the diagnosis of EoE.

We have identified reproducible methodologies for evaluating three potential biomarkers in differentiating LE from HE. Of the three, semi-quantitative assessment of extracellular MBP appears to be the most promising, with both mean and peak values performing well in terms of sensitivity and specificity on a group of patients with HE based on ROC curve analysis. It remains to be seen whether extracellular MBP can add to diagnostic differentiation in prospective studies, particularly histologically indeterminate cases, but this is certainly an important direction for future research. This is a very important topic for future studies. It would be necessary to correlate a sensitive and specific biomarker with clinical symptoms, disease activity, pathology findings, and treatment response. This initial validation of methodology study provides evidence for future studies. Future work in this area will help establish whether extracellular MBP, or other potential biomarkers for EoE, will be able to predict responses to treatment or prognosis in a prospective fashion to reduce diagnostic burden, evaluation costs, and sequelae associated with treatment delay in EoE.

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COMMENTS

Background

Inflammation in the esophagus can cause clinical symptoms of pain in the chest or upper abdomen, difficulty swallowing, vomiting or regurgitation. The most common type of inflammation in the esophagus is due to eosinophilic inflammation. While a number of disorders can cause eosinophilic inflammation, the two most common are reflux esophagitis and eosinophilic esophagitis. These two disorders have similar clinical complaints and are challenging to differentiate. These disorders have different treatment pathways so it is important to make not only an accurate, but also a timely diagnosis. Using biomarkers to help distinguish these two disease entities will allow for more efficient and accurate diagnoses. There have not been any established or validated biomarkers for these diseases to date.

Research frontiers

Identifying biomarkers for diagnosis and management of eosinophilic disorders of the esophagus is a current hotspot for research. A biomarker that is reproducible, inexpensive, non-invasive, and corresponds to the severity of disease would be a major advantage in the treatment of children with eosinophilic disorders of the esophagus.

Innovations and breakthroughs

To date, biomarkers for eosinophilic disease of the esophagus have been described; however, not validated for their reliability or reproducibility. This study is the first step in validation of methods to assess for potential biomarkers. Being able to differentiate between low eosinophil density vs high eosinophil density will aid in timely diagnosis of disease and decrease the need for multiple biopsies on separate occasions to better characterize the disorder.

Applications

This study demonstrated that extracellular major basic protein is the best predictor of determining the degree of esophageal eosinophilia at two extremes. This was both reliable and reproducible. This provides a basis for future studies to examine varying degrees of eosinophilia using extracellular major basic protein as a biomarker for eosinophilic disease of the esophagus.

Terminology

Eosinophils: Eosinophils are white blood cells that are normally produced in the body in response to allergic or parasitic conditions; Major basic protein: Major basic protein (MBP) is a granule protein released by the activated eosinophil. MBP also triggers degranulation of mast cells which, in turn, release inflammatory mediators such as cytokines and histamine. Immunohistochemical staining: Immunohistochemical staining is a process for detecting proteins by using antibodies that bind to specific antigens.

Peer review

The present study is an interesting study for validation of methods for assessing potential biomarkers for eosinophilic disease of the esophagus. Future work should include correlation of the validated methods with symptoms, disease severity and treatment response.

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Four-year follow-up of endoscopic gastroplication for the treatment of gastroesophageal reflux disease

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Abstract

AIM: To evaluate the long-term effect of Endocinch treatment for gastroesophageal reflux disease (GERD).

METHODS: After unblinding and crossover, 50 patients (32 males, 18 females; mean age 46 years) with pH-proven chronic GERD were recruited from an initial randomized, placebo-controlled, single-center study, and included in the present prospective open-label follow-up study. Initially, three gastroplications using the Endocinch device were placed under deep sedation in a standardized manner. Optional retreatment was offered in the first year with 1 or 2 extra gastroplications. At baseline, 3 mo after (re) treatment and yearly proton pump inhibitor (PPI) use, GERD symptoms, quality of life (QoL) scores, adverse events and treatment failures (defined as: patients using > 50% of their baseline PPI dose or receiving alternative antireflux therapy) were assessed. Intention-to-treat analysis was performed.

RESULTS: Median follow-up was 48 mo [interquartile

range (IQR): 38-52]. Three patients were lost to follow-up. In 44% of patients retreatment was done after a median of 4 mo (IQR: 3-8). No serious adverse events occurred. At the end of follow-up, symptom scores and 4 out of 6 QoL subscales were improved (all $P < 0.01$ compared to baseline). However, 80% of patients required PPIs for their GERD symptoms. Ultimately, 64% of patients were classified as treatment failures. In 60% a post-procedural endoscopy was carried out, of which in 16% reflux esophagitis was diagnosed.

CONCLUSION: In the 4-year follow-up period, the subset of GERD patients that benefit from endoscopic gastroplication kept declining gradually, nearly half opted for retreatment and 80% required PPIs eventually.

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Key words: Endoscopic therapy; Endocinch; Gastroesophageal Reflux; Gastroplication; Follow-up studies

Core tip: The long-term efficacy of the first commercially available endoluminal suturing device for the treatment of gastroesophageal reflux disease (GERD), Endocinch, was evaluated. In the 4-year follow-up period, the subset of GERD patients that benefit from endoscopic gastroplication kept declining gradually. Up to 80% of patients again required acid-suppressive medication, making this endoscopic treatment procedure unsuccessful for the majority of GERD patients.

Schwartz MP, Schreinemakers JRC, Smout AJPM. Four-year follow-up of endoscopic gastroplication for the treatment of gastroesophageal reflux disease. *World J Gastrointest Pharmacol Ther* 2013; 4(4): 120-126 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v4/i4/120.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v4.i4.120>

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a common disorder with a substantial impact on quality of life and healthcare resources. Often, it is a chronic ailment that requires maintenance medical therapy. Important in the pathophysiology of GERD are a low basal tone of the lower esophageal sphincter (LES), transient LES relaxations (TLESRs), and the presence of a hiatal hernia^[1]. Laparoscopic fundoplication normalizes esophageal acid exposure by tightening the LES and repairing the hiatal hernia. Five years after surgery the overall patient satisfaction rate is still as high as 88% and only 14% requires daily antisecretory medication^[2]. Indications for laparoscopic fundoplication are medication side-effects, incomplete relief of GERD symptoms despite medical treatment, or reluctance to take lifelong maintenance medication.

In the last two decades, the drawbacks inherent to a surgical procedure led to the development of endoscopic therapies for the treatment of GERD. These endoscopic procedures aimed to include the advantages of surgery, *i.e.*, cure of the disease, while avoiding the complications of a surgical procedure. Various types of endoscopic procedures (thermal ablation, injection or implantation techniques and suturing devices) were designed and implemented. Several have already been withdrawn from the market because of safety concerns or lack of efficacy. In general, suturing devices raised highest expectations. They were designed to place stitches at or just below the esophagogastric junction, thereby creating gastroplications that tightened the LES. The Endocinch device is one of the first generation devices that is still available for commercial use.

Only a few studies have published long-term results (18-41 mo)^[3-6]. In 2007 we completed a 3-mo, randomized, sham-controlled trial with a total follow-up duration of one year^[7]. During short to medium-term follow-up, symptoms improved moderately, with no significant effects on acid exposure compared to sham. Like others, we had concerns about the durability of the sutures^[8]. In a recent systematic review of randomized controlled trials and comparative studies on endoscopic treatments for GERD it was concluded that there is still not sufficient evidence to determine the long-term efficacy and safety of Endocinch^[9]. The present study followed up on the 3-mo sham-controlled trial and aimed to prospectively evaluate its long-term efficacy and safety.

MATERIALS AND METHODS

We conducted a prospective follow-up study until December 2008, which included all patients that were treated with Endocinch in the initial single-center, randomized trial that started in August 2003.

Approval for these studies was granted by the medical ethics committee of the University Medical Center Utrecht, the Netherlands. It was obtained prior to the start of the original study and once more during the

long-term follow-up period.

Study design

The single-center, double-blind, randomized, placebo-controlled trial included 60 patients that were allocated to 3 groups (Figure 1). Each group contained 20 patients. After 3 mo, the remaining untreated patients in the sham and observation groups were offered Endocinch treatment. 30 patients that agreed to cross over were successfully treated with Endocinch. Within the first year, patients that were classified as failures (defined as: unsatisfactory symptom response and/or < 50% reduction of their baseline dose of antisecretory medication) were offered retreatment. A total of 50 patients were included in the present follow-up study.

Endpoints were prospectively defined and included: GERD symptoms (heartburn and regurgitation), use of antisecretory medications, quality of life, adverse events, retreatment with Endocinch, and other reflux treatments. Patients were assessed on a yearly basis and these data were compared with pretreatment (baseline) and 3 mo post-treatment data.

The study objective was to establish whether the short-term effects would last. The hypotheses were that in the long-term: (1) symptoms; and (2) use of acid-suppressive medication would no longer be reduced compared with baseline values.

Patients

All randomized patients met the following inclusion criteria: persistent heartburn and/or regurgitation, and at least partial response to anti-secretory drugs and dependence on them for at least 1 year, unwillingness to take drugs lifelong, and esophageal pH results compatible with GERD diagnosis (> 5% of the time a pH < 4 or a 95% symptom association probability). Exclusion criteria were: < 18 years of age, severe esophageal motility disorder on manometry, hiatus hernia > 3 cm in length, history of thoracic or gastric surgery, reflux esophagitis grade C or D (LA classification), Barrett's epithelium, severe comorbidity (cardiopulmonary disease, portal hypertension, collagen diseases, morbid obesity and coagulation disorders), use of anticoagulant or immunosuppressive drugs, or a history of alcohol or drug misuse.

Before enrollment in the 3-mo trial each patient's eligibility was assessed. Patients underwent an upper endoscopy. The required daily dose of acid-suppressive medication to achieve optimal symptom control was recorded during a 1-mo run-in period. Subsequently, an esophageal manometry and ambulatory 24-h pH monitoring were performed after discontinuation of medication for 1 whole week (the results were published in a previous article)^[7]. Prior to randomization, written informed consent was obtained from all patients.

Baseline and follow-up assessment

The pre-treatment (baseline) questionnaire was completed at the end of the run-in period of the initial trial

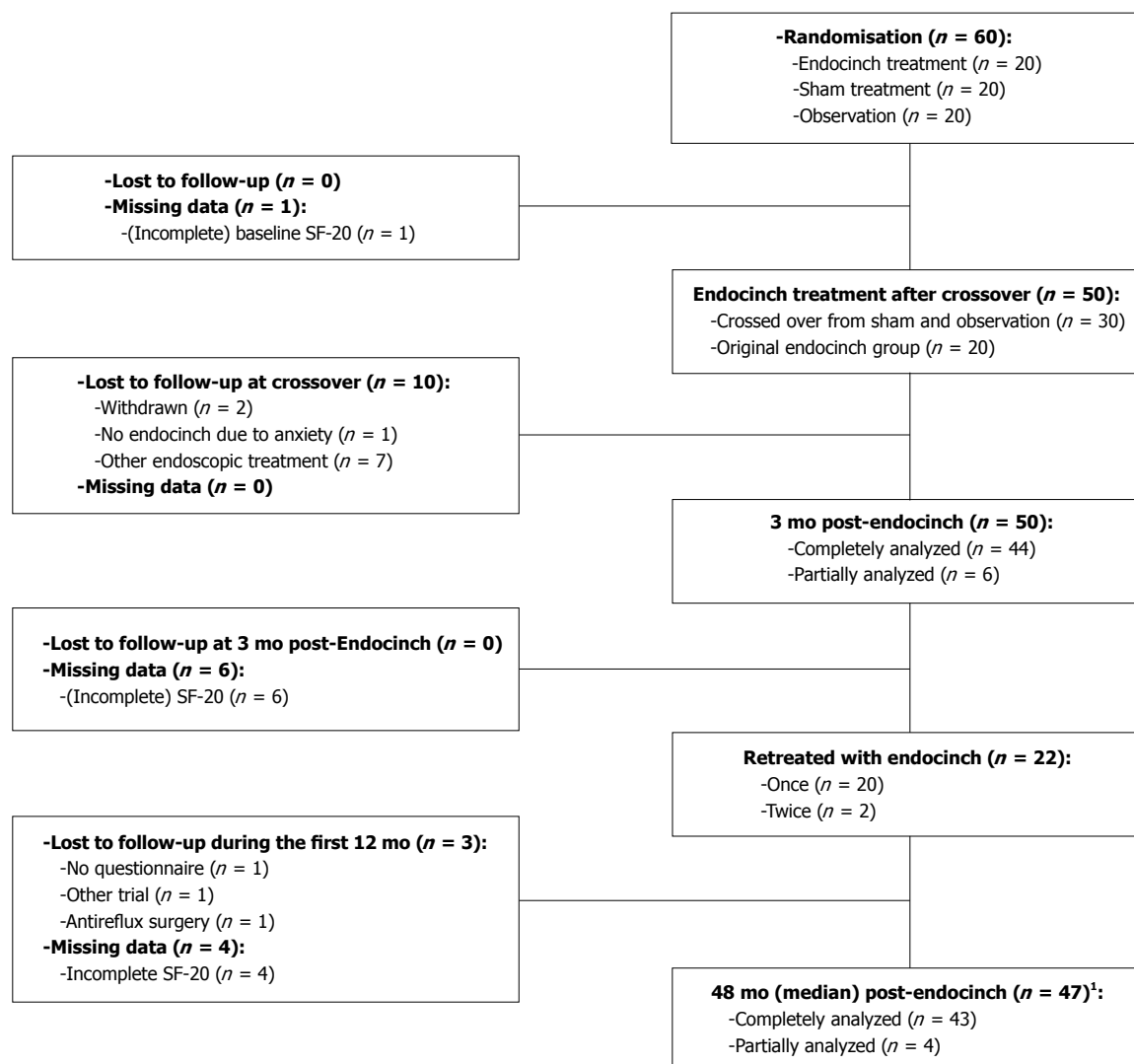


Figure 1 Flow diagram of follow-up. ¹Between 12 and 48 mo, 9 patients had antireflux treatment: 8 patients underwent antireflux surgery and 1 an alternative endoscopic treatment.

while patients were still off acid-suppressive medication. At 3 mo after randomization a second questionnaire was filled out (off medication). Esophageal manometry and ambulatory 24-h pH monitoring were repeated in the active and sham groups. Patients that were retreated underwent the same follow-up procedure. Additional reassessment took place at 6 and 12 mo. From 12 mo on, patients were reassessed with a yearly questionnaire. In accordance with intention to treat analysis, questionnaires from treatment failures were included.

Treatment procedure

Endoscopic suturing was carried out with the Endocinch suturing device (BARD Endoscopic Technologies, CR Bard, Billerica, MA, United States). The initial treatment aimed to create three gastroplications. During the first year of follow-up, patients with failure of therapy were offered retreatment consisting of one or two extra plications. All procedures were performed by the same endoscopist (MPS). The procedure was carried out using two video gastroscopes (type GIF-160, Olympus

Nederland BV, Zoeterwoude, The Netherlands) and the endoscopic suturing device. After endoscopic placement of an esophageal overtube, two stitches were placed adjacent to one other, using the same thread. The first gastroplication was positioned about 1.5-2.0 cm below the squamocolumnar line along the lesser curvature. The second endoscope was used to create a plication by tightly pulling the sutures together and placing a suture anchor. A second plication was placed 1 cm above the first, and a third plication was placed at the level of the second along the greater curvature. All procedures were carried out under deep sedation with a combination of midazolam and pethidin administered intravenously. Oxygen saturation was monitored during the procedure. Afterwards, patients were observed for a period of 4 h during which blood pressure and heart rate were measured hourly.

Questionnaire

GERD symptoms were scored utilizing a 6-point numeric scale measuring heartburn and regurgitation frequency

(< 1 per month, once monthly, once weekly, once daily, 25 times per day, > 5 times daily) and a 4-point numeric scale measuring severity (not, mildly, moderately and very severe)^[10]. The heartburn and regurgitation symptom scores were calculated by multiplying the frequency with the severity score. Quality of life assessments were done using the 20-item Short-Form Health Survey (SF-20). The SF-20 consists of 20 items that assess the following 6 aspects: limitations in physical, role and social activities due to health problems, perception of mental and general health, and bodily pain. Each individual scale was transformed to a 100-point scale, 0 being the worst and 100 the best score, except in bodily pain perception in which a higher score stands for experiencing more pain.

Statistical analysis

The mean SD is provided when data are normally distributed, the median [interquartile range (IQR)] when the distribution is not normal.

The Wilcoxon signed-ranks test (nonparametric two-related samples) was used to test whether post-treatment values differed from baseline values. Whether two variables were related to each other was explored with logistic regression analysis. Differences and relations were considered significant if $P < 0.05$.

RESULTS

Follow-up

Baseline characteristics are provided in Table 1. Follow-up and missing data are presented in Figure 1. The median duration of follow-up was 48 mo (IQR: 38-52). Three patients were lost to follow-up in the first 12 mo. Between 12 and 48 mo nine patients reached the following predefined endpoints: 8 patients underwent antireflux surgery and one patient received an alternative endoscopic treatment. Four SF-20 questionnaires were either incomplete or missing. Therefore, 43 patients were analyzed completely, and 4 partially. Twenty-two (44%) patients were retreated with a mean of 1.4 plications, after a median period of 4 mo (IQR: 3-8). The median follow-up period after retreatment was 33 mo (IQR: 15-48). Twenty-eight patients (60%), all with persistent complaints, underwent a postprocedure upper gastrointestinal endoscopy. A mean of 0.73 stitches per patient were judged as functional. In twelve patients (24%) erosive reflux esophagitis was diagnosed (grade A, $n = 7$; grade B, $n = 4$; grade C, $n = 1$).

Use of acid-suppressive medication and alternative treatment

Compared with baseline, acid-suppressive medication use decreased significantly. At 3 and 48 mo the median doses were 31% ($P < 0.001$) and 97% ($P < 0.001$) of the baseline dose (Figure 2). Twenty percent were completely off acid-suppressive medication at the end of follow-up. At the end of follow-up, 18 (36%) of the 47 remaining patients used 50% or less of their baseline dose and had

Table 1 Baseline patient characteristics¹ n (%)

Characteristic	Study group ($n = 50$)
Age (yr)	46 (11)
Male sex	64
Body mass index (kg/m ²)	27 (4)
GERD symptom score ²	
Heartburn	18 (15-20)
Regurgitation	15 (12-18)
SF-20 score ³	
Physical function	50 (17-75)
Role function	100 (0-100)
Social function	80 (60-100)
Mental health	76 (60-88)
General health	40 (18-70)
Bodily pain perception	75 (50-75)
PPI dose (mg) ⁴	40 (38-54)
Time pH < 4, %	8.4 (6.1-12.7)
LES pressure (kPa)	0.9 (0.3-1.5)
Hiatal hernia length (cm)	1 (1-2)
Esophagitis grade A/B with or without Barrett's metaplasia, n (%)	38

¹Data are presented for all patients included in the analyses, values enclosed in parentheses are means (SD) or medians (IQR); ²Frequency multiplied with severity; Scores range from 0 to 24 and were scored while off antisecretory drugs; ³Short-Form General Health Survey scores range from 0 to 100, with higher scores indicating better function, except for the bodily pain perception score; ⁴Proton pump inhibitor dose per day. GERD: Gastroesophageal reflux disease; PPI: Proton pump inhibitor; LES: Lower esophageal sphincter.

not received another treatment (Figure 3). As such, 64% were classified as treatment failures. If retreated patients were also considered to be treatment failures, irrespective of the effect of retreatment, the number of failures amounted to 36 patients (72%).

Symptoms

Compared with baseline, heartburn and regurgitation symptom scores were significantly decreased both at 3 mo and at the end of follow-up (Table 2). At 3 mo, heartburn and regurgitation scores decreased with 41% and 37%, respectively. At 48 mo, the heartburn score was reduced by 32% ($P < 0.001$) and the regurgitation score by 34% ($P < 0.001$).

Quality of life

At 3 mo, the SF-20 quality of life scores significantly improved in 5 of 6 subscales ($P < 0.026$, Table 2). Only the mental health subscale score had not changed significantly. At the end of follow-up, the same subscales remained significantly improved compared with baseline with the exception of role function.

At the end of follow-up, 17% of patients indicated that their GERD had completely been cured, 30% indicated that it had improved, 46% that it was unchanged and 7% that it had worsened.

Adverse events

One patient had a major hemorrhage immediately after the procedure and was hospitalized. After receiving endoscopic injection therapy to stop the bleeding and a

Table 2 Outcome at 3 mo post-treatment and at the end of follow-up (median period of 48 mo) *n* (%)

Variable	Baseline	3 mo		End of follow-up		P values ¹	
		Absolute values	% ²	Absolute values	% ²	3 mo	End
GERD symptom scores							
Heartburn score ³	16.4 (5.8)	9.7 (7.6)	-41	11.2 (8.5)	-32	< 0.001	< 0.001
Heartburn frequency	5.0 (1.3)	3.2 (2.1)	-35	3.4 (2.2)	-31	< 0.001	< 0.001
Heartburn severity	3.1 (0.9)	2.3 (1.3)	-26	2.5 (1.4)	-20	< 0.001	< 0.003
Regurgitation score ³	15.6 (4.8)	9.9 (7.8)	-37	10.2 (7.3)	-34	< 0.001	< 0.001
Regurgitation frequency	5.0 (1.1)	3.3 (2.2)	-33	3.4 (2.0)	-32	< 0.001	< 0.001
Regurgitation severity	3.1 (0.8)	2.2 (1.4)	-28	2.5 (1.2)	-20	< 0.001	< 0.002
SF-20 scores ⁴							
Physical health	46.3 (34)	64.0 (35)	38	60.1 (35)	30	< 0.009	< 0.02
Role function	59.2 (48)	76.7 (39)	31	73.9 (42)	25	< 0.023	< 0.09
Social function	71.4 (32)	81.3 (29)	14	83.2 (28)	16	< 0.026	< 0.006
Mental health	74.0 (18)	73.2 (17)	-1	76.1 (15)	3	NS	NS
General health	43.2 (27)	56.2 (28)	30	62.6 (28)	45	< 0.001	< 0.001
Bodily pain perception	67.4 (26)	48.9 (35)	-27	44.8 (34)	-34	< 0.003	< 0.001

¹Baseline data compared with 3 mo and end of follow-up data (median of 48 mo); ²Change in percentage compared with baseline, to calculate the change, the absolute values rounded to 2 decimal places instead of 1 were used; ³Frequency multiplied with severity. Scores range from 0 to 24; ⁴Short-Form General Health Survey scores range from 0 to 100, with higher scores indicating better function, except for the bodily pain perception score. GERD: Gastroesophageal reflux disease; NS: Not significant.

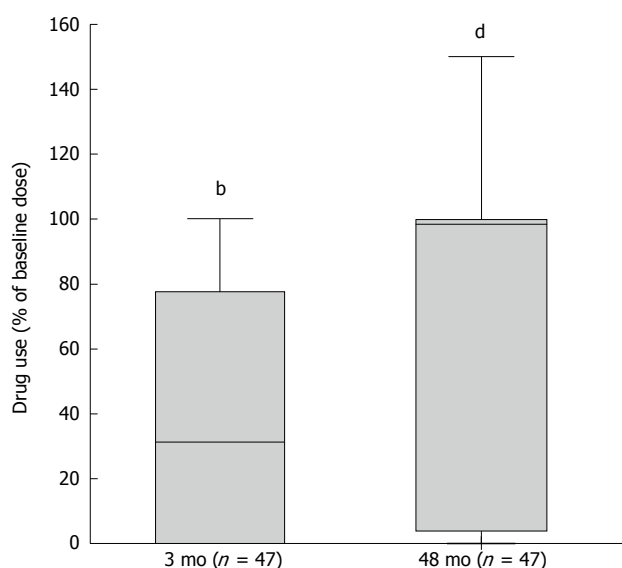


Figure 2 Medication use at 3 mo after endocinch and at the end of follow-up. Presented as percentage of the baseline dose. Interquartile range (IQR), range and median are shown; Median values were 31% and 97% for 3 mo and end of follow-up, respectively (^b*P* < 0.001, ^d*P* < 0.001, both compared with baseline).

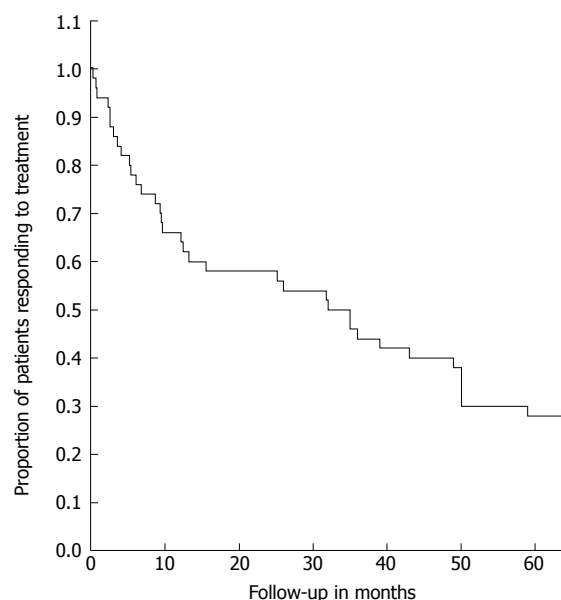


Figure 3 Kaplan-Meier survival curves. Analysis of proportion of patients responding to Endocinch during follow-up; All treatment failures (i.e., > 50% of baseline acid-suppressive dose or other antireflux treatment) were included.

blood transfusion, the patient made a speedy recovery. No other adverse events occurred either during treatments or follow-up.

DISCUSSION

Endoscopic treatment of GERD using Endocinch may improve GERD symptoms, decrease medication use and increase quality of life. However, after 4 years the treatment effect persists in less than half of treated patients. During the first follow-up year, the success rate progressively declined. This decline continued during the subsequent three years and it is likely that it will continue to do so. The percentage of treatment failures

rose to 64%. At the end of follow-up, 47% of patients indicated that GERD was still improved compared to baseline and the GERD symptom scores and quality of life scores remained significantly improved. Yet, 80% required PPIs.

The limited number of adverse events in this follow-up study demonstrated that the procedure is safe in the long-term.

One study reported that treatment failure occurred in 80% of the patients during the 18-mo follow-up study (*n* = 70)^[3]. Treatment failures were defined as in our study (unimproved heartburn symptoms or PPI dose exceeding 50% of the baseline dose), but patients were not re-treated. Only 6% of patients succeeded to stay off PPIs

after two years. The rate of 80% is comparable to the 72% found in the present study, when all retreated patients were also counted as treatment failures. However, in our study follow-up duration was much longer (48 *vs* 18 mo). Two other, multicenter studies from the US ($n = 85$) and Japan ($n = 48$) showed slightly better results with rates of 40% and 30%, respectively, of patients staying off PPI after two years^[4,5]. These results indicate that 6% is relatively low and that our 20% accurately reflects the percentage of patients that remain off medication in the long-term (≥ 4 years).

The loss of functional plications, due to too superficial (*i.e.*, not transmural) suturing, is generally accepted as the cause of loss of treatment effect^[11,12]. In the present study no systematic endoscopic evaluation of the functionality of sutures was performed, because only treatment failures underwent a follow-up endoscopy. Hence, the actual proportion of patients with functional sutures is unknown. Among treatment failures a mean of only 0.73 sutures were still considered to be functional. Increasing the initial number of plications will probably only temporarily prolong the treatment effect, comparable to performing a second procedure, as was shown by the present study and others^[6]. Also measurements to improve durability, such as cauterization of the mucosa of a gastroplication, as evaluated in a 2-year follow-up study ($n = 18$), will not have a beneficial long-term effect^[13]. Only modifications of the suturing technique can improve the depth of stitches, but up until now no improvements of the device have been made.

One can question whether the observed effect of treatment after 4 years is really the result of diminished esophageal acid exposure. After all, pH measurements were not repeated. Our initial study already showed that the improvement of esophageal acid exposure was not significantly greater than after sham treatment^[7]. This is consistent with the results in other studies that found no significant differences or only marginal differences between baseline and post-procedure pH-values^[3,6,14,15]. On the other hand, in our initial study a subgroup analysis (responders *vs* non-responders) made likely that the observed treatment effect was largely due to the reduction of esophageal acid exposure. We therefore believe that, although no repeated pH-metry was done, the effect after four years can also be attributed to esophageal acid reduction. It is highly unlikely that a placebo effect would still persist.

In summary, this is the longest prospective follow-up study after treatment with the endocinch procedure. It shows that GERD symptoms, medication use and quality of life improve in a subset of patients that gradually becomes smaller during follow-up. Endocinch can be carried out safely in an outpatient setting under conscious sedation. However, 44% had to undergo retreatment and eventually 80% needed PPIs again. In conclusion, in the long-term this procedure is not beneficial for the majority of GERD patients.

COMMENTS

Background

Gastroesophageal reflux disease (GERD) is a common, often chronic ailment with an important impact on quality of life and healthcare resources. Many patients require daily acid-suppressive medication or ultimately antireflux surgery. In recent years, several less invasive, endoscopic procedures have been developed. Endocinch is one of the first generation antireflux suturing devices, creating a barrier at or just below the gastro-esophageal junction against reflux.

Research frontiers

Many studies, including a sham-controlled trial of our group, have demonstrated the short-term safety and efficacy of the Endocinch procedure on symptoms, but often failed to show significant improvements in esophageal acid exposure. Several follow-up studies have raised questions about medium-term efficacy. Prospective data exceeding two years of follow-up are scarce.

Innovations and breakthroughs

Loss of treatment effect could be due to loss of functional sutures, as was suggested by some researchers. Retreatment has been shown to (at least temporarily) improve results. In the present study, retreatment was also offered in case of early treatment failure and prospective follow-up duration was extended up to a median of four years.

Applications

The results of this study suggest that the endocinch procedure improves GERD symptoms, quality of life and reduces the use of acid-suppressive medication in only a subset of patients. A subset that gradually becomes smaller during four years of follow-up. Percent of 44 patients had to undergo retreatment and eventually 80% required acid-suppressive medications again. The study can conclude that in the long-term this procedure is not beneficial for the majority of GERD patients.

Terminology

Gastroesophageal reflux disease is a chronic disease in which the lower esophageal sphincter (LES) allows gastric acids to reflux into the esophagus leading to symptoms as heartburn and acid indigestion. Endocinch is a commercially developed endoscopic device designed to place stitches at or just below the esophagogastric junction, creating so-called 'gastroplications' that tighten the LES.

Peer review

This is a well-designed prospective follow-up study in which the authors evaluated the long-term effect of endoluminal gastroplication with the Endocinch system for GERD. New important data have been provided that demonstrate that this procedure is not beneficial in the long-term for the majority of GERD patients.

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