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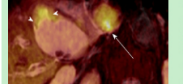
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World J Gastrointest Pharmacol Ther 2014 February 6; 5(1): 1-49





Contents

Quarterly Volume 5 Number 1 February 6, 2014

REVIEW

- 1 Clinical update for the diagnosis and treatment of *Clostridium difficile* infection
Oldfield IV EC, Oldfield III EC, Johnson DA
- 27 Role of chemoprophylaxis with either NSAIDs or statins in patients with Barrett's esophagus
Tsibouris P, Vlachou E, Isaacs PET

MINIREVIEWS

- 40 Aspirin, cyclooxygenase inhibition and colorectal cancer
Sostres C, Gargallo CJ, Lanas A

Contents

World Journal of Gastrointestinal Pharmacology and Therapeutics

Volume 5 Number 1 February 6, 2014

APPENDIX

I-V

Instructions to authors

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Clinical update for the diagnosis and treatment of *Clostridium difficile* infection

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Abstract

Clostridium difficile infection (CDI) presents a rapidly evolving challenge in the battle against hospital-acquired infections. Recent advances in CDI diagnosis and management include rapid changes in diagnostic approach with the introduction of newer tests, such as detection of glutamate dehydrogenase in stool and polymerase chain reaction to detect the gene for toxin production, which will soon revolutionize the diagnostic approach to CDI. New medications and multiple medical society guidelines have introduced changing concepts in the definitions of severity of CDI and the choice of therapeutic agents, while rapid expansion of data on the efficacy of fecal microbiota transplantation heralds a revolutionary change in the management of patients suffering multiple relapses of CDI. Through a comprehensive review of current medical literature, this article aims to offer an intensive review of the current state of CDI diagnosis, discuss the strengths and limitations of available laboratory tests, compare both current and future treatments options and offer recom-

mendations for best practice strategies.

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Key words: *Clostridium difficile*; Antibiotic-associated diarrhea; Fidaxomicin; Rifaximin; Fecal transplantation; Probiotics

Core tip: This paper seeks to explore the treatment and diagnosis of *Clostridium difficile* infection (CDI) through an extensive literature review of available laboratory techniques and new treatment options. For diagnosis, this includes the glutamate dehydrogenase of stool and polymerase chain reaction for gene toxin. For treatment this includes guidelines based on severity, newer antibiotics for the treatment of CDI, fecal microbiota transplantation, and several new experimental treatment options. Finally, this manuscript offers suggested clinical guidelines for how to optimizing diagnosis and treatment of CDI.

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INTRODUCTION

Clostridium difficile infection (CDI) continues to be a significant and increasing problem. By far, CDI remains, the most common cause of hospital acquired diarrhea with the number of hospitalized patients with any CDI discharge diagnosis doubling from 139000 in 2000 to 336600 in 2009 at a cost of \$1 billion annually^[1]. In fact, recently CDI has surpassed methicillin-resistance *Staphylococcus aureus* (MRSA) as the most common hospital-onset, healthcare facility-associated infection^[2]. Despite

significantly trailing MRSA in nosocomial deaths^[3], the CDI death rate has dramatically increased from 3000 per year in 1999-2000 to 14000 per year in 2006-2007^[4].

One of the most important developments has been the emergence of a new epidemic strain, which is resistant to quinolone antibiotics, such as ciprofloxacin. The first noted in 2001, the epidemic strain produces 16-fold more toxin A and 23-fold more toxin B than other *C. difficile* strains^[5]. In addition, the organism produces more spores which results in contamination of the environment and the potential for further spread. The epidemic strain has been associated with an increased incidence of complicated cases and mortality compared to other strains^[6]. Confusingly, the epidemic strain is referred to as 027 by polymerase chain reaction (PCR)-ribotyping, B1 by restriction endonuclease analysis (REA), Type 1 by pulse field gel electrophoresis (PFGE) and toxin type III by restriction fragment length polymorphism PCR. For continuity throughout the article, the epidemic strain will be identified only as B1. The B1 epidemic strain has now spread widely throughout the United States, however, very few clinicians are aware of its presence in their hospital because culture and identification of *C. difficile* strains is rarely, if ever performed.

In this update, we will review recent advances in the diagnosis of CDI, with a focus on laboratory methods, and also new advances in the treatment of CDI and relapses, including the rapidly expanding area of fecal transplants.

DIAGNOSIS OF CDI

Risk factors for CDI

The first issue in a patient with suspected CDI is to determine if there are associated risk factors. Antibiotic use increases the risk of CDI by 8-10-fold during and for one month after administration and 3-fold for the next 2 mo^[7]. Numerous studies have looked at risk factors for CDI with a consistent implication of ampicillin (or amoxicillin), clindamycin and cephalosporins (in particular the third generation cephalosporins (TGC), such as cefotaxime, ceftriaxone and ceftazidime). For the TGCs, an almost perfect correlation has been noted between increasing use and rising incidence of CDI^[8] and conversely a decrease in CDI with decreased use of TGCs^[9]. Increasingly, quinolones have been shown to be a significant risk factor for CDI, especially with the epidemic B1 strain^[6]. The use of multiple antibiotics and > 10 d of antibiotics have also been associated with increased risk (suggesting that therapeutic use of antibiotics poses a greater use than prophylactic use)^[10,11]. Antibiotics which have been less commonly associated with CDI include aminoglycosides, macrolides, sulfonamides and tetracyclines. Although the correlation with CDI is highest with certain antibiotics, all antibiotics, even vancomycin and metronidazole on rare occasion, have been reported to cause CDI. However, exposure to antibiotics is not necessary for acquisition of CDI. In one study, 24% of patients with CDI had no antibiotic exposure and 9%

Table 1 Characteristics of tests for *Clostridium difficile* infections

	Sensitivity	Detects toxin in stool	Time to test completion	Cost	Availability	Stand alone test
EIA Toxin A/B	++	Yes	h	+	++++	Yes
GDH ¹	++++	No ⁵	h	+	++++	No
PCR ²	+++	No ⁶	h	++++	+++	Yes
TC ³	+++++	No ⁶	d	+++	+ ⁷	Yes
CCCNA ⁴	+++	Yes	d	+++	+ ⁷	Yes

¹Glutamate dehydrogenase; ²Polymerase chain reaction; ³Toxigenic culture; ⁴Cell culture cytotoxicity neutralization assay; ⁵Detects presence of *Clostridium difficile* (*C. difficile* I) only, but not toxin producing capability, requires confirmatory testing; ⁶Detects toxin producing *C. difficile*, but not toxin in stool, false (+) in asymptomatic carriers; ⁷Only available in research laboratory. EIA: Enzyme immunoassay; GDH: Glutamate dehydrogenase; PCR: Polymerase chain reaction; TC: Toxigenic culture; CCCNA: Cell culture cytotoxicity neutralization assay.

had received 3 d or less^[12]. Of the patients without any antibiotic exposure, however, 75% were either hospitalized or had close contact with a person with diarrheal illness.

Antineoplastic agents have also been associated with CDI, including doxorubicin, cisplatin, cyclophosphamide, fluorouracil and chlorambucil^[13], with methotrexate most commonly implicated. The proposed mechanism behind the pathogenesis of chemotherapy related CDI is two-fold. First, the antineoplastic agents have been shown to alter the gut microflora in a manner similar to antibiotics, acting as the primary predisposing factor for developing CDI^[14]. The second, these agents are capable of inducing mitotic arrest in intestinal epithelial cells, subsequently causing necrosis and desquamation of the mucosal membrane^[15].

Immunocompromised patients may represent a special subset of CDI for which the incidence and treatment may be more challenging to approach, in particular those with solid organ transplantation. The incidence of CDI in transplant patients has been estimated at 3%-7% for liver recipients, 3.5%-16% for kidney recipients, 1.5%-7.8% in pancreas-kidney recipients, 9% in intestinal recipients, 15% in heart recipients, and 7%-31% in lung recipients^[16]. Further fulminant colitis is noted to occur in up to 8% of immunocompromised patients and 13% of solid organ transplant recipients with the highest incidence within the first 3 mo^[17,18]. The treatment of CDI in immunosuppressed patients should follow the same guidelines based on disease severity as those outlined in this paper. One important caveat to consider is the potential for drug interactions with metronidazole, in particular the potential for alteration in levels of tacrolimus^[19] (Table 1).

Increasing age has been a consistently noted risk factor, with a > 10-fold increased risk for those 60-90 years old^[11,20]. In fact, 90% of all deaths are in persons 65 and older^[11]. Other associated risk factors have included enemas, stool softeners and gastrointestinal stimulants^[21], and also enteral feedings (especially postpyloric), which have been associated with an 11-fold increased risk of CDI^[22].

Although rates of non-CDI diarrhea with enteral feedings have been reported in up to 60% of patients^[23], the increased risk of CDI would suggest that CDI is a significant problem for enterally fed patients. The significant risk related to postpyloric tube feeding may be related to the fact that gastric acidity has been shown to eliminate 99% of vegetative *C. difficile* cells^[24]. Rates have also been noticed to be increased after gastrointestinal operations up to 25-fold compared to controls, probably related to impaired motility, nasogastric tubes and preoperative antibiotics^[11].

Recently, several studies have found a higher risk of *C. difficile* infection in proton pump inhibitor (PPI) users. In theory, PPIs may increase the risk of *C. difficile* infection by increasing the ability of the spore to convert to the vegetative form and to survive in the lumen of the gastrointestinal tract. Several meta-analysis have found a significant relationship between PPI use and CDI with odds ratios ranging from 1.69 (95%CI: 1.395-1.974)^[25] to 2.05 (95%CI: 1.47-2.85)^[26]. Despite these results, the most recent studies offer conflicting viewpoints as to the association between PPI use and increased risk of *C. difficile* infection. These studies showed that while univariate analysis may show a statistically significant relationship between PPI use and CDI, multivariable analysis reveals no significant relationship^[27-29]. Further, the most recent review on detection, prevention and treatment of *C. difficile* does not include restriction or avoidance of PPIs in the recommendations for prevention of *C. difficile* infection^[30], nor is this recommended by multi society clinical practice guidelines^[31].

Although CDI is commonly felt to be a hospital-acquired infection, with up to 87% of infections nosocomially acquired, a significant number of cases are community acquired^[10]. In a prospective study of diarrheal pathogens, 20% of infections were community acquired. For an additional 15% of patients, CDI was acquired in the hospital, but diarrhea began after discharge at home for a total of 43% of cases with onset of symptoms at home^[16]. As many as 25% of all cases of CDI develop in nursing home patients^[1]. Suspicion should always be high for CDI whenever there is diarrhea in a resident of a long term care facility where there is a concentration of elderly, high use of antibiotics, CDI infection in other residents, or frequent exposure to hospitals. This increased risk is bidirectional: 20% of CDI with onset in the hospital are in residents of a nursing home and 67% of CDI in nursing home residents occurs in patients recently discharged from an acute care hospital^[1].

Even among asymptomatic patients many of these risk factors appear to be the same. During a 2-mo period, researchers at a tertiary care hospital in Minnesota performed PCR for toxigenic *C. difficile* on all consenting asymptomatic patients, who had greater than a 24 h stay without any known or suspected CDI, diarrhea or colitis. Of the 320 stool samples collected, 31 samples (9.7%) were positive for toxigenic *C. difficile*^[29]. Multivariate analysis revealed three main risk factors for *C. difficile* colonization: recent hospitalization within 3 mo (OR = 2.45,

95%CI: 1.02-5.84), chronic dialysis (OR = 8.12, 95%CI: 1.80-36.65), and corticosteroid use (OR = 3.09, 95%CI: 1.24-7.73).

Clinical presentation of CDI

There is a broad range of clinical manifestations from asymptomatic carriage (20% of culture positive patients) to colitis with or without pseudomembranes to fulminating colitis and toxic megacolon. In one series, a two-year institutional study of CDI revealed that “acute abdomen” was the presenting feature in 5% of patients with CDI, with 2 of 5 having no diarrhea prior to emergency laparotomy^[32]. This acute abdomen presentation without diarrhea may be particularly confusing in the postoperative patient. Onset is usually 5-10 d after antibiotic use, but ranges from 1 d up to 10 d after antibiotics are stopped. Frankly bloody diarrhea is uncommon (5%-10%)^[33]. In fact, only 26% have occult blood^[10]. Fever is noted in 30%-50%, usually low grade, not to exceed 102°F^[28].

Leukocytosis, hypoalbuminemia and elevation of baseline serum creatinine are highly suggestive of CDI. Elevated white blood cell (WBC) count is common (50%-60%), as well as increased band forms (47%) and may be marked elevated^[34]. Wanahita found a mean WBC of 15800/mm³ with 26% of patients having a WBC > 20000/mm³ and 6% > 30000/mm³. In fact, for all patients without a hematologic malignancy who had a WBC > 30000/mm³, 25% were found to have CDI^[34]. The elevation of WBC may even precede the onset of diarrhea or abdominal discomfort^[35] and may be responsible for up to 58% of cases of unexplained leukocytosis in hospitalized patients^[36]. In a series of patient with leukocytosis who were *C. difficile* toxin negative, empiric treatment for CDI led to resolution of leukocytosis^[36]. CDI results in a protein losing enteropathy with resultant hypoalbuminemia^[37]. Serum albumin of < 2.5 or a fall in albumin of > 1.1 have been associated with a poor prognosis^[38]. Bartlett has noted that hypoalbuminemia in persons with antibiotic associated diarrhea may be a clinical clue suggesting CDI^[37,39]. Fecal leukocytes have been found in 28%-40% of cases^[40]. Detection of fecal lactoferrin (typically used as an indicator of inflammatory bowel disease activity) has been shown to be almost twice as sensitive (75%) as fecal leukocyte detection by methylene blue stain^[41]; however, both tests lack sensitivity and specificity and add little to the diagnostic evaluation.

Radiologic diagnosis of CDI

Radiologic studies such as acute abdominal series have been of little value with non-specific findings. Plain films of the abdomen may reveal colonic dilation, (especially cecal), and non-obstructive related small bowel air fluid levels indicative of ileus pattern. Abdominal computed tomography (CT) has been reported to be normal in 39% of cases, but often reveals a thickened colonic wall, which may be focal or diffuse^[42]. With fulminant colitis, there may be mucosal thumbprinting and an “accordion” appearance with oral contrast trapped in the thickened mucosal folds.

Table 2 Comparison of American College of Gastroenterology 2013 and Society for Healthcare Epidemiology of America/Infectious Diseases Society of America 2010 Guidelines for Treatment of *Clostridium difficile* infection (Differences between the guidelines are in bold)

Severity	SHEA/IDSA 2010 ¹		ACG 2013 ²	
	Definition	Treatment	Definition	Treatment
Mild-to-Moderate	WBC < 15000 cells/ μ L or lower and serum Cr < 1.5 times the premorbid level	Metronidazole 500 mg 3 times/d by mouth for 10-14 d	Diarrhea plus any additional signs or symptoms not meeting severe or complicated criteria	Metronidazole 500 mg orally 3 times/d for 10 d. If no improvement in 5-7 d, consider change to vancomycin at standard dose
Severe	WBC > 15000 cells/ μ L or higher or a serum Cr > or equal to 1.5 times the premorbid level	Vancomycin 125 mg 4 times/d by mouth for 10-14 d	Serum albumin < 3 g/dL plus one of the following: WBC $\geq$ 15000 or abdominal tenderness	Vancomycin 125 mg orally 4 times/d by mouth for 10 d
Severe, complicated	Hypotension or shock, ileus, megacolon	Vancomycin 500 mg four times/d by mouth or by nasogastric tube, plus metronidazole 500 mg every 8 h intravenously. If complete ileus, consider adding rectal installation of vancomycin	Any of the following attributable to CDI: ICU admission, hypotension with or without the need for vasopressors, fever $\geq$ 38.5 °C, ileus or significant abdominal distension, mental status changes, WBC > 35000 cells/mm ³ or < 2000 cells/mm ³ , serum lactate > 2.2 mmol/L, end organ failure	Vancomycin 500 mg orally four times/d and metronidazole 500 mg IV every 8 h and vancomycin per rectum (500 mg in 500 mL saline as enema) four times a day

¹Society for Healthcare Epidemiology of America (SHEA)/Infectious Diseases Society of America (IDSA)^[31]; ²American College of Gastroenterology (ACG)^[71]. WBC: white blood cell; CDI: *Clostridium difficile* infection; ICU: Intensive care unit.

Endoscopic diagnosis of CDI

Endoscopy is usually reserved for special situations. The American College of Gastroenterology (ACG) guidelines recommend endoscopy when a rapid diagnosis is needed, when there is a delay in results of toxin assay or an initial negative toxin assay when CDI is strongly suspected, when there is an ileus and stool is not available and when other colonic diseases are in the differential^[43] (Table 2). Endoscopy is frequently normal with mild disease, but often reveals multiple typical yellowish-white plaques (pseudomembranes) elevated above the surrounding mucosa^[44]. The plaques vary from a few millimeters to 20 mm and may become confluent with advanced disease and may slough off leaving a denuded underlying mucosa. The intervening mucosa between the plaques may be normal or erythematous and edematous. Overall, pseudomembranes have been detected in 41% of cases of CDAD^[45]. Distal involvement of the colon is most common, making flexible sigmoidoscopy a reasonable initial test although in one series, false negative rate due to proximal involvement with rectal sparing was reported in 10% of cases^[46]. Histologically, the pseudomembranes, composed of fibrin, mucus, epithelial and inflammatory cells appear as “clouds” rising from points of superficial ulcerations. The lesions have been termed “volcano” lesions appearing like an eruption above underlying glandular lesions^[47]. In 22% of cases, pseudomembranes were visualized on endoscopy, but not present histologically^[48].

Laboratory diagnosis of CDI

The state of the art for best practice is controversial and confusing. Curry noted that “diagnosis of CDI remains one of the most vexing difficulties for hospital microbiology laboratories”, because there is no single accepted

gold standard^[49]. For many years, cell culture cytotoxicity neutralization assay (CCNA) was the accepted gold standard. By this method, stool filtrates are inoculated onto a monolayer of a cell culture in wells with and without *C. difficile* antitoxin. Rounding of the cells in the antitoxin-free well demonstrates a cytopathic effect and the presence of toxin. If there is no change in the antitoxin containing well, then the presence of *C. difficile* toxin in the stool is confirmed. CCNA is quite specific for CDI and can detect toxin in the stool as low as 10 picograms. However, the assay is expensive, has a slow turnaround time (2 d minimum), lacks standardization among laboratories and is generally unavailable outside the research setting. More recently, many investigators have considered toxigenic culture (TC) as the method of choice for diagnosis of CDI. With the toxicogenic culture method, stool is cultured for *C. difficile* on a selective differential medium (cycloserine, cefoxitin, fructose agar or CCFA). In the next step, the organism is tested for ability to produce toxin. Compared to TC, CCNA has only 67%-79% sensitivity^[49]. The Society for Healthcare Epidemiology of America (SHEA)/Infectious Diseases Society of America (IDSA) 2010 guidelines note that “the sensitivity and specificity of stool culture followed by identification of a toxigenic isolate (TC) as performed by an experienced laboratory provides the standard against which other clinical tests should be compared”^[31]. Despite the assertions of the superiority of TC as a gold standard, there are significant issues with using TC as a gold standard. The TC identifies the ability to produce toxin, but not actual toxin in stool. This can lead to false positives due to the fact that up to 7% of asymptomatic hospitalized patients may be colonized on admission with toxigenic *C. difficile*^[50]. Rates of asymptomatic colonization with toxin producing *C. difficile* can be even

higher among elderly patients in skilled nursing facilities, approaching 20%^[51]. Concern about using TC as the gold standard was raised by a recent study conducted by the National Health Service (NHS) Laboratories in the United Kingdom, which evaluated 12441 diarrheal fecal samples^[52]. The study showed that the presence of toxin in the fecal specimens was associated with poor clinical outcomes; however, culture of toxin producing *C. difficile* without detection of toxin in the diarrheal stool specimens was not associated with worse clinical outcomes than stools that were negative for toxigenic *C. difficile*. At best, which test should be the gold standard for diagnosis of CDI, TC or CCCNA, is currently undecided. One thing is clear if the gold standard being used is TC, then all the comparators, whether enzyme immunoassay (EIA), glutamate dehydrogenase (GDH) or polymerase chain reaction (PCR), will be less sensitive. If CCCNA is used as the gold standard, the comparators will appear more sensitive.

There is consensus that the EIA for toxin A/B, currently the primary test used in up to 90% of clinical laboratories^[53] is too insensitive and non-specific and no longer recommended as a stand-alone test^[54]. The EIA for toxin A/B has been adopted by most clinical laboratories because it is fast, convenient and inexpensive. Recent studies have shown however, that the sensitivity can be as low as 38%^[55]. The EIA requires 100-1000 picograms of toxin as compared to the ability of the CCCNA to detect less than 10 picograms of toxin^[53]. In addition to poor sensitivity, the EIA also has a positive predictive value (PPV) as low as 50% due to the low prevalence of *C. difficile* among all specimens submitted for testing from symptomatic patients^[54]. Historically, 15%-25% of antibiotic associated diarrhea has been felt to be due to *C. difficile*. However, most recent studies suggest a decreasing rate of positivity with only 5%-10% of samples testing positive^[54]. In 2001, 22% of samples tested were positive for toxin by EIA *vs* only 11% in 2007^[56]. If the prevalence of positive stools is 10%, then the PPV of a positive toxin EIA varies from less than 50%-90%. Falsely diagnosing a patient with CDI can lead to isolation of patients who are not infected. Isolation has been shown to have negative consequences, with a doubling of adverse events and days without a physician note and an increase in formal complaints by 8-fold^[57]. A false diagnosis of CDI can also lead to co-horting of uninfected patients with patients who have active CDI, particularly in skilled nursing facilities, as well as delay in finding the true etiology of the diarrhea and the unnecessary use of antibiotics. A systematic review of toxin detection kits concluded that the sensitivity and specificity of the different test kits were sufficiently heterogeneous between studies of the same test, such that meta-analytic methods could not be used to pool studies on a particular toxin EIA assay^[56]. They concluded that differences in test characteristics were most likely related to the threshold cutoff chosen for each test. Choosing a low threshold increased the sensitivity, but at the same time decreased specificity and vice versa. Overall, the au-

thors concluded that none of the EIA toxin assays had an acceptable predictive value and that a two-step testing strategy should be used.

The lack of sensitivity and specificity of the toxin A/B EIA assay has led to a search for more accurate test methods. The detection of GDH in stool has shown significant promise. The test is fast (15-45 min), convenient, inexpensive, and sensitive. The GDH is a common antigen expressed at high levels by all *C. difficile* strains. However, the test only documents the presence of *C. difficile*, but not the presence of a toxigenic strain (20% of *C. difficile* strains do not produce toxin) or the presence of toxin in stool^[58]. Therefore, GDH (+) stool requires confirmation of toxin production with a second test. Early studies reported sensitivities as high as 100% for detection of *C. difficile*^[59]. However, more recent studies have raised concern about the sensitivity of the GDH assay for non-epidemic B1 strains. For non-epidemic B1 strains, the sensitivity may be as low as 69%^[60].

The use of PCR to detect the gene for toxin production (*tdfB* gene) is promising as a stand-alone test for CDI. The PCR for the toxin gene is fast (2 h) and sensitive with a minimum detection limit of 105 per gram of stool^[61]. However, the cost can be 5-10 times greater than EIA for toxin A/B. Sensitivity has been 91% as compared to enzyme immunoassay at 67%^[62]. Overall, sensitivity has been 84%-94% in comparison to TC, similar to the CCCN^[60]. Many hospital laboratories will be able to offer ready availability of PCR testing with rapid turnaround.

There are currently four Food and Drug Administration (FDA) approved PCR assays, Gene Ohm (Becton Dickinson, San Diego), Gene Xpert (Cepheid, Sunnyvale, Ca.), which not only can identify the *toxin* gene but also the epidemic B1 strain, Progestro (Prodesse, Waukesha, Wi) and Simplexa (Quest Diagnostics, Madison NJ). In a meta-analysis of PCR *vs* TC, a pooled sensitivity of 92% and specificity of 94% was reported^[63]. However, as with TC mentioned earlier, the PCR detects the *toxin* gene, but does not detect toxin in stool raising concerns about over diagnosis by detecting asymptomatic carriers. In addition, the use of the PCR may increase CDI incidence rates by greater than 50%^[64]. This raises concern with mandatory reporting programs and inter-hospital comparisons. Some authors have noted an increase from 6.5% positive samples before the use of PCR to 15% after their laboratory changed to PCR for *C. difficile* detection^[65]. In addition, the PCR cannot be used for suspected relapse as up to 56% of patients will be positive by PCR at 1-4 wk after completion of therapy^[66]. However, despite its high sensitivity and specificity, at the recently noted prevalence of 10% of CDI among tested specimens, the positive predictive value may be only 63%^[65]. Despite these issues, some laboratories have now adopted PCR as a stand-alone diagnostic test for *C. difficile*^[67].

Another promising method for CDI diagnosis is detection of the *toxin* gene by loop-mediated isothermal amplification (LAMP), which does not require a large capital outlay for PCR^[63]. This non-PCR based gene amplification method detects the pathogenicity locus of

toxigenic *C. difficile*. The test is simple, rapid (1 h) and significantly less expensive than PCR based methods. The Illumigene (Meridian Bioscience, Europe) assay was found to have a 92% sensitivity, 98% specificity, 99% negative predictive value and 84% positive predictive value, respectfully^[68]. However, the same issues that raise concerns about TC and PCR, *i.e.*, detection of toxigenic *C. difficile*, but not toxin in stool, are true for LAMP.

The concerns with EIA for toxin A/B, PCR and GDH as stand-alone tests has led to the study of an algorithmic approach to the diagnosis of CDI, similar to HIV and syphilis testing. Larson *et al*^[69] studied a 3 step algorithm with the initial test being a glutamate dehydrogenase. If the GDH is positive, this was followed by confirmation of toxin in stool with an EIA for toxin A/B. If both are positive, the test is reported as positive for CDI. If the EIA toxin A/B is negative, the final result is determined with a PCR. Using this algorithm, they found a sensitivity of 84% and specificity of 99.7% with very high PPV of 97.5% and NPV of 99.7% compared to a modified gold standard using CCCNA and PCR. In the previously mentioned United Kingdom NHS study using 12441 diarrheal fecal specimens, Wilcox concluded that a two-step protocol with an EIA for GDH or a nucleic acid amplification test (NAAT), such as a PCR for *toxin* gene, followed by confirmation of stool toxin by a EIA for toxin A/B was the most effective testing algorithm in distinguishing patients with *C. difficile* infection from those who did not have CDI^[53]. This two-step algorithm has now become the standard in NHS laboratories in England as of April 2012^[70]. The combination optimizes specificity and positive predictive value (90%)^[52]. This same study found that using an algorithm that optimized for sensitivity such as a GDH followed by a PCR resulted in a 95% sensitivity, but a PPV that was only 60%. In other words, 4 of 10 positive tests did not really have CDI. This would be an optimal method for excluding CDI, but not a very good method for determining if CDI was really present. The American Society of Microbiology (ASM) recommends that if the toxin A/B EIA or CCCNA is used and is negative, specimens should be further tested by PCR or TC^[54]. The ASM noted that utilizing toxin A/B EIA for *C. difficile* diagnosis is insensitive and no longer recommended as a stand-alone test. The ASM also noted that laboratories can also use a PCR to detect *C. difficile toxin* genes as a stand-alone diagnostic test. The SHEA/IDSA guidelines suggested that an initial GDH test followed by confirmation with either TC or CCCN was an option^[31]. However, as previously noted, the last two tests are rarely available in clinical laboratories and results would not be available in time for clinical use. The 2013 ACG guidelines recommend a NAAT such as PCR as a standard diagnostic test for CDI. The guidelines also suggest that a GDH EIA can be used an initial screening test in a two-or three-step algorithm with subsequent confirmation of positive results with an EIA for toxin A/B. If the EIA for toxin A/B is negative, then a NAAT test should follow. How-

ever, the ACG guideline notes that the sensitivity is lower than a strategy based on an initial PCR^[71].

Repeat testing for CDI

One aspect of testing about which there is broad agreement is that there are limited indications for repeat testing. Yassin *et al*^[72] have suggested that performing the EIA for toxin on two or three samples can increase sensitivity to about 90%. However, Renshaw *et al*^[73] suggested that repeated assays accounted for 36% of all toxin assays ordered, but provided clinically useful information in only 1% of the cases and significantly increased cost. Aichinger *et al*^[74] found that repeat testing within 7 d by EIA for toxin A/B or by PCR for *C. difficile* toxin resulted in < 2% positive tests. In another study, repeat testing accounted for 17% of all tests ordered, but only 1% were positive^[75]. Peterson *et al*^[59] noted that with a sensitivity of 73% and a specificity of 97.6% that if the *C. difficile* EIA was negative on the first two tests, a positive result on the third test was three times more likely to be a false positive than a true positive due to decreasing pretest probability with consecutive negative tests. In fact, even on the second test after an initial negative, the positive predictive value is less than 50%, about as good as flipping a coin. The 2013 ACG guidelines make a strong recommendation that repeat testing not be performed.

There is clearly no indication for serial monitoring of stools or an end of treatment “test of cure” as 1/3 of patients will still have a positive assay at the end of successful treatment^[40]. Stool carriage has been noted to persist for 3-6 wk after successful treatment and has not been found to predict who will relapse^[76]. Requiring a negative test to come out of isolation or before transfer to a long term care facility is inappropriate. Again, the 2013 ACG guidelines make a strong recommendation that testing for cure should not be done.

Given the limitations of the available laboratory tests for CDI, a reasonable approach is: (1) if CDI is suspected on clinical grounds, perform *C. difficile* testing according to your hospital laboratory protocol. Be aware of the test or algorithm they are using. Many clinical laboratories are in the process of changing testing protocols; (2) if the test is positive, continue or initiate treatment, if not started empirically; and (3) if the test is negative, make a clinical decision on whether to treat based on the likelihood of CDI (recent exposure to antibiotics or prior CDI, elevated white blood count or elevated creatinine or decreased albumin, age or other risk factors). If CDI is still suspected after a negative test, empiric treatment is reasonable. Repeat testing yields minimal additional true positives and increases cost. The ACG Guidelines make a strong recommendation that “Repeat testing should be discouraged”^[71].

In summary, testing for CDI is in flux, confusing and controversial. As noted by Fang, “the clinical laboratory can place the perpetrator (*C. difficile*) at the scene of the crime, but only the clinician can establish whether a crime (CDI) has taken place”^[77].

THE CONTROVERSY OVER BASIC TREATMENT CHOICES

Despite numerous treatment trials for *C. difficile* infection, dating back to 1978, the drug of choice for CDI remains controversial. In fact, Pepin noted that “there are few common infectious diseases in developed countries for which the treatments used in 2006 are essentially the same as those recommended one-quarter of a century ago”^[78]. The same can be said for 2013 and the foreseeable future. The recent Cochrane Collaboration review of antibiotic treatment for CDI vividly illustrates the ongoing problems related to treatment decisions^[79,80]. The authors reviewed randomized, controlled trials of antibiotic therapy for CDI. There were 15 studies considered evaluable with 1152 patients involved. There was only one placebo controlled trial, which was considered to be of small size with poor methodological quality. The authors concluded that even the most basic question of whether any antibiotic is effective, much less which one, has not yet been answered. The authors stated, “this review cannot establish the efficacy of antibiotic therapy for CDI as the only placebo controlled trial is inadequate”. In fact, they noted that there is “uncertainty whether mild CDI needs to be treated”. Further, they noted that “this review cannot definitively make a specific antibiotic recommendation for the treatment of CDI”. When looking at particular antibiotics, they concluded that “no single antibiotic is clearly superior to others”. Although, they did note that teicoplanin was superior to vancomycin. Unfortunately, teicoplanin is not available in the United States.

Part of the reason that there have been so few changes in our treatment of CDI over the last 30 years may be due to the lack of development of significant resistance. Fortunately, a number of recent studies have not revealed resistance to the main standbys for treatment of CDI: metronidazole and vancomycin. Aspevall *et al.*^[81] studied 238 isolates of *C. difficile* collected from 2000 to 2001 and found no evidence of resistance to metronidazole or vancomycin. Hecht *et al.*^[82] studied 110 strains collected between 1983 and 2004. All strains were sensitive to metronidazole at less than or equal to 0.5 µg/mL. Bourgault *et al.*^[83] looked at 251 isolates collected during the outbreak in Quebec, Canada, which started in 2003. Of these, 69% were the B1 epidemic strain, while 11% were the NAP2 strain by PFGE. All isolates were sensitive to metronidazole and vancomycin. There was no increase in MIC's compared to historical isolates.

Unfortunately, the same cannot be said for other antibiotics. Recently, the complete genome of *C. difficile* has been sequenced revealing a significant potential for development of antibiotic resistance^[84]. Significant portion of the genome (11%) consists of mobile genetic elements, mainly conjugative transposons, which can be used to transfer genetic material between bacteria. These mobile genetic elements are often involved in the transfer of antimicrobial resistance and virulence factors.

Bourgault *et al.*^[83] found that for the B1 epidemic strain the quinolones, macrolides and other commonly used antibiotics have succumbed to the antibiotic resistance mechanisms of *C. difficile*. All strains were resistant to bacitracin, ciprofloxacin, levofloxacin and clarithromycin, while 80% were resistant to gatifloxacin, moxifloxacin and ceftriaxone. All historical NAP1 isolates were resistant to quinolones, suggesting that the epidemic may be more associated with the increased use of fluoroquinolones, as opposed to the recent development of quinolone resistance by the epidemic strain. Of note, 69% of the B1 epidemic strains were sensitive to clindamycin, while only 11% of the non-epidemic strain strains were sensitive to clindamycin.

METRONIDAZOLE OR VANCOMYCIN

Having summarized the murky state of the evidence based treatment of CDI, it would be reasonable to look at the pros and cons of metronidazole and vancomycin. The oft-quoted reasons for metronidazole assuming the status of preferred agent for treating CDI has been the potential for development of vancomycin-resistant enterococci (VRE) and the higher cost of oral vancomycin. In contrast to this notion, a small study looking specifically at the issue of developing VRE found no patients developed VRE while being treated with oral vancomycin^[85]. Unfortunately, vancomycin capsules (Vancocin HCl Pulvules) are extraordinarily expensive, with an average wholesale price of \$31.83 per capsule or \$127.32 per day for a dose of 125 mg *qid* *vs* \$2.19 per day for generic metronidazole 500 mg *tid*^[86]. Further, retail costs are much higher. Most hospitals avoid the extraordinary cost of vancomycin capsules by using the generic intravenous formulation and compounding it in water as a liquid vancomycin solution. One pharmacy, close to our clinic, sells vancomycin intravenous formulation for \$5.85 per 500 mg *vial*. If this 500 mg of vancomycin powder is reconstituted in 20 cc of water (often with flavoring to hide the bitter taste of vancomycin), the cost of vancomycin approaches \$1.50 per dose. Stability of the vancomycin solution in the refrigerator (4 degrees C) is at least 75 d and at least 26 d at room temperature (25 degrees C)^[87].

Despite issues related to fostering VRE and cost, prior comparative studies of metronidazole and vancomycin have not revealed a statistically significant difference between the two antibiotics^[88,89]. In one study, 95% were cured with metronidazole *vs* 100% with vancomycin^[88]. In the second study, the cure rates were identical at 94% in each group^[89]. However, the number of patients was small and neither study was stratified by severity of disease.

Despite similar response rates, there are significant pharmacologic concerns related to metronidazole, which tilt the balance in favor of vancomycin. Metronidazole is rapidly absorbed from the gastrointestinal tract and excreted through the biliary system, with only about 14% of the drug excreted in the stool^[90]. Fecal metronidazole

levels have been noted to increase with colonic inflammation, probably from transudation into the lumen, but these levels decrease as inflammation subsides and are undetectable upon recovery^[37,91]. More recently, Musher noted a failure rate of 22% with standard doses of metronidazole^[92]. This was not due to resistance, as those strains tested, were all sensitive to metronidazole. Interestingly, in this study there was no difference in outcomes between those who were continued on metronidazole despite clinical failure compared to those who were changed to vancomycin. Musher *et al.*^[92] suggested that patients with severe disease could have decreased blood flow to the colon, which would result in less transudation of metronidazole into the lumen and either a slower response or clinical failure^[93]. Despite this potential for low metronidazole levels, *in vitro* the drug has been shown to be very rapidly bactericidal at 8-times the minimum inhibitory concentration (MIC), a level which is usually reached in the colon. This rapid bactericidal effect can be compared to vancomycin, which has been shown to be only inhibitory of bacterial growth^[40]. As opposed to the poor pharmacokinetics of metronidazole, vancomycin has near perfect characteristics for a drug used to treat an infection limited to the lumen of the colon. Vancomycin achieves levels in the colon of about 1000 µg/mL in stool due to the fact that there is limited or no absorption from the colon. Al-Nassir *et al.*^[94] have shown that vancomycin is much more effective than metronidazole in removing *C. difficile* from the stool as measured by *C. difficile* density cultures^[94]. By day 5 of treatment, patients treated with vancomycin were 3.3 times more likely to have undetectable *C. difficile* than metronidazole ($P = 0.015$). In this study, 10 of 34 patients were switched from metronidazole to oral vancomycin between days 2 and 10 due to suboptimal clinical response, of whom 8 of the 10 had less than a one log decrease in *C. difficile*. Once they were switched to oral vancomycin, 7 of these 8 patients had undetectable *C. difficile* by culture. Freeman *et al.*^[95,96] confirmed the favorable characteristics of oral vancomycin in a human gut model composed of three vessels operating in a weir cascade system in an oxygen free nitrogen atmosphere. They found that cytotoxin titers were unaffected by metronidazole, while vancomycin resulted in a marked decrease in toxin and the *C. difficile* vegetative form, leaving only spores which do not produce toxin. Another issue which may decrease the effectiveness of metronidazole is inactivation by *Enterococcus faecalis*, which has been shown to allow protection of organisms which would normally be killed by metronidazole^[97]. There also appears to be a higher failure rate with metronidazole when the physician is forced to continue the offending antibiotics, which is often the case. In one series, all patients who could have the offending antibiotic discontinued had resolution of diarrhea by 14 d when treated with metronidazole^[98]. However, 41% of the patients who had antibiotics continued failed to have symptomatic resolution of diarrhea by day 14 ($P = 0.02$).

Because rifampin has been shown to have markedly

superior *in vitro* activity in comparison with other antimicrobials against *C. difficile*^[99] combination therapy has been studied as a means to improve outcomes with metronidazole therapy. Lagrotteria *et al.*^[100] conducted a prospective, randomized, single-blind study of metronidazole alone *vs* metronidazole plus rifampin^[100]. There was a similar time to improvement, similar proportion of relapses, but significantly more deaths in the combination group as compared to metronidazole alone (32% *vs* 5%, $P = 0.04$). The authors concluded: “there is no role for rifampin as an adjunct to treatment with metronidazole.”

TREATMENT DECISIONS BASED UPON STRATIFICATION BY DISEASE SEVERITY

A concern with all of the preceding comparative studies of vancomycin with metronidazole has been that there was no stratification by disease severity. One of the most important recent advances in the treatment of CDI has been the development of scoring systems, which allow the physician to determine which patients are at highest risk for severe CDI. The development of scoring systems was started by Pepin *et al.*^[78] who developed local recommendations, because of the overwhelming epidemic in Quebec caused by the new epidemic B1 strain. In January of 2004, they developed local recommendations for the use of oral vancomycin: a WBC greater than 20000 cells/mm³ and a serum creatinine greater than or equal to 200 µmol/L. This recommendation was based upon a reduction of complicated CDI by 79% if vancomycin was the initial treatment compared to metronidazole^[101].

Zar *et al.*^[102] conducted the first randomized, double-blind, placebo controlled trial comparing metronidazole and vancomycin in the treatment of CDI that stratified patients at study entry based upon severity of disease. The authors developed a scoring system giving 1 point each for the presence of age greater than 60 years, temperature greater than 38.3 degrees centigrade, albumin less than 2.5 mg per deciliter, or a WBC count greater than 15000 cells per mm³. They also gave 2 points for endoscopic evidence of pseudomembranous colitis or treatment in an intensive care unit setting. Mild disease was defined as 0 or 1 points and severe CDI was defined as greater than or equal to 2 points. Clinical cure was noted in 90% of those with mild CDI randomized to metronidazole and 90% of those randomized to vancomycin. For those with severe CDI, clinical cure was noted in 76% who received metronidazole *vs* 97% who received vancomycin ($P = 0.02$). Recurrences were similar for both groups at 15% and 14% for the metronidazole and vancomycin groups, respectively. The authors concluded that metronidazole and vancomycin are equally effective for the treatment of mild CDI; however, vancomycin is superior for treating patients with severe CDI. Critiques of the Zar *et al.*^[102] article were that one of the criteria for failure was persistent toxin positivity at day 6 and 10 of therapy. In addition, there was exclusion of 8 patients

Table 3 Comparative average wholesale price for antibiotics used in the treatment of *Clostridium difficile* infection

Antibiotic	Cost per dose ¹	Usual regimen	Cost per treatment ¹
Metronidazole	\$0.73		
Vancomycin capsules (Vancocin HCL pulvules)	\$31.83	125 mg 4 times/d × 10 d	\$1273.20
Vancomycin intravenous formulation (generic)	\$5.00/g (\$0.62 per 125 mg dose)	125 mg 4 times/d × 10 d	\$25.00
Fidaxomicin (Dificid)	\$168.00	200 mg 2 times/d × 10 d	\$3360.00
Rifaximin (Xifaxan)	\$19.02 400 mg	400 mg 3 times/d × 20 d ²	\$1141.20

¹Average Wholesale Price (AWP); Anon, edition. Red Book online. ²Dose as a “chaser” after a course of oral vancomycin for recurrent CDI^[151]. Via Drugdex System (internet database) Greenwood Village, CO: Thompson Healthcare, 2011^[86]. CDI: *Clostridium difficile* infection.

with early death^[103]. When the 2 patients who were judged as having failed therapy solely on the basis of persistent toxin positivity and the 8 early deaths were included, vancomycin was still superior to metronidazole for those with severe disease with a 90% cure rate for vancomycin *vs* 71% for metronidazole ($P = 0.04$).

The 2010 IDSA guidelines recommend oral metronidazole for CDI with a WBC 15000 cells/mm³ and < 50% increase in serum Cr from baseline. The guidelines define severe disease as CDI with a WBC 15000 cells/mm³ or a 50% increase of serum Cr from baseline. For severe CDI, they recommend starting therapy with oral vancomycin 125 mg *qid*^[50]. Most recently, the ACG has updated its practice guidelines to include summary recommendations based on CDI severity^[71]. Mild-to-moderate disease is defined as diarrhea plus any additional signs or symptoms not meeting severe or complicated criteria. Notably, the ACG classification for severe disease has been redefined from the IDSA guidelines to use only three criteria: a serum albumin < 3 g/dL plus one of either WBC 15000 cells/mm³ or abdominal tenderness. The choice to limit the guidelines to these three criteria was based on a prospective observational study by Fujitani *et al*^[104] which found that the only independent risk factors for severe CDI were abdominal distention, fever, WBC > 20000 cells/mm³, and serum albumin < 3 mg/dL. The ACG guidelines recommend the same initial treatments of metronidazole 500 mg orally three times daily for 10 d for mild-to-moderate disease and vancomycin 125 mg orally four times daily for severe disease.

NEWER ANTIBIOTICS FOR CDI

Rifaximin

Rifaximin (Xifaxan, Salix Pharmaceuticals, Inc. Raleigh, NC) is a broad spectrum, non-absorbable antibiotic used for the treatment and prevention of traveler's diarrhea. The drug is not inactivated by gastric fluids and is also poorly absorbed, thereby largely excreted unchanged in the feces reaching concentrations up to 8000 g/gm of stool after 3 d of therapy^[105]. Rifaximin treatment has demonstrated survival rates in animal models equivalent to vancomycin. Rubin *et al*^[106] conducted an open label pilot study to assess the efficacy of rifaximin as an initial treatment option in patients without recurrent CDI. Of the 8 patients who completed the 10-d course of rifaximin 400 mg three times daily, 7 (88%) had symptom

resolution with 10 d of rifaximin treatment with no relapse within 2 wk. Additionally, Boero *et al*^[107] compared the efficacy of rifaximin 200 mg *tid* and vancomycin in a study of 20 patients. Response rates were 90% and 100% for rifaximin and vancomycin, respectively. One concern about rifaximin is the potential for resistance, especially given the lack of sensitivity testing outside of a research laboratory. A study of rifaximin susceptibility of 80 different *C. difficile* isolates found resistance among 14 isolates, of which 64% were the epidemic B1 strain^[108]. At this point, it is difficult to ascertain the clinical impact of these findings, especially given the extremely high fecal concentrations achieved with rifaximin. While these small studies suggest a potential application for rifaximin for the initial treatment of CDI, more attention has been placed on a rifaximin “chaser” in the treatment or prevention of recurrent CDI (see section on Recurrent CDI) (Table 3).

Nitazoxanide

Nitazoxanide (Alinia, Romark Laboratories, Tampa, Florida) is a broad-spectrum antiparasitic agent currently approved for the treatment of giardiasis and cryptosporidiosis^[109]. Nitazoxanide is highly active *in vitro* against *C. difficile*. Studies have shown that two-thirds of the drug is excreted in the stool as an active metabolite with activity against *C. difficile* comparable to the parent compound^[110]. Nitazoxanide has been shown to prevent colitis in the hamster model^[111]. Further, nitazoxanide has been shown to very active against a panel of 127 *C. difficile* isolates from the United Kingdom's *C. difficile* Ribotyping Network at an MIC range of 0.03-0.5 mg/L^[112]. A recent prospective, randomized, double blind study by Musher *et al*^[113] compared metronidazole 250 mg *qid* for 10 d to nitazoxanide 500 mg *bid* for 7 or 10 d^[113]. After 7 d of treatment, the metronidazole response was 82% compared to 90% for nitazoxanide. At 31 d after starting treatment, a sustained response was noted for 58% of patients treated with metronidazole *vs* 66% for the 7-d course of nitazoxanide and 74% for the 10-d course ($P = 0.34$). Musher *et al*^[114] also reported the use of nitazoxanide in 35 patients that failed to respond to metronidazole after 14 d of therapy or who had prompt recurrence on at least two occasions after an initial response. They noted that 74% of patients responded, however, 7 of the 26 recurred, leaving an overall cure rate of 54%.

Most recently, Musher *et al*^[115] completed a randomized, double-blind study of nitazoxanide *vs* vancomycin. After 10 d of treatment, resolution of CDI occurred in 20 of 27 vancomycin patients (74%) and 17 of 22 nitazoxanide patients (77%). For those completing therapy, both treatments had similar times to resolution with response rates of 87% for vancomycin and 94% for nitazoxanide. Subsequently, 2 vancomycin patients and 1 nitazoxanide patients relapsed, leaving a sustained response rate of 78% for vancomycin and 89% for nitazoxanide. The authors noted that, while the small sample size may not have the power to prove noninferiority *vs* vancomycin, as the first randomized control trial their results suggest nitazoxanide may be equally effective.

Fidaxomicin

Fidaxomicin (Dificid®, Optimer Pharmaceuticals, San Diego, CA) is a macrocyclic antibiotic with a narrow spectrum of activity against gram-positive cocci. Fidaxomicin has been 100% protective in a hamster model of CDI^[116]. Importantly, fidaxomicin has been shown to have a comparable safety profile to vancomycin^[117], have undetectable serum levels while achieving high fecal concentrations, averaging greater than 10000 times the MIC for *C. difficile*^[118], a bactericidal mechanism of action^[119], preserve the intestinal microbiome (by sparing of *Bacteroides* sp.), reduce both toxin reexpression and CDI recurrence^[120], and reducing the acquisition of VRE and *Candida* species during CDI treatment^[121,122].

Much of the attention centered on fidaxomicin has been based on findings from two prospective, multicenter, double-blind, randomized Phase III trials demonstrating non-inferiority to vancomycin. The first trial (003 in the United States and Canada) of 629 patients randomized to receive either fidaxomicin 200 mg twice daily (with intervening placebo) ($n = 302$) or vancomycin 125 mg four times daily ($n = 327$), revealed no significant difference in the clinical cure rates: 88.2% for fidaxomicin and 85.8% for vancomycin^[123]. Another interesting observation that arose from the 003 trial was that overall recurrence rates, as defined by the reappearance of more than three diarrheal stools per 24-h period within 4 wk after cessation of therapy, were lower in the fidaxomicin group at 15.4% compared to 25.3% in the vancomycin group ($P = 0.005$). However, recurrence rates with the epidemic B1 strain were similar between fidaxomicin and vancomycin with 24.4% and 23.6% recurrences, respectively. The second Phase III trial (004 conducted at 45 sites in Europe and 41 sites in the United States and Canada) also found fidaxomicin to be non-inferior with cure rates of 91.7% *vs* 90.6% for vancomycin (one sided 95%CI: -4.3)^[124].

Most recently, a post-hoc intent to treat meta-analysis was performed on the results of the combined 003/004 Phase III trials. Of the 1164 patients included, fidaxomicin when compared to vancomycin was associated with a 40% reduction in persistent diarrhea, recurrence, or death through day 40 (95%CI: 26%-51%; $P < 0.0001$)^[125]. Subgroup analysis limited to the epidemic B1 strain, revealed

a 22% non-significant reduction in persistent/recurrent diarrhea (95%CI: 44% reduction to 8% increase, $P = 0.14$). The authors point out that with only 292 of 814 strains testing positive for B1, the results from this analysis are too underpowered to conclude fidaxomicin lacks beneficial effect for the B1 strain.

One important aspect of fidaxomicin remains, cost. At \$168 per 200 mg tablet, a twice-daily 10-d treatment course costs \$3360 for a 10 d course^[86]. The pharmaceutical company selling this medication has recently developed several strategies to help reduce the patient cost if the medication is needed.

COMPLICATED CDI

Complicated CDI is defined in the 2010 IDSA guidelines as severe CDI plus intensive care unit (ICU) admission, need for colectomy, ileus, toxic megacolon, hypotension or colonic perforation^[31]. The 2013 ACG guidelines define severe complicated CDI as any of the following: admission to the ICU, hypotension with or without the need for pressors, fevers $> 38.5^{\circ}\text{C}$, ileus or significant abdominal distension, mental status changes, $\text{WBC} \geq 35000 \text{ cells/mm}^3$ or $< 2000 \text{ cells/mm}^3$, serum lactate $> 2.2 \text{ mmol/L}$, or end organ failure^[71].

For severe complicated CDI, the IDSA guidelines recommend high dose oral vancomycin 500 mg *qid* (by nasogastric tube, if necessary) and/or metronidazole 500-750 mg q8h intravenously. Metronidazole and vancomycin combination has been shown to be synergistic *in vitro* for 68% of *C. difficile* isolates^[99]. Apparently, the increased dose of vancomycin for complicated CDI is related to a delay in attaining adequate fecal levels with 125 mg *vs* a higher dose when given orally^[126]. For complete ileus, metronidazole intravenously plus vancomycin administered by retention enema is recommended. The critical point is that the vancomycin, needs to be retained and distributed in the colon to be effective. Specific orders should detail the administration lest the vancomycin be administered as a plain enema, providing no benefit for the patient and creating a hazard for nursing staff. The vancomycin should be administered using a # 18 French Foley catheter with a 30 mL balloon. The Foley catheter should be inserted into the rectum, the balloon inflated and the vancomycin instilled. The catheter is then clamped; some authors recommend turning the patient on their right side to assist distribution of the vancomycin solution throughout the colon. After 60 min, the balloon is deflated and the catheter is removed^[127]. Because there have been no controlled trials of vancomycin by intracolonic installation, the optimal dose and interval are unclear. Apisarnthanarak *et al*^[128] reported a descriptive case series of nine consecutive patients treated with intracolonic vancomycin as adjunctive therapy for severe CDI. Eight of nine patients had failed five to 7 d of standard therapy for CDI and had evidence of a severe ileus with resultant cessation of diarrhea. Further evidence of the severity of the colitis was suggested by the fact that six of the nine patients were hypotensive at the time CDI

was diagnosed. They administered intracolonic vancomycin 0.5-1.0 g in one to two liters of normal saline as a retention enema. Because this was a retrospective collection of cases, the dosing interval and duration of therapy were variable. Two patients received intra-colonic vancomycin at 4 h intervals, two at 6 h, two at 8 h and three at 12 h. The authors noted complete resolution of colitis in eight of nine patients with no relapses and no surgical interventions. As a note of caution, four patients were colonized with VRE prior to the intracolonic vancomycin and two of these 4 developed VRE bacteremia. However, none of five patients who were not colonized with VRE before therapy developed subsequent colonization.

The 2013 ACG guidelines offer slight changes in recommend therapy for severe and complicated CDI^[71]. Initial therapy for severe and complicated CDI without any significant abdominal distention is vancomycin orally 125 mg *qid* plus intravenous metronidazole 500 mg *tid*. For severe and complicated CDI with ileus, toxic colitis, or significant abdominal distention, the recommended therapy is vancomycin delivered both orally 500 mg *tid* and per rectum 500 mg in volume of 500 mL *qid* plus intravenous metronidazole 500 mg *tid*. Of note, the author's discuss the potential for development of electrolyte imbalances with the use of saline for delivery of the vancomycin enema, in particular hyperchloremia. In such a situation, the authors propose the use of Ringer's Lactate, which contains a lower concentration of chloride^[71].

Tigecycline is a broad-spectrum glycylcycline antibiotic with reportedly low MIC values against *C. difficile*, along with evidence that it does not promote growth or toxin production in both a mouse and human model^[82,129,130]. To date no clinical trials have been performed on the use of tigecycline; however, several case reports have reported the successful use of IV tigecycline in severe or severe complicated CDI in which patients failed prior treatment with metronidazole and vancomycin^[131]. There has also been a case report noting the successful treatment of severe refractory CDI using a combination of tigecycline (50 mg IV every 12 h for 10 d) and rifaximin (400 mg twice daily for 17 d)^[132].

SURGICAL INTERVENTION

Failure to respond to maximal medical management, including unrelenting sepsis, cecal dilatation greater than 10 cm and bowel perforation have been considered indications for surgical intervention. In large series, 0.4%-3.6% of patients have required surgery, with an overall mortality of 30%-80%^[133-135]. Series of severe CDI repeatedly emphasize how difficult the diagnosis may be. In a report of 14 patients requiring surgical intervention, only 50% had a preoperative diagnosis of CDI, because they required laparotomy before results of *C. difficile* testing became available^[136]. Of note, the survival was better (86% *vs* 33%) in those with a preoperative diagnosis of CDI, which may have been due to the surgeon being more aware of the need for a total colectomy. Longo *et al*^[137] noted some of the difficulties

in the diagnosis of severe CDI in a series of 67 patients who required colectomy; 37% of the patients had no history of diarrhea, 45% presented in shock and 64% presented as an acute surgical abdomen^[137]. Dallal *et al*^[17] in a review of 64 patients who required a colectomy or died directly from CDI noted that 20% of the patients presented without diarrhea due to ileus. Of note, in this study 35% of diagnoses of severe CDI were found only at autopsy and the author suggested that a significant number of ICU deaths from "sepsis" may actually be CDI. Overall, 13% were *C. difficile* EIA toxin assay negative. Longo *et al*^[137] found false-negative *C. difficile* cytotoxin assay in 18% of CDI severe enough to require colectomy^[17]. Better diagnostic accuracy for severe CDI has been reported for the abdominal CT (89%-100% positive) and colonoscopy (100% positive)^[17,136,137]. Of note, intravenous and oral contract were not required for a correct diagnosis with CT of the abdomen. Flexible sigmoidoscopy was falsely negative in 25% (2 of 8), one due to poor prep and one due to right sided colitis^[17].

Lamontagne conducted a retrospective review of 165 cases of CDI which required ICU admission during the epidemic in Quebec between January 2003 to June 2005^[138]. Of note, 24% of these ICU admissions resulted from relapse of previously diagnosed CDI, confirming how serious relapses can be. Predictors of 30 d mortality included a WBC of greater than 50000, age greater than 75-year-old, requirement for vasopressors and immunosuppression. Thirty eight patients underwent colectomies, 15 because of shock despite vasopressors, 11 with toxic megacolon, 10 with a lack of response to medical therapy and 2 because of perforation. The authors noted a significant decrease in mortality in those who had a colectomy *vs* those who were treated medically, with an adjusted odds ratio of 0.22, suggesting a 78% reduction in mortality. The major surgical benefit was found in those patients greater than 65 years of age who were immunocompetent with a WBC greater than 20000 and a lactate between 2.2 and 4.9 mm per liter. No surgical benefit was found in those with a white blood cell count less than 20000, less than 65 years of age and those with a normal lactate.

Recent surgical series have revealed conflicting data on which surgical procedure is preferred. Koss *et al*^[139] presented a retrospective review of 14 patients who required surgery. The indications were systemic toxicity (*n* = 10), progressive toxic colonic dilatation (*n* = 4), and one with both colonic dilation and bowel perforation. Overall, mortality was 36%. Of those who underwent a total colectomy, mortality was 11% compared to 100% mortality in those whose surgical procedure was limited to a left hemicolectomy. Of note, at the time of surgery the exterior surface of the colon frequently was noted to be unremarkable, but all were distended and edematous. Longo *et al*^[137] conducted a population based study from all 159 Department of Veterans Affairs Hospitals of patients who required colectomy for fulminant CDI between 1997 and 2001. For the 67 patients, the postoperative 30 d mortality was 48%. Of those who underwent

segmental colectomy, the mortality was 14%, while the mortality was 57% for those who underwent total colectomy (80% of the cases). At surgery, 58% of the patients were noted to have perforation or colonic infarction. As opposed to the Koss study, 12 of 14 patients who underwent hemicolectomy survived, probably because the colitis was restricted to the involved segment. A study by Dallal *et al.*^[17] confirms the possibility of segmental colectomy. This study was a retrospective review of 64 patients who died or underwent colectomy for pathologically proven CDI drawn from 2334 hospitalized patients with CDI, who were hospitalized between January 1989 and December 2000. There were 44 patients who required surgical intervention. Of those undergoing a right hemicolectomy, 100% survived. This was a select subgroup of 4 patients, all of whom had intraoperative colonoscopy confirming the fact that the colitis was restricted to the right hemicolon. Overall, in this study 89% of patients underwent a total colectomy, with a mortality of 63%. Most predictive of perioperative mortality was vasopressor requirement preoperatively, which increased postoperative mortality by four-fold. The authors suggested that hypotension requiring vasopressors may be too late a point for successful intervention. They noted that a white blood cell count greater than 30000 with a left shift almost always preceded the onset of shock and may be used as an early indicator of fulminant CDI, which may require surgical intervention.

Most recently, Neal *et al.*^[39] have studied an alternative to total colectomy advocating a diverting loop ileostomy with colonic lavage. They studied 42 patients with severe, complicated CDI. Their surgical approach was creation of a laparoscopic loop ileostomy followed by intraoperative colonic lavage with a warmed polyethylene glycol/electrolyte solution thru the ileostomy. They also performed postoperative antegrade instillation of vancomycin solution through the ileostomy. Compared to well matched historical controls mortality was reduced from 50% to 19%. Delayed reversal of the ileostomy, after recovery from the acute episode, resulted in preservation of the colon in 93% of cases. Based on these improved outcomes, they suggested that all patients with severe CDI should be considered for surgical management.

The 2013 ACG guidelines also defined signs and symptoms in complicated CDI which warrant surgical consultation, including: hypotension requiring vasopressor therapy, clinical signs of sepsis and organ dysfunction, mental status changes, WBC 50000 cells/mm³, lactates 5 mmol/L, or complicated CDI with failure to improve on medical therapy after 5 d^[71]. The suggested operative management is subtotal colectomy and end-ileostomy, which has been associated with reduced mortality in fulminant CDI^[140].

RECURRENT CDI

Recurrence of CDI after initial successful treatment has been a significant problem. On average, recurrence can be expected in 20%-30% of cases. Once there has been

an initial recurrence, relapse may occur in up to 65% of patients^[141]. Risk factors associated with recurrence include older age (greater than 65), longer hospital stays (greater than 16 d), the presence of comorbidities and another course of antibiotics^[142,143]. The new epidemic strain has been associated with an even higher rate of recurrence; rates may be as high as 47%^[143]. Some authors have postulated that recurrence may be related to inability to mount an adequate antibody response as manifested by low IgG directed against toxin A^[50].

The severity of recurrent episodes of CDI should not be underestimated. Pépin *et al.*^[144] reviewed the outcomes of a first recurrence of CDI with the epidemic strain during the Quebec outbreak^[144]. They noted that 11% of patients with a first recurrence had at least one severe complication of CDI, including shock, colectomy, megacolon, perforation or death within 30 d. Complicated recurrent CDI was strongly associated with three factors: older age, elevated white blood cell count and renal failure. For those patients greater than 65 years of age, 13% developed recurrent CDI that was severe *vs* 7.5% for those 18-64 years of age. Subgroup analysis revealed recurrent CDI with a white blood cell count > 20000 was associated with a 38.9% incidence of complicated CDI *vs* only 10.6% when the white blood cell count was 10000-19000^[144]. The long term negative impact of CDI was also explored by Musher *et al.*^[145], who reviewed outcomes for 103 patients who were considered to be cured without recurrence at 90 d after completion of therapy. They found that 22% of these patients developed recurrent diarrheal disease more than 90 d after the initial episode, 83% of whom were toxin positive.

Clinical approach to recurrent CDI

Most authors have recommended, repeating a course of the antibiotic used in the initial treatment, usually metronidazole, as the first step in the treatment of a recurrence. This sentiment is backed by the 2013 ACG guidelines^[71]. For additional recurrences, a combination of a prolonged taper of the antibiotic with oral vancomycin, followed by pulsed dosing is often used. The original reports of tapered dosing utilized oral vancomycin as the preferred drug, since levels in stool are high, over 1000-fold higher than the level needed to inhibit *C. difficile* and do not decrease as diarrhea resolves^[146]. Early suggested courses were vancomycin 125 mg *qid* for 7 d, tapering to 125 mg *bid* for 7 d, then daily for 7 d^[147]. After the taper has been completed, pulsed dosing can begin. The pulsed dosing of vancomycin is thought to allow time for germination of residual spores during the days off antibiotics, with killing of the vegetative form when the antibiotic is given again. Although there is no standard well studied pulsing regimen, one suggestion has been to give vancomycin 250 mg every 2 or 3 d for 3 wk^[148]. Bartlett has noted that he always utilizes a 6 wk course as this is the approximate time for return of normal flora^[149]. More recently, some authors have recommended continued lengthening of the pulsing interval until the vancomycin is given only once every

10 d^[150]. Rare patients may require chronic pulsed dosing every 3-4 d, relapsing each time they try to lengthen the interval or discontinue the vancomycin. The 2013 ACG guidelines recommend a simplified pulsed dosing only regimen with vancomycin 125 mg orally every 3 d for 10 doses without tapering of the vancomycin (Conditional recommendation, low-quality evidence). For patients with more than 3 recurrences, the ACG guidelines now suggest considering fecal microbiota transplant (FMT)^[71].

A new approach to relapsing CDI using a rifaximin “chaser” has been described. Johnson *et al*^[151] conducted an empirical trial of a 14-d course of rifaximin following a variety of different treatments, mainly using vancomycin, for the treatment of recurrent CDI. The authors studied eight women from their clinical practices, who had suffered from 4-8 episodes of CDI. The patients ranged in age from 43-88 years of age, with six of the eight being greater than 65 years old. The onset of recurrences varied from 1-59 d (mean of 10.5 d) after completion of treatment for CDI. For five of the patients, recurrences were as early as 1 d after treatment ended. The patients had been treated with 79-372 d with a variety of different treatments including metronidazole, vancomycin tapered and/or pulsed, probiotics and vancomycin plus rifaximin. Rifaximin was used as a “chaser” when the patients were asymptomatic, immediately at the end of the vancomycin treatment. Six of the patients received 400 mg *bid* for 14 d. Rifaximin was well tolerated without side effects. Seven of the eight patients had no further recurrence, with follow up that varied from 51-431 d. The one patient who was noted to have a recurrence was immediately retreated while symptomatic (the only deviation from their basic protocol) for 14 d. This patient was noted to develop resistance to rifaximin. More recently, a randomized, double-blind, placebo controlled trial was conducted on the efficacy of the rifaximin “chaser”. Patients completing a standard antibiotic regimen for CDI were assigned to receive either placebo or 400 rifaximin mg 3 times daily for 20 d. Recurrent diarrhea occurred in 49% of placebo patients and 21% of rifaximin patients ($P = 0.018$). Actual CDI recurrence rates, as assessed by positive toxin assay, were 31% (11 of 35) in the placebo group and 15% (5 of 33) in the rifaximin group ($P = 0.11$)^[152]. Although the difference between rifaximin and placebo was not significant, the study was underpowered to exclude a statistically significant difference.

Rifaximin as a stand-alone treatment for recurrent CDI has also been a focus of interest. A retrospective study examining 32 patients with recurrent CDI who had undergone an average of 4.4 antimicrobial treatment courses for CDI, found treatment with 400 mg twice-daily rifaximin for 14 d was successful in preventing relapse in 53% (17 of 32) of cases^[153]. Interestingly, the authors empirically noted the success of rifaximin treatment appeared to be related to the MIC of the particular isolate, and that B1 isolates (30% in the study) had the highest MICs among those tested. There was, however, no statistically significant difference ($P = 0.11$) in relapse rates among those with the B1 strain, 42% (8 of 19) compared

to 53% overall. Among the proposed mechanisms for this increased efficacy in treatment and prevention of recurrent CDI are rifaximin’s anti-inflammatory properties; rifaximin has been shown to induce epithelial cell changes that alter bacterial attachment and internalization, while also reducing the release of inflammatory cytokines^[154]. Lastly, with the increasing prevalence of the B1 strain, clinicians should be aware of the potential for rifaximin resistance given the lack of commercial testing availability. At this point, however, it is difficult to ascertain the clinical impact of these findings, in particular when rifaximin has been noted to achieve such high fecal concentrations. The most recent consensus from the ACG notes that there is no convincing evidence at this point in time for the use of rifampin or rifaximin in the treatment of recurrent CDI^[71].

Fecal Transplantation

Rapidly emerging onto the scene, FMT represents the most promising candidate among non-antibiotic treatment options for patients suffering from multiple relapses or recurrences. Borody *et al*^[155] in an article subtitled “Toying with Human Motions”, reviewed the use of the ultimate natural probiotic, transplanted human stool. Although noted to be “aesthetically unpleasing”, the use of stool transplant from one individual, usually a close relative, to the patient with relapsing CDI has had a high success rate. They reviewed the published literature of the use of fecal transplantation in 84 patients, noting a rapid response without recurrence in 86%. The authors also reviewed the use of stool transplantation for inflammatory bowel disease and irritable bowel syndrome and provide a detailed method for donor screening, preparation and administration.

Since then, the potential impact of FMT in the treatment of recurrent CDI has been more clearly elucidated and now represents a focal point of ongoing research. A systematic review of published studies between 2000-2011 identified 124 patients in seven studies with recurrent or refractory CDI who underwent FMT^[156]. Among these patients, 83% reported immediate improvement following the procedure and further remained diarrhea free for months to years. The results from early studies all varied in protocol for pre-transplant antibiotic use, methods of delivery, amount of material delivered, long-term follow up, and none were controlled trials. Nonetheless, this systematic review of the early studies highlights the potential impact of fecal transplant for the treatment of recurrent or refractory CDI.

Brandt *et al*^[157] in a multicenter long-term follow up study of 77 patients undergoing colonic FMT for recurrent CDI monitored both primary and secondary cure rates for individuals undergoing the procedure. A primary cure was defined as resolution of symptoms without recurrence within 90 d of treatment, while a secondary cure was resolution of symptoms with one further course of vancomycin. Follow up revealed a primary cure rate of 91% and a secondary cure rate of 98%. Of interest, the study addressed, through patient surveys, one of the

major drawbacks to FMT: the fact that the procedure is inherently aesthetically unpleasing. The survey results of these 77 patients revealed that 97% of patients would undergo another FMT for a CDI recurrence and that 53% of the patients would choose FMT as their first treatment option. This represents a promising finding that the unappealing nature of FMT may eventually be overcome by the predictable efficacy of FMT for patients facing the debilitating consequences of multiple CDI recurrences.

One of the most pressing question that has not been fully elucidated about FMT remains, how does it compare with other treatment options? While no double-blind randomized controlled trials have been completed to this date, new evidence has emerged from an interim analysis of an open-label, randomized, controlled trial in the Netherlands^[158]. This study of recurrent CDI infection assigned patients to receive one of three treatments: initial vancomycin regimen (500 mg four times daily for 4 d) followed by bowel lavage and subsequent nasoduodenal infusion of donor feces, standard vancomycin regimen (500 mg four times daily for 14 d) with bowel lavage, or standard vancomycin regimen alone. With a primary endpoint measured as cure without relapse within 10 wk, the overall cure rate with FMT was 94% (15 of 16). Of these 16, 13 achieved cure on their initial treatment, with 2 more achieving cure after treatment with a different donor stool. This was compared to 31% (4 of 13) in the vancomycin alone group and 23% (3 of 13) in the vancomycin and lavage group. Lastly, post-FMT analysis of patient feces showed increased bacterial diversity, similar to that of the healthy donors.

Overall, the current literature suggests a promising future for the application of FMT in the treatment of recurrent CDI, however, some issue still remain, namely, the lack of a consensus protocol and viable sources of the donor feces. The majority of early FMT procedures utilized donor feces from spouses, intimate partners, or close family members, while potentially safer, also possesses many practical challenges in gathering the sample and administration. New evidence suggests that there may be equally efficacious alternatives to these close family donors. Between 2004-2010, a group of 32 patients with relapsing CDI at the Stockholm South General Hospital underwent FMT by either enema or colonoscopy using a fecal transplant suspension reconstituted from a single donor specimen obtained in 1994^[159]. Among the patients, 69% (22 of 32) had a durable cure. These findings suggest that, in the future, it may be possible to establish a donor bank of prescreened individuals or specimens, thereby improving the ease, efficiency and safety of the process. Perhaps even more promising are results from a proof-of-principle study demonstrating that a stool substitute was capable of curing an antibiotic-resistant hypervirulent strain of *C. difficile*, ribotype 078. Researchers in this “RePOOPulating” study extensively cultured a stool sample from a 41-year-old healthy female donor to make a synthetic sample consisting of 33 different purified isolates, which was then used to treat 2 patients who had failed traditional therapy^[160]. Both patients

returned to normal bowel patterns within 2-3 d and remained symptom free at 6 mo. The authors of the study highlight numerous potential benefits of synthetic stool over donor stool including, the ability to control and alter the exact bacterial composition, the ability to replicate the procedure with an identical specimen, increased stability of donor stool sample, improved safety from knowledge of the exact sample composition, and the ability to adjust the sample for antimicrobial sensitivity.

A systematic review and meta-analysis by Kassam *et al*^[161] provides new insight into variation between methods of FMT delivery and from donor type. This review of 11 studies including 273 CDI patients treated with FMT, performed a subgroup analysis comparing lower gastrointestinal delivery with upper gastrointestinal delivery. Lower gastrointestinal delivery (colonoscopy or enema) had clinical resolution rates of 91.4% (203/222) compared to upper gastrointestinal delivery (nasogastric/nasojunal tube and gastroscopy) resolution rates of 82.3% (42/51). Further comparison between anonymous *vs* patient selected donors did not reveal a significant difference in clinical outcomes regardless of the follow-up time.

In April 2013, the United States FDA determined that FMT is a biologic product and drug that is regulated by the FDA. The FDA ruled that an investigational new drug (IND) application, a cumbersome and time consuming process, was needed for the use of FMT for any indication. In response to vocal and unified opposition by the gastrointestinal specialty societies, the FDA rapidly reversed this requirement and provided that the “treating physician obtains adequate informed consent from the patient or his or her legally authorized representative for the use of FMT products. Informed consent should include at a minimum, a statement that the use of FMT products to treat *C. difficile* is investigational and a discussion of its potential risks”^[162].

In conclusion, as more evidence continues to become available, fecal transplantation is becoming an increasingly viable option for the treatment of recurrent or relapsing CDI, in particular given the recent recommendation for FMT to treat 3 CDI recurrences in the 2013 ACG guidelines^[71]. While there remains no optimal protocol for administration or consensus on the ideal source of the transplant sample, future studies, including an NIH-funded blinded RCT and the pending FDA IND process, may provide valuable insight for these questions.

ADJUNCTIVE THERAPY: INTRAVENOUS IMMUNOGLOBULIN AND ANIONIC BINDING RESINS

There has been significant interest in the use of intravenous immunoglobulin (IVIG) to treat severe refractory and recurrent CDI. This interest is based upon the fact that development of *C. difficile* antitoxin antibody has been associated with protection from the development of CDI after colonization with *C. difficile*^[50,163]. Small se-

ries and case reports have suggested a possible response to IVIG^[164,165]. Of note, all immunoglobulin lots tested contained IgG against toxins A and B and were capable of neutralizing cytotoxicity in one series^[166]. However, with the cost of IVIG approaching \$10000 for an individual treatment course, proof of efficacy is important. McPherson conducted a retrospective review of 14 patients with either severe, refractory or recurrent CDI^[167]. They used an IVIG dose of 150-400 mg/kg. Nine of these 14 patients responded in a median of 10 d, a relatively slow response, and 3 of these 9 patients had recurrent CDI after initial resolution. The most instructive study on the use of IVIG for severe CDI was conducted by Juang *et al*^[168] at the University of Pittsburgh Medical Center. Because of the severity of CDI at their institution, a committee developed eligibility criteria for IVIG which was then used in a prospective manner to choose patients eligible for IVIG. Eighteen patients received IVIG at a dose of 200-300 mg/kg and these patients were pair matched by propensity scoring with other patients with severe CDI. There was no difference in mortality (3 patients in each group), colectomy (3 patients in each group) or length of stay. Although this study is not definitive, the results do not support the use of IVIG for severe CDI. The 2013 ACG guidelines addressed the use of IVIG in the treatment of recurrent CDI, and concluded that it does not have a role as sole therapy; however, they noted that it may be helpful in patients with hypogammaglobulinemia. This recommendation is based on the predisposition for CDI in patients following solid organ transplantation.

Anion binding resins, like cholestyramine and colestipol, have been used to treat CDI. The non-absorbable resin binds to *C. difficile* toxin removing 99% of the cytotoxic activity^[169]. However, concerns have been raised about the use of these toxin-binding agents, because they also bind to vancomycin^[170]. Thus, combination therapy should be used carefully, if at all, with separation of the anion binding resin and vancomycin by at least 2-3 h. Other sources have recommended giving the vancomycin either 1 h before or 4-6 h after the cholestyramine dose^[171].

PREVENTATIVE THERAPY

One of the most important issues related to CDI from the perspective of the practicing clinician is the approach to the patient with a known history of *C. difficile*, who requires a subsequent course of antibiotics for an infection such as urinary tract infection or pneumonia or who cannot stop the antibiotics which induced the original episode of CDI. The use of metronidazole or vancomycin in this setting can be referred to as preventative therapy. Unfortunately, there is no data from systematic studies of the use of preventative therapy. However, Miller noted that “on the basis of no prospective evidence but, often, a large body of clinical experience, some clinicians now start a parallel course of oral metronidazole or vancomycin along with treatment with the

potentially CDI-inducing antimicrobial, to prevent the appearance of symptomatic CDI”^[172]. He goes on further to note “that despite absence of guidelines for this approach, there is remarkable homogeneity in the approaches used by most clinicians, in that clinicians who practice this prophylactic strategy use oral metronidazole or vancomycin during the entire course of antimicrobial therapy and for an additional 7 d after the end of the administration period”. This preventative approach to *C. difficile* seems an intuitively reasonable approach, which can be utilized pending results of future clinical trials that would validate its effectiveness.

Probiotics

Probiotics, defined by the World Health Organization as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host,” have seen a recent surge in interest and use^[173]. Current estimates for sales of probiotics, as both supplements and foods, was estimated to be \$770 million in the US alone for 2012, with worldwide sales at \$2.25 billion, a 79% increase since 2010^[174,175]. Further, recent estimates have projected worldwide spending on probiotics to reach \$4 billion annually by 2016^[176]. Despite all the interest and sales of probiotics, their utilization for the prevention or treatment of CDI remains controversial and unproven.

Heavy marketing campaigns and choice labeling of products have helped fuel the dramatic growth of the probiotics markets. Further helping to shape the consumer image, clinical evidence for the use of probiotics in the prevention of antibiotic-associated diarrhea (AAD) appears promising. Systematic reviews and meta-analyses were completed for the use of probiotics in any AAD. In one analysis, the pooled results from 63 randomized control trials revealed a RR of 0.58 (95%CI: 0.50-0.68, $P < 0.001$) with an number needed to treat (NNT) of 13^[177]. Among those studies, a subset of 14 were randomized controlled trials for the prevention of CDI and pooled analysis revealed a RR of 0.29 (95%CI: 0.17-0.48, $P < 0.001$) with an NNT of 25. However, it was noted that poor adherence and limited reporting of the number of samples tested may have skewed the results. Another meta-analysis of 34 studies including 4138 patients showed a 0.53 relative risk for the development of antibiotic-associated diarrhea in the probiotics *vs* the placebo group (95%CI: 0.44-0.63), with an NNT of 8^[178]. Importantly, the authors of this study chose to omit any trials involving the use of probiotics for the prevention or treatment of CDI.

Although some may wonder why a variant of baker's yeast, which is not a part of the normal microflora of the gut, would be effective in preventing or treating CDI, there is some theoretical support for the use of *Saccharomyces boulardii*, which has been shown to prevent toxin A binding and also to inactivate toxins A and B by proteolytic digestion^[179,180]. Further, in the hamster model, *S. boulardii* has been shown to be effective in preventing deaths from acute disease^[181]. Other mechanisms

by which *Saccharomyces* may prevent CDI include inhibition of *C. difficile* adhesion, cellular protection from histologic damage and inhibition of pro-inflammatory cytokine gene expression^[182-184]. In fact, Czerucka used the term “immunobiotic” to describe *S. boulardii*^[182].

The clinical efficacy of *S. boulardii* has shown mixed results in a number of reviews and meta-analyses of randomized, controlled trials of CDI. Dendukuri *et al*^[185] concluded that the “studies conducted to date provide insufficient evidence for the routine clinical use of probiotics to prevent or treat CAD”. Szajewska *et al*^[186] found a reduction in antibiotic associated diarrhea of 57%, but no reduction in CDI. Katz in reviewing the use of probiotics for the prevention of CDI developed a proposed guideline which noted no evidence to support efficacy in the primary prevention of *C. difficile*, but suggested that “*S. boulardii* can be used to decrease recurrences of *C. difficile*”^[187]. McFarland *et al*^[188] found that *S. boulardii* was not effective in preventing recurrence after an initial episode of CDI. The authors did find, however, a 50% reduction among patients who had had a previous recurrence. A second study of the use of *S. boulardii* in patients with recurrent CDI confirmed a decrease in recurrences, but only when combined with a high dose of oral Vancomycin (500 mg *qid*). There was no reduction in recurrent CDI with lower doses of vancomycin or metronidazole^[189]. McFarland later conducted a meta-analysis and noted that “from six randomized trials, probiotics had significant efficacy for CDD”^[190]. Unfortunately, he combined the 2 studies using *S. boulardii* with studies using a variety of *Lactobacillus* preparations, which could lead to significant misinterpretation of the data. As noted by Gerding; “A recent meta-analysis suggested that probiotics are effective; nevertheless, because of the heterogeneity of study methods and patient populations, it is not scientifically possible to conduct a meta-analysis of findings in the probiotic literature”^[191].

Another aspect of *S. boulardii* in the treatment or prevention of CDI is the risk for adverse events. While generally considered safe, there have been increasing reports of fungemia due to *S. boulardii*, especially in those with intravascular catheters and antibiotic therapy. Of the 37 patients with *S. boulardii* fungemia in one report, use of *S. boulardii* as a probiotic was considered to be the source of infection in 64%^[192]. Of note, an additional five cases were reported in patients who were not receiving a probiotic. In these cases, there was evidence of healthcare associated acquisition from other patients who were being treated with *S. boulardii*. The authors suggested that special caution should be taken with probiotics in critically ill and immunocompromised patients. Segarra-Newnham in a review of the use of probiotics for CDI concluded that “there were numerous unanswered questions”^[193]. She also noted that “given the potential for complications in debilitated immunosuppressed patients, the risk may outweigh the benefits”. Czerucka went even further suggesting “the presence of indwelling catheters is a contraindication for the administration of *S. boulardii*”^[182]. Further evidence that the safety of probiotics cannot

be assumed comes from a recent double blind, placebo controlled trial of a multispecies probiotic (mostly *Lactobacillus* sp. and *Bifidobacterium* sp.) in the treatment of severe acute pancreatitis^[194]. In the probiotic group, 16% of patients died *vs* 6% in the placebo group. Nine patients (8 with fatal outcomes) developed bowel ischemia. Eight involved the small bowel. The authors concluded “probiotics can no longer be considered to be harmless adjuncts to enteral alimentation, especially in critically ill patients”.

While the early literature focused primarily on the application of *S. boulardii* for prevention and treatment of CDI, more recently there has been a shift towards to the use of *Lactobacillus* sp. preparations, such as Lactinex (Becton Dickinson, San Diego, Ca) or *Lactobacillus* GG (Culturelle, Bloomfield, Ct.). Early support for *Lactobacillus* came from a randomized, double-blind, placebo controlled trial, published by Hickson *et al*^[195] reporting the use of Actimel (Danone, France) in the prevention of CDI. In the United States, a similar product would be DanActive by Dannon. No patients in the probiotic group developed CDI, while 17% (9 of 53) in the placebo group developed CDI ($P = 0.001$). The authors concluded that “this has the potential to decrease morbidity, health care cost, and mortality if used routinely in patients aged over 50”. Unfortunately, the article by Hickson *et al*^[195] adds little substantive new data to the argument, because of its very poor generalizability. The extraordinarily high exclusion rate resulted in only 6.4% of screened patients being evaluable in the efficacy analysis. Of the 1760 patients assessed for eligibility, 1625 (92%) were excluded and a further 148 refused to participate, leaving only 135 patients to be entered in the study. Of these, 16% were lost to follow up, leaving only 6.4% of the patients eligible for analysis. As noted in a Letter to the Editor “I was astounded to read in the study method that Hickson *et al*^[195] had excluded high risk antibiotics (as well as some misclassified low risk antibiotics). To do so is akin to performing a trial of an agent that claims to prevent type 2 diabetes, but excluding obese patients”^[196].

Since that time a significant number of trials have been conducted with varying levels of support for probiotics. A 2008 Cochrane Review of the use of probiotics in the treatment of CDI in adults identified 4 randomized control trials meeting inclusion criteria, all of which were noted to be small in size and have methodological problems^[197]. Of these studies, only one was found to have a statistically significant benefit for probiotics, the previously mentioned study by MacFarland *et al* on *S. boulardii*. The most promising evidence to date for probiotics comes from a systematic review and meta-analysis involving pooled data from 20 studies and 3818 patients, which revealed a pooled RR of 0.34 (95%CI: 0.24-0.49), in other words a reduction in the incidence of CDI of 66%^[198]. Calculating the optimal information size, which is the number of patients required for an adequately powered study, using the worst-plausible-assumption and applying a 5% population incidence of antibiotic-associated CDI, the authors suggest this moderate-quality

evidence predicts probiotics prophylaxis would prevent 33 episodes of CDI per 1000 persons. Additionally, while their study indicated a larger risk reduction in the use of multiple species preparation over single species, this was likely accounted for by heterogeneity between studies.

The newest evidence surrounding the use of probiotics for prevention of CDI comes from the PLACIDE trial, a multi-center, randomized, double-blind, placebo controlled trial for the use of lactobacilli and bifidobacteria in the prevention of AAD and CDD, for which inpatients over the age of 65 were randomized to either a microbial preparation or placebo. Relative risks between the groups were RR 1.04 for AAD (95%CI: 0.84-1.28) and RR 0.71 for CDD (95%CI: 0.34-1.57)^[199]. The authors concluded no evidence that multi strain preparation of lactobacilli and bifidobacteria was effective in the prevention of AAD or CDD.

Overall, interpretation of results from probiotic studies present many challenges. Lawrence, who conducted a study of a *Lactobacillus* preparation to prevent recurrent CDI noted that a number of problems were faced in attempting to determine the efficacy of probiotics^[200]. He noted the high percentage of patients receiving systematic antibiotics (66.7%) and a high number of patients receiving gastric acid suppression, both of which might interfere with the efficacy of a probiotic. Other problems with studies of probiotics for the prevention of recurrences of CDI, include the lack of randomization of the type or dose of the antibiotic used with the probiotics, which may have altered the outcomes. Doses of probiotics were not standardized and may have been too small or the preparations may have become nonviable after manufacture or may have a different strain than advertised. A number of authors have found that the number of colony forming units can be much lower than what is advertised on the label^[201]. The 2013 ACG guidelines concluded that there is insufficient evidence that probiotics prevent CDI (Strong recommendation, low quality evidence)^[71]. In summary, there is much more enthusiasm than data for the use of probiotics in the prevention or treatment of CDI.

The newest approach in the prevention of CDI focuses on targeting the infective spore to prevent germination into the vegetative toxin producing form. Since only the vegetative form produces toxin, theoretically prevention of spore germination would prevent symptomatic infection. Howerton *et al*^[202] demonstrated that a cholate meta-benzene sulfonic derivative (CamSA) is a strong competitive inhibitor of taurocholate-mediated *C. difficile* spore germination. Subsequently, they administered a single 50 mg/kg dose of CamSA to mice infected with *C. difficile* spores and were able to prevent any signs of CDI^[203]. The authors also noted that CamSA gave complete protection against an “unnaturally massive” *C. difficile* spore infection, equivalent to human ingestion of hundreds of grams of infective spores. While still early in the investigative process, CamSA represents an entirely new approach to preventing CDI.

FUTURE TREATMENT OPTIONS FOR CDI

With the increased virulence and decreased response to standard treatment, combined with an increase in recurrences, both due to relapse and acquisition of epidemic strains in hospitals, the need for newer approaches to the treatment of CDI becomes even more important. There are a number of exciting new antibiotics being studied for treatment of CDI, including rifalazil, ramoplanin and non-antibiotic based approaches, such as tolevamer, monoclonal antibodies against toxin A, and a vaccine.

Rifalazil

Rifalazil is an experimental, absorbable antibiotic in the rifamycin class, related to rifampin with a broad spectrum of activity against a wide range of organisms, including *Mycobacterium tuberculosis*, *Chlamydia*, *Helicobacter pylori* and *C. difficile*^[204]. Testing of rifalazil against 110 isolates of *C. difficile* collected from 1983-2004 revealed excellent activity with an MIC 90 of 0.03 µg/mL, with only one isolate from the United States found to be resistant^[82]. In a study of *C. difficile* in the hamster model, all animals treated with rifalazil or vancomycin were protected from disease. Histologically, the rifalazil treated animals had less edema and neutrophil infiltration than the vancomycin treated animals. When vancomycin was discontinued, 65% of the animals developed disease, while none of the rifalazil treated animals had positive toxin assays or disease^[202]. Future trials of rifalazil in humans with CDI are eagerly anticipated, especially given the low relapse rate in animal models.

Ramoplanin

Ramoplanin is an experimental broad spectrum, non-absorbable glycolipodepsipeptide. In the same study mentioned above, all isolates of *C. difficile* were sensitive to ramoplanin with an MIC 90 of 0.5 µg/mL^[204]. In another study, which included *C. difficile* isolates with reduced susceptibility to vancomycin and resistance to metronidazole, no resistance was found to ramoplanin^[205]. In a hamster model of CDI, both ramoplanin and vancomycin were uniformly effective in resolution of symptoms^[96]. In the vancomycin group, 100% of animals had spores detected *vs* only 30% treated with ramoplanin after 2 d of treatment. Ramoplanin was noted to have a profound effect on both the vegetative and spore forms of *C. difficile* with complete eradication of both forms of the organism by 24 h. Vancomycin, on the other hand, had no effect on spores. The authors hypothesized that the efficacy against spores may be related to the binding of lipid II. A related antibiotic, nisin, which has been used as a food preservative for decades, had been noted to inhibit transformation from spore to the vegetative form in *Bacillus* and other *Clostridial* species^[206].

REP3123

REP3123 is a novel inhibitor of methionyl tRNA synthetase, which is required for bacterial growth. REP3123 inhibits toxin formation, is active in animal models,

prevents death of human cells exposed to *C. difficile* toxin and decreases spore formation. REP3123 has shown activity against 108 different *C. difficile* isolates, including the B1 strain, with an affinity for bacterial MetRS over 1000 times that of human mitochondrial or cytoplasmic MetRS^[207]. In addition, REP3123 is highly selective for gram positive bacteria which may spare much of the normal colonic flora^[208]. Clinical trials are eagerly awaited.

Tolevamer

Tolevamer (Genzyme Corp. Cambridge, MA) is a high molecular mass, non-absorbable polymer that has been shown to be a potent neutralizer of *C. difficile* toxins A and B, with each polymer molecule irreversibly binding 3-4 toxin molecules^[209]. A proposed advantage of non-antibiotic approaches for the treatment of CDI is the fact that there is no disturbance of the normal intestinal flora, potentially decreasing the risk of recurrent disease. Louie, et al. reported a randomized, double blind trial of tolevamer in patients with mild to moderate CDI^[210]. The patients were randomized to 3 or 6 g of tolevamer for 14 d or vancomycin 125 mg *qid*. If the 6 patients who had recurrence of diarrhea while still on treatment with tolevamer (4 in the 3 g/d group and 2 in the 6 g/d group) are included in the efficacy analysis, resolution of diarrhea was found in 60% of the tolevamer 3 g group, 79% in the 6 g group and 91% with vancomycin. Recurrence rates were 10% in the tolevamer 6 g group *vs* 19% in the vancomycin group. The major side effect of tolevamer was noted be hypokalemia, found in 23% of those in the 6 g group *vs* 7% of those treated with vancomycin. Because tolevamer is an anionic polymer capable of binding cations in colonic fluid, the hypokalemia is not surprising. Addressing this issue, the next study on tolevamer utilized a modified product, which is liquid with potassium added, to allow net-neutral potassium balance. This randomized Phase I trial tested tolevamer at 6, 9, 12 and 15 g/d, normal potassium was maintained with the new product and researchers reported that tolevamer was generally safe and well tolerated in patients at doses up to 15 g/d^[211].

Despite its demonstrated safety with the reformulated drug, two subsequent studies challenged the efficacy of tolevamer for the treatment of CDI. The first was a Phase III randomized trial of 544 patients on either tolevamer (3 g, 3 times a day for 14 d), vancomycin (125 mg, 4 times a day for 10 d), or metronidazole (375 mg, 4 times a day for 10 d)^[212]. Of the 278 patients on tolevamer only 42% achieved clinical success, thereby failing to demonstrate noninferiority to the 73% success rate of vancomycin. One interesting finding, however, was the patients on Tolevamer had a decreased rate of recurrence (6%) when compared to the vancomycin group (18%; $P = 0.009$) and metronidazole group (19%; $P = 0.006$). The authors attributed the decreased rate of recurrence to the flora-sparing activity of tolevamer. As a follow-up to the findings of this Phase III trial, researchers in the UK studied the neutralizing effects of tolevamer on the

C. difficile cytotoxins in an *in vitro* human gut model^[213]. In contrast to previous studies, these researchers found that tolevamer was not associated with loss of the *C. difficile* cytotoxic effect. These results support and may explain the poor results for the primary endpoint in the previously described Phase III trial.

Monoclonal antibodies

The proposed mechanism behind the use of monoclonal antibodies (MAbs) in CDI is the potential ability to directly modulate the effects of *C. difficile* cytotoxins A and B. In animal models, MAbs have been shown to reduce the severity and duration of diarrhea, death rate, and rate of recurrence^[214]. Literature concerning the administration of a single MAb against either toxin A or toxin B seems to be conflicting; one early study reports that a MAb against toxin A was sufficient to protect from death, while a MAb against toxin B had no effect^[215]. In contrast, it was more recently suggested that the MAb against toxin B was protective against CDI^[216]. Given these conflicting reports, clinical application of MAb therapy appears to be adopting a dual administration of MAbs for both toxin A and toxin B. A randomized, double-blind Phase II placebo controlled trial of MAbs against toxin A (CDA1) and toxin B (CDB1) was able to demonstrate a lower recurrence rate with the administration of a single infusion of 10 mg/kg of MAb compared to placebo in patients also receiving either metronidazole or vancomycin^[217]. Overall, recurrence rates were 7% for the MAb group *vs* 25% for the placebo group (95%CI: 7-29, $P < 0.001$), while for patients with more than one previous episode of CDI the recurrence rates were 7% for the MAb group compared to 38% for the placebo group ($P = 0.006$).

Many questions remain about the application of MAb therapy in the treatment of CDI. Concern has been raised that MAb therapy does not decrease the severity of diarrhea, duration of hospitalization, or time to resolution^[218]. Additionally, the clinical applications of the current studies may not be appropriate given differences in course of illness between different patient populations, in particular the elderly^[219]. Some of these questions may be answered by two Phase III trials currently underway^[220,221]. Also likely to emerge in the future is the application of new MAbs that specifically bind to epitopes in the neutralizing regions of toxins A and B. These MAbs, known as PA-50 and PA-41, were shown to confer a dramatically increased survival rate in a hamster model, where the administration of a dual PA-50/PA-41 MAb revealed long term survival rate of 95% *vs* 0% for placebo^[222].

Vaccine

Interest in a vaccine is based upon the fact that development of *C. difficile* antitoxin antibody has been associated with protection from the development of CDI after colonization with *C. difficile*. A vaccine against toxins A and B, that has been efficacious in animal models as

well as humans, and demonstrated successful prevention of recurrence in 3 case reports^[223]. More recently, six Phase I trials on 200 individuals have been completed by Sanofi Pasteur with a bivalent formalin-inactivated vaccines against toxins A and B showing seroconversion of 75% of participants by day 70^[224]. A Phase II trial of this vaccine, currently underway in the US, is being conducted to assess primary CDI prevention in 650 at risk adults^[225]. Also in development is a chimeric antitoxin vaccine using an endotoxin free expression system from *Bacillus metaerium*, which was capable of producing neutralizing antitoxins and preventing spore-induced relapse in CDI^[226].

CONCLUSION

The impact of CDI infection is significant. This infection places a tremendously onerous burden on the health care system worldwide and has major adverse clinical and economic impact. This topic will be a continued high priority for national guidelines and clinicians will need to pay close attention to any forthcoming revisions for diagnosis and management. Presently, best practice recommendations would be as follows: (1) only patients with diarrhea (a stool that takes the shape of the container) should be tested for CDI; (2) initial testing should be done with glutamate dehydrogenase or nucleic acid amplification test for CDI, without repeat testing unless high suspicion for infection and initial GDH testing is done; (3) patients with resolution of diarrhea should not be rested to document cure of CDI; (4) initial antibiotic treatment for patients with mild/moderate CDI infection should be metronidazole 500 mg *tid* orally (provided no drug allergy contraindication); (5) initial treatment for severe CDI or failure to respond to 5-7 d of metronidazole should be vancomycin 125 mg *qid* orally. If severe or complicated CDI, intravenous metronidazole 500 mg *tid* should be added; (6) in patients with severe ileus or complicated CDI, best antibiotic plan is intravenous metronidazole 500 mg *tid* plus vancomycin 500 mg *qid* (oral) plus vancomycin 500 mg in 500 cc fluid *qid* (rectal by retention enema); (7) use of intravenous formulation compounded by pharmacy into oral solution offers significant cost advantage; (8) the first recurrence of CDI can be treated with the initial regimen if it induced appropriate clinical response; (9) the second recurrence of CDI should be treated with pulsed vancomycin; (10) the third recurrence or unresponsive severe CDI, fecal microbiota transplant should be considered; (11) current data suggests limited if any value, of probiotics for CDI treatment or prevention of relapse. The use of these agents in patients with central venous catheters should be avoided given possible infectious complications; and (12) high level disinfection of environmental surfaces for bathroom and if inpatient, contact surfaces is recommended. We routinely have patients discard toothbrush and change any device or implement that may allow oral contact ingestion of aerosolized spores in patients with CDI.

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Role of chemoprophylaxis with either NSAIDs or statins in patients with Barrett's esophagus

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Abstract

The incidence of esophageal adenocarcinoma, a poor prognosis neoplasia, has risen dramatically in recent decades. Barrett's esophagus represents the best-known risk factor for esophageal adenocarcinoma development. Non-steroidal anti-inflammatory drugs through cyclooxygenase-2 inhibition and prostaglandin metabolism regulation could control cell proliferation, increase cell apoptosis and regulate the expression of growth and angiogenic factors. Statins can achieve equivalent effects through prenylation and subsequently control of cellular signaling cascades. At present, epidemiological studies are small and underpowered. Their data could not justify either medication as a chemo-preventive agent. Population based studies have shown a 43% reduction of the odds of developing an esophageal adenocarcinoma, leaving out or stating a 25% reduction in patients consuming non-aspirin nonsteroidal anti-inflammatory drugs and a 50% reduction in those patients consuming aspirin. They have also stated a 19% reduction of esophageal cancer incidence when statins have been used. Observational studies have shown that non-steroidal anti-inflammatory drugs could reduce the

adenocarcinoma incidence in patients with Barrett's esophagus by 41%, while statins could reduce the risk by 43%. The cancer preventive effect has been enhanced in those patients taking a combination of non-steroidal anti-inflammatory drugs and statins (a 74% decrease). Observational data are equivocal concerning the efficacy of non-steroidal anti-inflammatory drug subclasses. Non-steroidal anti-inflammatory drugs clearly have substantial potential for toxicity, while statins are rather safe drugs. In conclusion, both non-steroidal anti-inflammatory drugs and statins are promising chemopreventive agents and deserve further exploration with interventional studies. In the meanwhile, their use is justified only in patients with cardiovascular disease.

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Key words: Esophageal adenocarcinoma; Barrett's esophagus; Non-steroidal anti-inflammatory drugs; Aspirin; Statins; Cancer chemoprevention

Core tip: Esophageal adenocarcinoma remains a major burden upon health. Experimental studies have suggested that non-steroidal anti-inflammatory drugs and statins may have useful actions against esophageal cancer cells. This review of observational studies shows that non-steroidal anti-inflammatory drugs reduced adenocarcinoma incidence in patients with Barrett's esophagus by 41%, while statins reduced the risk by 43%. The cancer preventive effect is enhanced in those patients taking a combination of non-steroidal anti-inflammatory drugs and statins (a 74% decrease). Non-steroidal anti-inflammatory drugs clearly have substantial potential for toxicity, while statins are rather safe drugs. Their combination offers promise for chemoprevention and further interventional studies are warranted.

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with either NSAIDs or statins in patients with Barrett's esophagus. *World J Gastrointest Pharmacol Ther* 2014; 5(1): 27-39 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v5/i1/27.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v5.i1.27>

INTRODUCTION

A rapid increase in incidence and mortality from esophageal adenocarcinoma (EAC) has been observed over the past four decades in the Western world^[1,2]. Although the absolute incidence of EAC varies dramatically by gender and race, few demographic groups have been spared from the increases^[3]. Moreover, survival of persons with EAC remains abysmal, with most succumbing to the disease within a year^[4], while the 5-year survival rate is less than 15%^[5].

Barrett's esophagus (BE), replacement of the squamous esophageal mucosa by metaplastic columnar epithelium due to prolonged reflux^[6] of the gastric content into the esophagus, represents the best-known risk factor for EAC development^[7]. The annual incidence of EAC development in patients with BE is 0.2%-0.5%^[8,9].

Clinical and demographic factors that have shown some promise in being predictive of malignant transformation in BE are male gender^[10,11], increasing age^[11], length of Barrett's segment^[12-14], duration of BE^[13] and size of hiatal hernia^[14]. There is little evidence to suggest that total alcohol consumption or specific alcoholic beverages modifies the risk of EAC in the general population^[15,16], while smoking^[16-19] and obesity^[16,20] raise the risk for neoplastic progression.

According to the most popular theory, carcinogenesis in BE patients is completed in three stages. During the first, a distinct stem cell population develops in the bone marrow of genetically predisposed patients with gastroesophageal reflux disease. Those cells migrate during the second stage to the gastroesophageal junction and lower esophagus, producing a macroscopically visible BE. The inflammatory milieu in the lower esophagus produces the driving force for stem cell migration. Repeat call for repair in the hostile environment of lower esophagus in BE patients leads to increase cell proliferation and frequent mutations. As noxious mutations sum up by a multistep process, metaplastic epithelium evolves into low-grade dysplasia, high-grade dysplasia, early EAC and ultimately invasive cancer^[21].

Although cancer surveillance is performed in most institutions, once diagnosis of BE is rendered, the true cost-benefit ratio of this endeavour is still essentially unknown^[22]. Surveillance does not interfere with the neoplastic process and could not affect the pre-neoplastic stem cell population generated in the bone marrow. Thus, there is a quest for global and more interventional strategies. Chemoprevention is attractive, especially for the high-risk group of BE individuals, since it can affect the neoplastic process from its early beginning. Moreover, because it could be effective even under insufficient gas-

tric acid suppression^[23], it may be superior to BE ablative techniques that presuppose adequate acid suppression to prevent BE recurrence^[24] and may prove too expensive^[25]. Finally, since BE surveillance cost-effectiveness has been undermined by recent data suggesting a low risk of malignant transformation^[26,27], chemoprevention seems to represent an attractive alternative^[28]. At present, there are no proven chemo-preventive agents, although non-steroidal anti-inflammatory drugs (NSAIDs) and statins appear to offer the most attractive combination of risks and benefits.

This review is to assess current experimental and epidemiological data that NSAIDs and statins could reduce the risk of developing EAC in BE. Moreover, we aim to clarify how existing findings could be included in the EAC etiological models, as well as any side effects, that would follow clinical application of NSAIDs and statins for cancer prevention.

NSAIDS AND EAC CHEMOPREVENTION

Numerous *in vitro* and animal studies support the possible chemo-preventive effect of cyclooxygenase-2 (COX-2) inhibition in BE. COX-2 inhibitors, either drugs or naturally occurring in plant foods, could produce significant suppression of cell proliferation and induce cell cycle arrest in cultured EAC cells^[29]. Selective COX-2 inhibitors have a similar effect in cell cultures from endoscopic biopsies taken from BE patients^[30]. Adding COX-2 inhibitors in rat diet after esophagojejunostomy had reduced progression to EAC^[31,32] in some studies, while indomethacin, but not selective COX-2 inhibitors, produced a similar effect in others^[33].

Several case control studies comparing EAC patients to healthy controls have shown that NSAID use can effectively prevent EAC. A meta-analysis of all human studies published prior to 2003, showed an overall 43% reduction of the odds of developing an EAC in NSAID takers, comprising a 25% reduction in patients consuming non-aspirin NSAIDs and 50% reduction in aspirin users^[34], but the analysis included only one small case-control study comparing BE and EAC patients^[35] and no prospective study. Thus, it cannot differentiate whether any beneficial effect of NSAID use is produced before or after BE appearance. In 2009, a questionnaire based study that included approximately 300000 members of the American Association of Retired Persons found no significant association between EAC and the use of aspirin or non-aspirin NSAIDs^[36].

Since 2003, several observational studies comparing BE and EAC patients have been published (Table 1). These were either case-control retrospective^[23,37,38] or cohort studies^[39-43]. Prospective chemoprevention trials are underway to evaluate the efficacy of aspirin and NSAIDs. In the United Kingdom, the AsPECT trial is currently evaluating the combination of high-dose proton pump inhibitors and aspirin in minimizing the risk of progression to cancer in 9000 BE sufferers^[44]. A similar

Table 1 Available epidemiological evidence of benefit of non-steroidal anti-inflammatory drugs and aspirin in prevention of esophageal adenocarcinoma in patients with Barrett's esophagus

Ref.	Type of study	Size-follow-up	Effect on EAC rate	Beneficial effect
Abnet <i>et al</i> ^[36]	Population based	31115 AARP members	Aspirin OR = 1.1 (0.78-1.57) Non-aspirin NSAIDs OR = 0.90 (0.55-1.43)	None
Tsibouris <i>et al</i> ^[23]	Case-control	BE: 382 EAC: 114	Daily use of non-aspirin NSAIDs OR = 0.30 (0.10-0.91) Daily use of low-dose aspirin OR = 1.21 (0.52-2.83)	Non-aspirin NSAIDs
Beales <i>et al</i> ^[37]	Case-control	BE: 170 EAC: 85	Statins + aspirin OR = 0.31 (0.04-0.69)	Statins + aspirin
Nguyen <i>et al</i> ^[38]	Case-control	BE: 696 EAC: 116	All NSAIDs OR = 0.64 (0.42-0.97)	All NSAIDs
Vaughan <i>et al</i> ^[39]	Cohort	BE: 350 1731 PY	All NSAIDs OR = 0.20 (0.10-0.41)	All NSAIDs
Kastelein <i>et al</i> ^[40]	Cohort	BE: 570 4.5years	Non-aspirin NSAIDs OR = 0.50 (0.26-0.97) Aspirin OR = 0.67 (0.31-1.46)	Non-aspirin NSAIDs
Nguyen <i>et al</i> ^[41]	Cohort	BE: 344 2620 PY	All NSAIDs OR = 0.51 (0.25-1.04)	None
Gatenby <i>et al</i> ^[42]	Cohort	BE: 650 3683 PY	Non-aspirin NSAIDs OR = 0.90 (0.34-2.37) Aspirin OR = 0.72 (0.41-1.31)	None
Kantor <i>et al</i> ^[43]	Cohort	BE: 411 2805 PY	All NSAIDs OR = 0.46 (0.34-1.10)	None

PY: Patient years; EAC: Esophageal adenocarcinoma; BE: Barrett's esophagus; NSAIDs: Non-steroidal anti-inflammatory drugs.

prospective study is running in the United States^[45]. In the only prospective interventional study published today, Heath *et al*^[46] randomized 100 patients who had either low or high-grade dysplasia and BE to receive either a COX-2 selective NSAID (celecoxib) or placebo. After 48 wk of treatment, there was no significant difference between the 2 groups in the proportion of esophageal biopsy specimens showing dysplasia or cancer^[46]. This study has limitations (*e.g.*, the use of dysplasia as the primary outcome, the use of a low dose of celecoxib) that prevent definite conclusions on the utility of NSAID chemoprevention.

Although all case control studies have shown that

NSAID use is beneficial, there is considerable diversity concerning NSAID subclasses that could reduce EAC risk. Our case control study has shown that daily use of non-aspirin NSAIDs was beneficial; while a daily low dose, as well as infrequent use of either aspirin or non-aspirin NSAIDs, was not^[23]. Beales *et al*^[37] found that statin and aspirin combination reduced incidence of EAC and Nguyen *et al*^[38] that all NSAIDs are beneficial, without a separate report of NSAID subclasses.

Cohort study results are more diverse. Vaughan *et al*^[39] found that, comparing current NSAID users to those who never used, NSAIDs had a significantly decreased risk of EAC. Kantor *et al*^[43] reported that non-aspirin NSAID use reduced the risk of neoplastic progression but not aspirin use. The other 3 cohort studies were negative^[41-43], although Kantor found that NSAID use was beneficial only for patients with high-grade dysplasia. A pooled analysis of 6 population-based studies within the Barrett's and Esophageal Adenocarcinoma Consortium have shown that daily NSAID use can reduce the risk of developing EAC by more than 40% (OR = 0.56, 95%CI: 0.43-0.73, $P < 0.001$)^[47]. A meta-analysis of all published observational studies calculated the pooled effect size for COX-inhibitors to 0.59 (95%CI: 0.45-0.77) with minimal heterogeneity^[48].

Many of the observational studies have inherent limitations because not all confounding variables (such as socioeconomic status, tobacco and alcohol use, *H. pylori* status, dietary intake) have been taken into account, especially in case-control studies. Use of aspirin and/or NSAIDs may have been associated with certain patient-led behaviors that have an influence on risk. Such behaviors may include vitamin supplementation^[49] and dietary habits. Furthermore, patients on aspirin may indeed have been more health conscious and might have been more likely to have their cancers detected than others. Finally, it is likely that those with upper gastrointestinal symptoms such as heartburn and regurgitation, risk factors for EAC, are less likely to have been prescribed NSAIDs or aspirin. The use of acid-reducing agents with the sole aim of reducing BE has not been proven in a long-term controlled trial^[45]. Although most studies suggest a synergy between sufficient acid suppression and NSAIDs chemopreventive effect^[38,40], we have shown that NSAIDs could be effective despite financially driven reduction of proton pump inhibitor treatment^[23].

Typically, diagnosis of BE is made in men older than 50 years of age, a group with elevated frequency of cardiovascular disease. Low-dose aspirin is beneficial for primary cardiovascular events in men older than 50 years of age who are at risk of developing coronary artery disease^[50-52]. Today, data concerning BE patients with ischemic heart disease are scarce. We have reported that low-dose aspirin could reduce the risk of EAC in BE patients with ischemic heart disease, but it had no beneficial effect in patients without cardiovascular co-morbidities^[53], possibly due to cofactors common to the etiology of ischemic heart disease and EAC, such as alcohol, tobacco, diet and exercise.

Limited data suggest that biomarkers might have a role in identifying those patients with BE who are most likely to benefit from chemopreventive therapies. In BE patients with DNA content abnormalities, such as 17p loss of heterozygosity (LOH), and/or 9p LOH in their esophageal biopsy specimens, NSAID use was associated with a significant reduction in the risk of EAC after 6-10 years of follow-up. In contrast, no beneficial effect was seen in patients without those abnormalities^[54].

MECHANISMS OF NSAID CHEMOPREVENTIVE EFFECT

Esophageal carcinogenesis is mainly related to the inflammatory process in macroscopically visible Barrett epithelium, due to persistent gastroesophageal reflux^[47] in addition to angiogenesis up-regulation^[55]. The inflamed mucosa produces several inflammatory intermediates. Interleukin-1 and tumor necrosis factor induce nuclear factor (NF)- κ B over-expression^[56]. After activation, NF- κ B translocates to nucleus, where it activates gene transcription^[57]. In BE patients, NF- κ B binds the promoter region of COX-2 gene, increasing COX-2 expression^[58]. Most observational studies suggest that NSAIDs, in doses adequate to suppress COX-2, can effectively prevent BE progression to EAC^[23,37-40].

Reactive oxygen species may damage DNA, RNA, lipids and proteins, leading to increased mutation and altered functions of enzymes and proteins (*e.g.*, activation of oncogene products and/or inhibition of tumor suppressor proteins). They also related to cellular immunity, signal transduction and modification of extracellular matrix. In normal esophagus, low levels of reactive oxygen species are produced in non-phagocytic cells and are thought to be by-products of aerobic metabolism^[59]. Pulsed acid treatment and bile significantly increases H₂O₂ production in BE cells *via* NADPH oxidase NOX-5-S over-expression. It also increases calcium ion influx and cyclic adenosine monophosphate (AMP) reactive element binding protein^[60,61]. Increased cellular calcium ion influx causes up-regulation of NADPH oxidase NOX5-S^[62]. Overproduction of reactive oxygen species derived from up-regulation of NADPH oxidase NOX5-S, as well as H₂O₂ overproduction, can up-regulate NF- κ B^[63] and as a result leads to COX-2 over-expression^[64].

Because acid and bile contents of refluxate represent the main driving forces for COX-2 over-expression in BE patients^[65] and because proton pump inhibitors enhance COX-2 anti-proliferated effect *in vitro* and prevent vascular endothelial growth factor overexpression^[66], acid suppression should be an essential cofactor of EAC chemoprevention. This suggestion is also supported by epidemiological data^[38,40,45].

COXs (or prostaglandin H synthases) are a family of myeloperoxidases located at the luminal side of the endoplasmic reticulum and nuclear membrane, which catalyze the rate-limiting step of prostaglandin biosynthesis from arachidonic acid^[67]. COX-2 induction or over-expression

is associated with an increased production of prostaglandin E₂ (PGE₂), which is known to modulate cell proliferation, cell death and tumor invasion in many types of cancer. In addition to COX overexpression, pulsed acid exposure can up-regulate microsomal PGE synthase 1 and through it, PGE₂ production and cell proliferation. Acid-induced microsomal PGE synthase 1 over-expression depends on NADPH oxidase 5S activation and NF- κ B1 over-expression^[68] and it is regulated through the increase of cytosolic calcium^[69]. Epidemiological data suggest that the acid related route of PGE₂ production is of minor importance since COX-2 inhibitors can be effective even under inadequate acid suppression^[23].

PGE₂ acts through different membrane receptors called EP receptors (EP1, EP2, EP3 and EP4). These receptors are all located on the cell surface but trigger different signaling pathways. Thus, it is known that EP1 signaling acts through phospholipase C/inositol triphosphate signaling, leading to intracellular mobilization of calcium. EP2 and EP4 receptors are coupled with G proteins and activate adenylate cyclase, leading to an increase of intracellular cyclic AMP^[70]. Cyclic AMP is then able to activate various kinases, such as protein kinase A, phosphoinositide-3 kinase and glycogen synthetase kinase-3, leading to an activation of β -catenin, a pathway regulating cell proliferation^[71]. Contrary to EP2 and EP4, EP3 is coupled with Gi protein, leading to an inhibition of adenylate cyclase and decreases of cAMP inside the cells^[70]. Dietary elements entering arachidonic acid metabolism can interfere with PGE₂^[49] and therefore they should not be overlooked. Unfortunately, almost all epidemiological studies ignore this parameter^[23,37-43].

Cell cycle regulatory mechanisms form checkpoints where the cell cycle can be stopped after cellular damage in order to allow repair and to maintain cellular integrity or, alternatively, to eliminate mutated and potentially dangerous cells. Different serine-threonine kinase proteins called cyclin-dependent kinases (Cdk) are important cell cycle regulators. They interfere with the cell cycle by phosphorylating many substrates^[72]. The inhibitors of cyclin kinase 4 (INK4) family (p16, p15, p18 and p19) and the Cip/Kip family (p21, p27 and p57)^[72,73] are key regulators of cell transition from G1 to S phase. INK4 family inhibits Cdk4 and Cdk6, whereas Cip/Kip family inhibits all Cdk. After DNA damage, p53, a tumor suppressor gene, activates transcription of p21, which inhibits cyclin E phosphorylation, leading to hypophosphorylation of retinoblastoma protein^[71]. After phosphorylation, retinoblastoma protein releases transcription factor E2F activating genes involved in the S phase-like proliferating cell nuclear antigen^[74]. p53 also regulates cell transition from G₂ to M phase through cyclin B-Cdk 2 complex activation. Cyclin B-Cdk 2 complex accumulates during the previous step of the cell cycle. It is inactivated by phosphorylation at tyrosine 15 and threonine 14 by Wee 1 and Myt 1 and can be reactivated when these phosphate groups are removed by the phosphatase CDC25A, a cyclin related phosphatase, when cells enter mitosis^[75].

COX-2 up-regulation increases Barrett's epithelium

and esophageal adenocarcinoma cell proliferation by induction of retinoblastoma tumor suppressor protein phosphorylation and up-regulation of cyclins, cyclin-dependent kinases^[76] and p53 LOH^[77]. NSAIDs could also increase the proportion of Barrett cells in G₀-G₁ phase and reduce those in S and G₂-M phase^[78,79].

Two major cascades of intracellular events are commonly involved in mediating apoptosis. (1) The intrinsic pathway, also called the mitochondrial or stress-induced apoptotic pathway, which is activated in response to damaging stresses; and (2) the extrinsic, or physiological, apoptotic pathway. Typical hallmarks of the intrinsic pathway are mitochondrial outer membrane permeabilization, accompanied by a collapse of the mitochondrial membrane potential^[80]. These events lead to the release of cytochrome *c* into the cytosol and the death complex formation by apoptotic protease activating factor-1 and procaspase-9. Once recruited, procaspase-9 is cleaved to its activated form (caspase-9) to further activate the executor caspase-3 and to finalize the apoptotic program. The intrinsic pathway can be triggered upon binding of specific ligands to death receptors characterized by the presence of a death effector domain^[81]. Ligands include cytokines, such as tumor necrosis factor α , tumor necrosis factor-related apoptosis inducing ligand-induced apoptosis or Fas. After binding, death inducing silencing complex is formed. The adaptor proteins, tumor necrosis factor receptor-associated death domain and Fas associated death domain, form the death inducing silencing complex that is able to recruit and activate pro-caspase-8. The latter activates caspase-3 in order to trigger the final steps of apoptosis.

Cross talks between the two pathways take place. The extrinsic apoptotic pathway can activate the intrinsic pathway *via* truncation of the BH3-only protein Bid by caspase-8. BH3-only protein Bid interacts with mitochondria, by favoring the activation of the pro-apoptotic Bcl-2 family members Bak and Bax, thus leading to mitochondrial outer membrane permeabilization and caspase-9 activation^[80]. The intrinsic apoptotic pathway may, in turn, activate caspase-8, downstream to caspase-3^[82]. NSAIDs can inhibit programmed cell death in BE cells *via* prevention of Bcl-2 suppression^[83], a key checkpoint in COX-2 controlled apoptotic cascade^[84].

Anoikis is a form of apoptosis mediated by the loss of cell anchorage. This pathway plays a fundamental role during development and maintenance of tissue homeostasis by killing damaged cells or detached cells in order to maintain tissue architecture. It is dependent on caspase activation and cytochrome *c* release by mitochondria and is regulated by Bcl-2 family members^[71]. Cell anchorage is due to cell-cell and cell-matrix interactions. Cell-cell interactions are mainly mediated by integrins, transmembrane receptors located at the cell surface^[85]. Many intracellular signals can act downstream to integrins, which, correctly switched on, can ensure cell survival. Some of them are mediated by kinases such as focal-adhesion-kinase or integrin-linked kinase. Focal-adhesion-kinase is phosphory-

lated upon integrin adhesion, leading to activation of other signaling pathways like phosphoinositide 3 kinase and mitogen-activated protein kinase (MAPK)^[71].

NSAIDs can up-regulate MAPK signaling cascade^[86] through Cl/HCO₃ membrane exchange channel after intracellular acidification^[87]. COX-2 inhibitors can regulate mesenchymal-epidermal cross talk^[88]. In non-dysplastic Barrett, COX-2 is selectively increased only in stromal cells, while in adenocarcinoma it is also increased in neoplastic epithelium^[89,90].

COX-2 can also regulate the expression of angiogenic factors, especially vascular endothelial growth factor^[90], mainly through a MAPK dependent pathway^[91]. Because reactive oxygen species are overproduced in the ischemic tissue^[92] and various angiogenic factors are abundant in patients with cardiovascular diseases^[93], NSAIDs are expected to be more effective in this patient group. Nevertheless, non-aspirin NSAID are not effective in BE patients with ischemic heart disease, while aspirin is especially effective in this patient group^[53].

Although low-dose aspirin clearly prevents EAC when prescribed in healthy controls, it suppresses COX insufficiently^[34]. Thus, apart from COX-related, there are also other mechanisms implicated in NSAID chemopreventive action. Epidemiological data doubt the significance of COX-independent mechanisms^[23,37-43].

Independently to COX, NSAIDs can bind and inhibit protein kinase B/Akt, an important mediator of cell proliferation and in apoptosis. Protein kinase B is able to phosphorylate Cdk inhibitors, such as p21 and p27, leading to proliferating cell nuclear antigen activation^[94]. Moreover, it inhibits apoptosis by phosphorylating the pro-apoptotic protein Bad and by inhibiting caspase-9 cleavage^[80]. Independently to COX, NSAIDs can also activate the extrinsic apoptotic pathway by modulating the sensitivity of several tumor cells to Fas and tumor necrosis factor-related apoptosis inducing ligand^[95]. They can also up-regulate Bax expression and mitochondrial cytochrome *c* translocation^[96]. Finally, NSAIDs are able to decrease intracellular content of glutathione, the most important intracellular non-protein antioxidant defense against free radicals and, in such a way, affect both cell proliferation and apoptosis^[97].

Although *in vitro* studies suggest that NO-aspirin is more effective than aspirin to prevent Barrett cell hyperproliferation^[98], this did not prove to be the case in a clinical study^[23].

COST-EFFECTIVENESS AND SIDE EFFECTS OF NSAIDS CHEMOPREVENTION

Assuming that aspirin use can reduce EAC development risk by 50% in BE patients, the cost of the chemopreventive intervention was calculated to 40000 Euros for every quality year of life saved^[28].

NSAIDs clearly have substantial potential for toxic-

ity, including serious gastrointestinal and cardiovascular side effects that should be balanced with their potential cancer-preventive effects. Generally the risk for low-dose aspirin is low. A meta-analysis of randomized controlled trials comparing low-dose aspirin (75-325 mg) and placebo for cardiovascular prophylaxis found that the absolute annual increase in risk attributable to aspirin was only 0.13% (95%CI: 0.08-0.20) for major bleeding, 0.12% (95%CI: 0.07-0.19) for major gastrointestinal bleeding, and 0.03% (95%CI: 0.01-0.08) for intracranial bleeding^[99]. Moreover, concomitant proton pump inhibitor therapy could reduce the risk of gastrointestinal bleeding by a factor of 2 to 9^[100,101].

We have shown that complications, including upper gastrointestinal bleeding, esophageal ulcers and benign esophageal strictures, were no more common in NSAID users with BE than NSAID non-users. Moreover, the majority of those complications were acid related and could be prevented by adequate acid suppression, preferentially with high dose proton pump inhibitors. On high dose proton pump inhibitors, only 14% of BE patients consuming NSAIDs presented with any complication^[23]. In accordance to our findings, Hillman *et al.*^[102] have shown that esophageal ulcers and stenosis can be effectively prevented with adequate acid suppression^[102].

Because thromboxane biosynthesis depends on sustained inhibition of COX-1, several NSAIDs present serious cardiovascular side effects. In the two meta-analyses published today, major vascular events were increased by about a third for COX-2 selective and non-selective NSAIDs, with the exception of naproxen. Analyses showed that the excess risk was mainly attributable to an increase of about three quarters in the risk of major coronary events. Vascular death increased by about two-thirds, heart failure risk roughly doubled, while risk for stroke was not affected^[103,104].

Nitro-NSAIDs represent an NSAID subclass with lower risk for gastrointestinal bleeding^[105]. We have shown that combination of NSAID use to nitrates in BE patients neither affected EAC risk nor improved NSAID safety profile^[23].

STATINS AND EAC CHEMOPREVENTION

Cellular effects of statins on EAC cell lines have been evaluated in three *in vitro* studies. All reported anti-proliferative and pro-apoptotic effects^[106-108]. Qresearch, a prospective study based on 24 general practice research databases from England and Wales, have shown that statins were protective against esophageal cancer development in both men and women. The risk of esophageal carcinoma decreased in both men and women prescribed simvastatin, as well as in men prescribed atorvastatin. There were inadequate data for other statins. There was some evidence of a dose-response associated with simvastatin in men only^[109]. A more recent analysis of the same database revealed no protective effect from statin use^[110]. An analysis of General Practice United Kingdom Research

Database in 2002 that included only 9 esophageal carcinoma cases revealed no protective effect related to statin use^[111]. Bhutta *et al.*^[112] case control study found that statin use was negatively associated with the development of esophageal carcinoma. Both lipophilic and hydrophilic statins were protective. The magnitude of this negative association was similar for time periods extending beyond one year. When statin use and cancer development was accessed through a health care program database from northern California, esophageal carcinoma was more common among statin users^[113]. Population studies published today, although they have been adjusted for many covariates including age, body mass index, smoking, do not differentiate between EAC and squamous carcinomas and do not allow evaluation of statin use in EAC prevention.

Three population studies^[110-112] were included in the meta-analysis of risk of esophageal carcinoma among general population cohorts with statin use. The pooled effect size was 0.86 (95%CI: 0.78-0.94, $P = 0.001$) with minimal heterogeneity^[114]. A recent meta-analysis of all published studies calculated the pooled effect size for statins to 0.81 (95%CI: 0.75-0.88) with substantial heterogeneity^[48].

Several observational studies evaluating NSAIDs chemopreventive effect have also analyzed the utility of statin use (Table 2). In their case control study, Beales *et al.*^[37] found that regular statin use was associated with a significantly lower incidence of EAC. Longer duration of statin use and higher doses were both associated with a significantly greater reduction in EAC. Kastelein *et al.*^[40] reported that statin use for greater than one mo was associated with a statistically significant inverse risk for neoplastic progression, although this was only observed in men over 60 years of age. The concomitant use of both statins and NSAIDs was associated with a greater risk reduction. Nguyen *et al.*^[41] reported that having any filled statin prescription was associated with 45% lower risk of EAC. Patients with a cumulative filled statin prescription for > 12 mo have a reduced risk of EAC compared to those with ≤ 12 mo or those with no statin prescription. Kantor *et al.*^[43] found that statin use was not associated with a reduced risk of neoplastic progression in BE patients. Nevertheless, when the analysis was limited to persons with high-grade dysplasia at baseline, a subgroup at particularly high risk of EAC development, statin use was definitively protective. The combination of statins and NSAIDs was also protective. The main drawback of most observational studies was that authors did not adjust for important covariates, namely body mass index and smoking^[40,41,43].

Kastelein *et al.*^[40] and Nguyen *et al.*^[41] cohort studies were included in the meta-analysis of risk of EAC. The pooled effect size was 0.53 (95%CI: 0.36-0.78, $P = 0.001$) with minimal heterogeneity^[115]. A recent meta-analysis, including all 5 studies published today, calculated the pooled effect size for statins to 0.57 (95%CI: 0.43-0.75) with minimal heterogeneity. For the combination of statins and COX inhibitors, pooled effect size was 0.26 (95%CI: 0.10-0.68)^[48].

Table 2 Available epidemiological evidence of benefit of statin use in prevention of esophageal adenocarcinoma in patients with Barrett's esophagus

Ref.	Type of study	Size-follow-up	Effect on EAC rate	Beneficial effect
Hippisley-Cox <i>et al</i> ^[109]	Population based	General population 2004692 cases	Statins Men ¹ OR = 0.78 (0.66-0.91) Women ¹ OR = 0.68 (0.52-0.88) Simvastatin Men ¹ OR = 0.69 (0.50-0.94) Women ¹ OR = 0.82 (0.68-0.99) Atorvastatin Men ¹ OR = 0.73 (0.55-0.96) Women ¹ OR = 0.73 (0.47-1.13) Statins ¹ OR = 0.88 (0.77-1.01)	Statins ¹
Vinogradova <i>et al</i> ^[110]	Population based	General population 2004692 cases	Statins ¹ OR = 0.88 (0.77-1.01)	None ¹
Kaye <i>et al</i> ^[111]	Population based	Esophageal cancer: 9	Statins ² OR = 0.8 (0.3-1.8)	None ²
Bhutta <i>et al</i> ^[112]	Population based	Esophageal cancer: 4242 Controls: 17233	Statins ¹ OR = 0.84 (0.73-0.95) Lipophylic statins ¹ OR = 0.86 (0.75-0.98) Hydrophilic statins ¹ OR = 0.71 (0.51-0.98)	Statins ¹
Beales <i>et al</i> ^[37]	Case-control	BE: 170 EAC: 85	Statins OR = 0.57 (0.28-0.94)	Statins
Kastelein <i>et al</i> ^[40]	Cohort	BE: 570 4.5 years	Statins OR = 0.46 (0.21-0.99) Statins + NSAIDs OR = 0.22 (0.06-0.85)	Statins Statins + NSAIDs
Nguyen <i>et al</i> ^[41]	Cohort	BE: 344 2620 PY	Statins OR = 0.55 (0.36-0.86)	Statins
Kantor <i>et al</i> ^[43]	Cohort	BE: 411 2805 PY	Statins OR = 0.68 (0.30-1.54) Statins + NSAIDs OR = 0.41 (0.13-1.26)	Statins + NSAIDs

¹Results pertain to esophageal carcinoma; ²Results pertaining to all cancers. PY: Patient years; EAC: Esophageal adenocarcinoma; BE: Barrett's esophagus; NSAIDs: Non-steroidal anti-inflammatory drugs.

At present, there are no published observational studies evaluating statin chemopreventive effect in BE patients in the general population. Moreover, there are no

interventional studies underway.

MECHANISMS OF STATIN CHEMOPREVENTIVE EFFECT

Statins competitively inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-limiting enzyme in the biosynthesis of cholesterol. Although this is their most appreciated biological action, statins have several other important roles. They inhibit biosynthesis of L-mevalonate^[116], a precursor of cholesterol, and they produce two isoprenoid intermediates: farnesyl pyrophosphate and geranylgeranyl pyrophosphate^[117]. Farnesyl pyrophosphate and geranylgeranyl pyrophosphate attach to several cellular proteins including G proteins by a posttranslational modification termed isoprenylation. The isoprenylation of G proteins is crucial for membrane attachment and normal functioning. The low molecular weight G proteins, including Ras, Rho, Rab and Cdk 42, play crucial roles in signal transduction and therefore influence important cellular functions, such as proliferation, apoptosis and differentiation. Ras represents the most important G protein and is predominantly farnesylated, while all other GTPases are predominantly geranylated. Ras mutations in preneoplastic cells determine their susceptibility to statin treatment^[118]. Because Ras in EAC cells is very susceptible to statin treatment^[106], statins are very effective in all available epidemiological studies^[37,40,41,43].

Ras pathway down-regulation could reduce phosphoinositide 3 kinase/Akt and extra-cellular signal regulating kinase activities, enhancing cell proliferation and modulating cell-cell interactions. Moreover, it up-regulates the pro-apoptotic proteins Bad and Bax through phosphoinositide 3 kinase/Akt pathway, preventing cell apoptosis^[106,107,118-120]. Through Ras modification, statins attenuate total cellular and cell-surface intracellular adhesion molecule-1 expression and activate NF- κ B^[121].

Through a G-protein independent mechanism, statins can suppress angiogenesis. Angiogenesis inhibition is a result of the inhibition of the expression or activity of monocyte chemoattractant protein-1, inhibition of metalloproteinase, angiotensin-2, preproendothelin gene, as well as inhibition of actin filament and by focal adhesion molecules formation^[122]. Finally, they present with an anti-inflammatory effect by reducing tumor necrosis factor- α ^[107] and intracellular adhesion molecule-1 (a critical adhesion molecule involved in transendothelial tumor cell migration)^[123,124].

Because statins do not interfere with various proliferation pathways, such as MAPK pathway and transcription factor AP1/c-jun terminate kinase^[106], NSAIDs can enhance statin chemopreventive effect by blocking those metabolic routes^[125]. As a result, whenever statins and NSAIDs are combined, lower doses of either chemopreventive agent are necessary, leading to a reduction of side effects^[106]. Current epidemiological data unanimously verify NSAID and statin synergy^[37,40,41,43].

Because adiponectin and ghrelin can interfere in vitro with EAC cell apoptosis^[126], obesity, a parameter overlooked by most observational studies^[40,41,43], mandates further attention.

SIDE EFFECTS OF STATIN CHEMOPREVENTION

Statins are generally safe medications. Out of the various adverse effects of statins, only liver and muscle-related toxicity is consistently reported^[127]. Between 1987 and 2001, the Food and Drug Administration (FDA) recorded 42 deaths from rhabdomyolysis induced by statins, translated to one death per million prescriptions (30 day supply). Although 5%-10% of patients complain of muscle symptoms, only 1%-3% of them are actually statin related. Muscle symptoms usually occur within the first 6 mo of starting statins but can occur months or years after the initiation of statin therapy and automatically resolve within 2 mo of discontinuing statin therapy^[128]. The incidence of statin-associated myopathy is quite low (approximately 0.01%) and rhabdomyolysis even lower (0.002%)^[129]. Fatal rhabdomyolysis has been estimated to occur in approximately 1.5 in 10 million prescriptions^[130].

Post-marketing surveillance studies of statins revealed that elevation in hepatic aminotransferases are dose related, mild and unrelated to low-density lipoprotein lowering effect. Thus, most hepatologists no longer consider statins to have any significant hepatotoxicity^[131]. Although serious hepatotoxicity is rare, 30 cases of liver failure associated with statin use were reported to the FDA between 1987 and 2000, the rate being about one case per million person-years of use. Thus, the occurrence of acute liver failure thought to be caused by statins is well below the background rate of idiopathic acute liver failure in the general population^[132].

Evidence from four cohort studies and case reports suggest that statins cause reversible peripheral neuropathy. Nevertheless, the attributable risk is small (12 per 100000 person-years) and no change in cognitive function was found in randomized trials of statins in elderly patients^[130].

Because BE patients are usually old with various multi-systemic comorbidities^[36], increased toxicity is expected^[133] with statin use. No study today has specifically addressed statin toxicity in BE patients.

FUTURE DIRECTIONS

The poor prognosis of patients diagnosed with EAC presents a challenge to the clinician. Consequently there is burgeoning interest in potential chemo-preventive strategies. Considerable evidence of medium quality is available of a protective effect of NSAIDs, yet because of their side-effect profile, widespread use cannot be currently justified. Although statin safety profile is good, epidemiological and animal data are limited to justify their use as chemo-preventive agents. Because mortality due to

cardiovascular disease is high in BE patients, "technical review on the management of Barrett's esophagus today" suggests screening for cardiovascular factors in BE patients and aspirin and statin use as warranted^[45]. Because we have shown no benefit for non-aspirin NSAID use in BE patients with ischemic heart disease^[53] and substantial cardiovascular side effects are expected^[103,104], use of non-aspirin NSAIDs should be withheld in patients with BE and cardiovascular co-morbidities, at least until more clinical data might justify their use.

Large randomized control trials in the near future are expected to safely evaluate NSAIDs and statins as chemopreventive agents and possibly introduce their widespread use in patients with BE. Because of their synergistic effect^[106], such trials ought to test either and both medications against proton pump inhibitors alone.

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Aspirin, cyclooxygenase inhibition and colorectal cancer

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Abstract

Colorectal cancer (CRC) is the third most common type of cancer worldwide. Screening measures are far from adequate and not widely available in resource-poor settings. Primary prevention strategies therefore remain necessary to reduce the risk of developing CRC. Increasing evidence from epidemiological studies, randomized clinical trials and basic science supports the effectiveness of aspirin, as well as other non-steroidal anti-inflammatory drugs, for chemoprevention of several types of cancer, including CRC. This includes the prevention of adenoma recurrence and reduction of CRC incidence and mortality. The detectable benefit of daily low-dose aspirin (at least 75 mg), as used to prevent cardiovascular disease events, strongly suggests that its antiplatelet action is central to explaining its antitumor efficacy. Daily low-dose aspirin achieves complete and persistent inhibition of cyclooxygenase (COX)-1 in platelets (in pre-systemic circulation) while causing a

limited and rapidly reversible inhibitory effect on COX-2 and/or COX-1 expressed in nucleated cells. Aspirin has a short half-life in human circulation (about 20 minutes); nucleated cells have the ability to resynthesize acetylated COX isozymes within a few hours, while platelets do not. COX-independent mechanisms of aspirin have been suggested to explain its chemopreventive effects but this concept remains to be demonstrated *in vivo* at clinical doses.

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Key words: Aspirin; Colorectal cancer; Cyclooxygenase inhibition; Mechanisms; Risk; Benefits

Core tip: Colorectal cancer (CRC) is a major cause of morbidity and mortality worldwide. Currently, CRC screening programs are not widely available and need to be improved. New prevention strategies are therefore necessary. Daily low-dose aspirin, as given for the prevention of cardiovascular disease events, has demonstrated benefits in clinical and basic studies in terms of preventing adenoma recurrence and decreasing the incidence of CRC and attributable mortality. These findings indicate that the antiplatelet action of aspirin plays a central role in its antitumor effect. Cyclooxygenase-dependent and independent mechanisms have been suggested to explain this effect. Extensive translational medical research is mandatory for future progress in CRC prevention.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common can-

cer worldwide, accounting for an estimated 9.8% of all new cancers (1.2 million cases annually) and 8.1% of all cancer mortality^[1]. It arises through the cumulative effects of inherited genetic predisposition and environmental factors. Genomic instability is an integral part of the transformation of normal colonic or rectal mucosa into carcinoma. Three molecular pathways have been identified: chromosomal instability, microsatellite instability and CpG island methylator phenotype pathways. These pathways are not mutually exclusive, with some tumors exhibiting features of multiple pathways. Germline mutations are responsible for hereditary CRC syndromes (accounting for less than 5% of all CRC), while a stepwise accumulation of genetic and epigenetic alterations results in sporadic CRC.

Today it is well known that screening reduces CRC mortality and is recommended, beginning at age 50, for average risk individuals, although compliance is far from adequate and screening is not widely available in resource-poor settings^[2,3]. Primary prevention strategies are therefore still necessary to reduce the risk of CRC, especially because of the limitations of population-based secondary prevention programs that rely on detection and removal of adenomas.

Aspirin has demonstrated its efficacy in the prevention of adverse events related to cardiovascular disease (CVD). It is one of the most widely used drugs in the world. One survey suggested that over one-third of the United States adult population use low-dose aspirin (LDA) regularly^[4]. In England in 2007, over 30 million primary care prescriptions were issued for aspirin^[5]. Hence, both physicians and patients are largely familiar with the long-term use of aspirin for chronic disease management. In addition, CRC and CVD share the same risk factors, such as older age, being overweight/obesity and physical inactivity.

Today, a large body of clinical and experimental evidence indicates that aspirin can protect against different types of cancer, in particular CRC^[6]. A role of the anti-platelet effect of aspirin in its anti-cancer effect is also supported by several studies.

In this review we will discuss clinical results related to the impact of aspirin on the risk of CRC. Then, we will explain the pharmacology of aspirin at low doses in order to provide a mechanistic interpretation of aspirin action as a chemopreventive agent for CRC, in particular the selective inhibition of platelet cyclooxygenase (COX)-1 activity.

CLINICAL EFFECTS OF ASPIRIN ON SPORADIC CRC

Evidence from epidemiological studies

Most case-control and cohort studies have found that regular aspirin use was associated with reduced risk of CRC^[7]. A systematic review of case-control studies published in 2012 showed a statistically significant reduction of long-term risk of developing CRC (OR = 0.62,

Table 1 Summary of the associations between regular use of aspirin and risk of colorectal cancer in case-control and cohort studies

Study type	n	Aspirin	Controls	OR (95%CI)	P value
Case-control					
Any ASA	26	10464/25618	28300/47834	0.67 (0.60-0.74)	< 0.0001
Maximum reported ASA	17	1551/12659	2664/18153	0.62 (0.58-0.67)	< 0.0001
ASA ≥ 5 yr	10	971/7682	1534/10029	0.68 (0.63-0.75)	< 0.0001
Daily ASA	4	165/1254	349/1523	0.49 (0.40-0.60)	< 0.0001
Daily ASA ≥ 5 yr	1	66/1668	121/1973	0.63 (0.46-0.86)	0.004
Standard cohort					
Any Aspirin	11	3791/2764414	3623/2514652	0.85 (0.82-0.89)	< 0.0001
Maximum reported ASA	8	661/664475	1858/1374905	0.78 (0.71-0.84)	< 0.0001
ASA ≥ 5 yr	4	889/1 022 192	1311/1304760	0.76 (0.70-0.82)	< 0.0001
Daily ASA	5	741/658536	1115/819288	0.80 (0.73-0.88)	< 0.0001
Daily ASA ≥ 5 yr	1	60/38302	420/232116	0.68 (0.52-0.90)	0.0060
Nested case-control					
Any ASA	6	2215/8926	65 099/109526	0.87 (0.75-1.00)	0.0700
Maximum reported ASA	5	206/4457	8302/40948	0.67 (0.58-0.77)	< 0.0001
ASA ≥ 5 yr	1	116/228	23704/37935	0.62 (0.48-0.81)	< 0.0001
Daily ASA	1	53/165	8744/22975	0.77 (0.55-1.07)	0.1400
Daily ASA ≥ 5 yr	1	29/141	7274/21505	0.51 (0.34-0.76)	0.0120

Modified from Algra *et al*^[8]. Estimates from standard cohort studies are based on results adjusted for age and other baseline clinical characteristics. ASA: Aspirin.

95%CI: 0.58-0.67) in regular aspirin users compared with non-users, as well as a significant reduction in the proportion of cancers with distant metastasis at diagnosis (OR = 0.69, 95%CI: 0.57-0.83)^[8] (Table 1). An analysis of 662424 men and women enrolled in the Cancer Prevention Study II cohort showed that daily use of aspirin for at least 5 years was associated with a 32% reduction in risk of CRC^[9]. Two cohort studies of United States health professionals (47363 men and 82911 women) showed that regular aspirin users (≥ 2 times/wk) had 21% and 23% lower risk of CRC, respectively, during follow-up periods of 18 and 20 years respectively^[10,11]. Moreover, in a separate analysis of a Nurses Health Study cohort, regular aspirin use also reduced the risk of death from CRC by 28% and risk of death from any type of cancer by 12%^[12].

Evidence from clinical trials

In 2010, Rothwell *et al*^[13] obtained long-term follow-up data on cancer outcomes from four randomised trials that were originally designed to evaluate the effect of aspirin on the prevention of CVD events (Table 2). These trials studied diverse populations with CVD, including men at low risk ($n = 10,224$) and men and women at high risk ($n = 3809$). Dosage ranged from 75-1200 mg/d, median treatment duration was 6 years and median follow-up was 18.3 years. Treatment with aspirin (75-500 mg/d) reduced the 20-year risk of CRC by 24% and CRC-associated

Table 2 Characteristics of trials included in Rothwell *et al* study and details of post-trial follow-up

	Thrombosis prevention trial	Swedish aspirin low dose trial	UK-TIA aspirin trial	British doctors aspirin trial
ASA comparison	75 mg/d vs placebo	75 mg/d vs placebo	300 mg vs 1200 mg/d vs placebo	500 mg/d vs placebo
Recruitment period	1989-1992	1984-1989	1979-1985	1978-7199
Median duration of scheduled treatment in original trial (yr)	6.9	2.7	4.4	6
Year post-trial follow up extended to	2009	2007	2006	2002

Modified from Algra *et al*^[8]. ASA: Aspirin.

mortality by 35%. The benefit increased with longer duration of treatment and seemed to be higher for proximal CRC compared to distal CRC. An absolute reduction of 1.76% ($P = 0.001$) in 20-year risk of any fatal CRC after 5 years of daily treatment with aspirin (75-300 mg) was observed. Subsequently, the same authors published a study that examined the effects of daily aspirin on long-term risk of death due to all cancers. They included data from eight randomised trials (25570 patients, 674 cancer deaths) and concluded that aspirin use reduced the risk of death due to cancer (pooled OR = 0.79, $P = 0.003$), but the benefit was only apparent after 5 years of treatment. Absolute reduction reached 7% in 20-year risk of death due to cancer for patients aged ≥ 65 years. In the 3 trials reporting data on the specific site of cancer occurrence with treatment duration of 5 years or longer and long-term follow-up, patients randomized to aspirin showed a statistically significant 20 year risk reduction of death due to CRC of 40% (HR = 0.60; 95%CI: 0.45-0.81, $P = 0.0007$)^[14].

Although these data are compelling, it should be taken into account that these studies were secondary analyses of CVD prevention trials and therefore they were not originally designed to examine CRC incidence or mortality. In addition, there are two large randomized trials of alternate-day aspirin treatment in healthy subjects: the Physician's Health Study (PHS)^[15] and Women's Health Study (WHS), which showed no effect of aspirin on the incidence of CRC over a 10-year follow-up period^[16]. The PHS determined the effect of aspirin 325 mg every other day on CVD in 22,071 healthy male physicians. In this study, the relative risk of CRC over a 10-year follow up was 1.03 (95%CI: 0.83-1.28). The WHS examined the effect of 100 mg every other day in 39876 healthy women. The relative risk of CRC was 0.97 (95%CI: 0.77-1.24). There are several plausible explanations for the discrepancy in results between the meta-analyses performed by Rothwell and incidence data of the PHS and WHS trials. Firstly, both trials used alternate-day dosing regimens in contrast to daily dosing used in the studies included in both meta-analyses. Secondly, in the PHS and WHS tri-

Table 3 Clinical effects of aspirin on sporadic colorectal cancer (clinical trials)

	Rothwell <i>et al</i> meta analysis	Physician's health study	Women's health study
ASA dosage	75-1200 mg/d	325 mg per every other day	100 mg per every other day
Duration of follow up (yr)	≥ 20	10	10
Relative risk of CRC over follow up (HR)	0.76 (95%CI: 0.60-0.96)	1.03 (95%CI: 0.83-1.28)	0.97 (95%CI: 0.77-0.24)

Comparison of findings from meta-analysis performed by Rothwell *et al*^[13] and incidence data of Physician's Health Study and Women's Health Study studies^[13,15,16]. CRC: Colorectal cancer; ASA: Aspirin.

als, the duration of follow-up was shorter and may have been insufficient to detect the aspirin effect. Finally, in the WHS trial, the equivalent daily dose of aspirin was 50 mg, lower than the 75 mg/d shown to be the minimum effective dose in the Rothwell meta-analyses^[13] (Table 3).

The precursors of CRC are colorectal adenomas in most cases. It would be expected that the chemopreventive effects of aspirin should begin before the development of CRC. The long duration of aspirin treatment required to show a preventive effect against invasive CRC probably reflects the time required for the development of cancer from precursor lesions (5-10 years). To date, four randomized double-blind placebo-controlled trials with 2967 participants have evaluated aspirin versus placebo for the secondary prevention of colorectal adenomas (in patients who had had colorectal adenomas or CRC)^[17-20]. Doses ranged from 81 to 325 mg/d and median follow-up was 33 mo. The meta-analysis of these randomized trials^[21] showed a statistically significant 17% reduction of the risk of developing adenoma with any dose of aspirin vs placebo (RR = 0.83; 95%CI: 0.72-0.96). This corresponded to a significant absolute risk reduction of 6.7%. For any advanced lesion, a significant relative risk reduction of 28% for aspirin at any dose was observed. This preventive effect emerged rather quickly (1 year) after the initiation of aspirin use (Table 4).

CLINICAL EFFECTS OF ASPIRIN IN HIGH-RISK POPULATIONS: FAMILIAL ADENOMATOUS POLYPOSIS AND LYNCH SYNDROME

To date, there are two controlled randomized trials that primarily evaluated the efficacy of aspirin in high-risk CRC patients: the Colorectal Adenoma/Carcinoma Programme (CAPP)¹^[22], which included 206 young individuals with a diagnosis of familial adenomatous polyposis (FAP), and CAPP2^[23], which studied 1009 patients with Lynch syndrome. Both studies compared aspirin (600 mg/d), with or without resistant starch or resistant starch placebo. Data from CAPP1 patients were only analyzed

Table 4 Clinical effects of aspirin in incidence of sporadic colorectal adenomas (clinical trials)

Study	Patients	Treatment	RR (95%CI)	Ref.
AFPPS trial	Patients with a recent history of histologically documented (removed) adenomas	ASA (81 or 325 mg/d) or folic acid (1 mg/d) or placebo for 2.7 years	Any adenoma 0.81 (0.69-0.96), ASA 81mg <i>vs</i> non ASA 0.96 (0.81-1.13), ASA 325 mg <i>vs</i> non ASA Advanced lesion 0.59 (0.38-0.92), ASA 81 mg <i>vs</i> non ASA 0.83 (0.55-1.23), ASA 325 mg <i>vs</i> non ASA 0.65 (0.46-0.91)	[17]
CAPS trial	Patients with a histologically documented colon or rectal cancer with a low risk of recurrent disease	ASA 325 mg/d or placebo for 2.6 years		[18]
APACC trial	Patients with a history of colorectal adenomas	ASA 160 or 300 mg/d or placebo for 1 and 4 years	0.73 (0.52-1.04) for both doses, after 1 year 0.96 (0.75-1.22), for both doses, after 4 years	[20]
ukCAP trial	Patients with an adenoma removed in the 6 mo before recruitment	ASA (300 mg/d) plus placebo or ASA plus folic acid (0.5 mg/d) or folic acid plus placebo or double placebo for about 2.6 years	Any adenoma 0.79 (0.63-0.99), ASA <i>vs</i> non ASA, Advanced adenoma 0.63 (0.43-0.91), ASA <i>vs</i> non ASA Ongoing	[19]
J-CAPP trial	Patients with previous sporadic colorectal tumors	ASA 100 mg/d or placebo for 2 years		

ASA: Aspirin.

Table 5 Clinical effects of aspirin in high risk population (clinical trials)

Study	Patients	Treatment	RR or HR (95%CI)	Ref.
CAPP1 trial	FAP young patients (10 to 21 years of age)	ASA (600 mg/d) plus placebo or resistant starch (30 g daily) plus placebo or double placebo for 17 years	RR = 0.77 (0.54-1.10), ASA <i>vs</i> non ASA	[22]
CAPP2 trial	Hereditary non-polyposis colon cancer or HNPCC	ASA (600 mg/d) or ASA placebo or resistant starch (30 g daily) or starch placebo for up to 4 years	HR = 0.63 (0.35-1.13), for the entire post-randomization period (ASA <i>vs</i> placebo) HR = 0.41 (0.19-0.86), for ≥ 2 years of treatment (ASA <i>vs</i> placebo)	[23]
J-FAPP II trial	FAP patients (≥ 16 years of age)	Placebo <i>vs</i> enteric coated ASA (100 mg/d) for 6-10 mo	Ongoing	[25]

ASA: Aspirin; FAP: Familial adenomatous polyposis.

if they had received treatment for at least 1 year. CAPP2 patients received aspirin for a mean of 29 mo.

The CAPP1 trial showed that the mean size of the largest polyps was significantly reduced in aspirin users. Despite a trend to fewer polyps in the rectum and sigmoid colon in aspirin versus non-aspirin users at the end of intervention (from 1 to 12 years), the difference was not significant.

CAPP2 was the first clinical trial that had cancer prevention as a primary endpoint. At the end of the intervention phase, analysis showed that aspirin treatment did not reduce the risk of developing new adenomas (RR = 1.03; 95%CI: 0.7-1.4) or CRC. The study design involved post-intervention follow-up^[24]. Over a mean follow-up of 55.7 mo, 48 aspirin users had developed 53 primary CRC, whereas in intention-to-treat analysis of time to first CRC there were no differences (HR = 0.63; $P = 0.12$) and the per-protocol analysis of patients completing 2 years of intervention yielded a HR of 0.41 (0.19-0.86, $P = 0.02$) (Table 5).

In Japan, two chemoprevention studies are currently being performed: one in patients with previous sporadic colorectal tumors [Japan Colorectal Aspirin Polyps Prevention (J-CAPP study)] and the second in patients with

familial adenomatous polyposis (J-FAPP study II). Both are double-blind randomized controlled trials with low-dose aspirin (100 mg/d) and study the effect of aspirin in colorectal carcinogenesis^[25].

CLINICAL EFFECTS OF ASPIRIN IN PATIENTS WITH PREVIOUS CRC

It has been suggested that aspirin may prevent recurrence or death in CRC patients. In a placebo-controlled randomized trial of patients with a history of non-metastatic CRC after resection, daily treatment with LDA was associated with a 35% reduction in risk of recurrent adenoma or carcinoma at 36 mo^[18]. In a cohort study of health professionals diagnosed with stage I - III CRC, regular use of aspirin after diagnosis was associated with higher CRC specific survival compared with non-users^[26].

DOSING FOR CHEMOPREVENTION

Because most aspirin-related adverse effects are dose-dependent, to find the minimum effective dose required for CRC prevention remains a critically important issue.

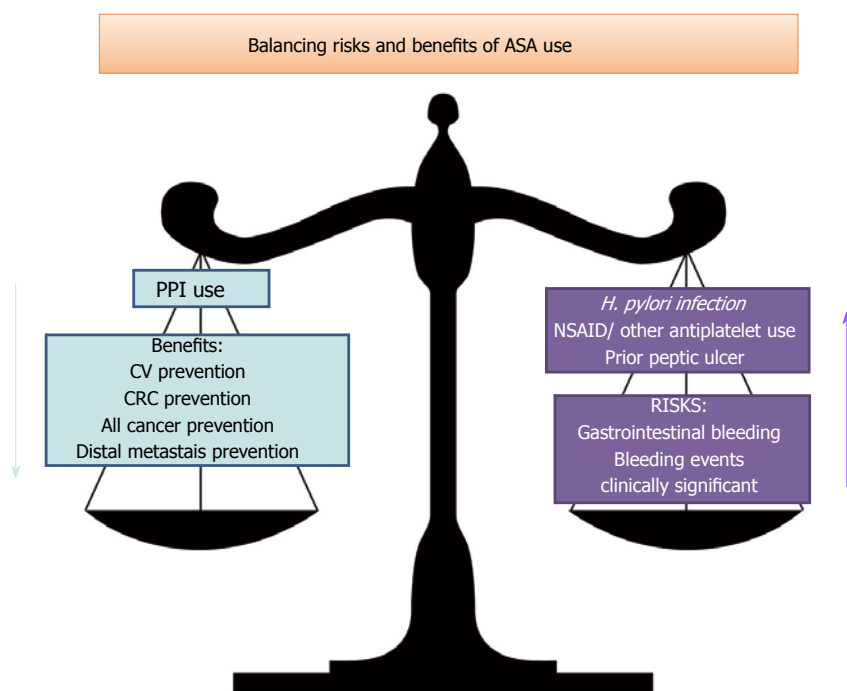


Figure 1 Balancing risk-benefits for the use of low dose aspirin. CRC: Colorectal cancer; NSAID: Nonsteroidal anti-inflammatory drug; CV: Cardiovascular; PPI: Proton pump inhibitor; ASA: Aspirin.

The Rothwell meta-analysis found that daily LDA regimens for the prevention of CVD-related events (75-325 mg) were as effective as daily high-dose aspirin^[13]. However, the short-term follow up data from the PHS^[15] (aspirin 325 mg every other day) and the WHS^[16] (aspirin 100 mg every other day) did not show a reduction in risk of CRC. These negative findings could be attributed to alternate day dosage and/or short follow up and/or the lower dose, especially in the WHS trial. The adenoma trials (REFS) also indicate that LDA (81-325 mg/d) reduces the risk of developing adenomas and advanced adenomas.

Although follow-up of the randomized trial of daily aspirin in CVD prevention and adenoma prevention trials demonstrated that daily LDA of 75-81 mg may be sufficient for CRC prevention, the results of observational studies are controversial. Some suggested that 300-325 mg may be necessary for CRC prevention but most provided incomplete information regarding the dose and duration of aspirin treatment^[7,8,10,11,27].

Therefore, taking the clinical trial and observational information together, there is very strong evidence that long-term LDA (75-325 mg/d) reduces the risk of CRC. Importantly, for the prevention of CVD-related events, LDA (75-81 mg/d) seems to be as effective as high-dose aspirin (300-325 mg/d) and, moreover, LDA has a better safety profile. However, daily aspirin at any dose may show greater benefit in patients with CVD than in those at risk of CRC.

BALANCING RISKS AND BENEFITS

Based on current evidence, treatment with LDA for 5 years in patients at risk of CVD-related events will probably prevent between 12 and 40 myocardial infarctions per 1000 patients treated, assuming an overall 10% risk of

CVD-related events in this population^[28]. Unfortunately, LDA use is also associated with 2-4 upper gastrointestinal bleeding events per 1000 patients^[28]. However, the risk of adverse events differs according to patient characteristics (gender, age, history of ulcer, *etc.*). It is of course possible to reduce gastrointestinal risk with proton pump inhibitors, but we cannot reduce the risk of intracranial bleeding. Given the risk of bleeding, clinical guidelines (2007) recommended against the routine use of aspirin for CRC prevention in average-risk individuals^[29]. However, the accumulating evidence from randomized clinical trials provides an exciting opportunity to reconsider the potential role of aspirin in cancer prevention; therefore, future practice guidelines recommendation for primary prevention in average-risk individuals for aspirin prophylaxis may also consider the prevention of cancer and not only the benefits of aspirin for the prevention of CVD-related events (Figure 1).

MECHANISM OF ACTION OF ASPIRIN

Aspirin, like other nonsteroidal anti-inflammatory drugs (NSAIDs), has the capacity to reduce prostanoid generation by inhibiting the activity of COX isozymes. Prostanoids are biologically active derivatives of arachidonic acid (AA) released from membrane phospholipids through the activity of different phospholipases^[30,31]. There are two isoforms of COX, named COX-1 and COX-2^[32]. Both COX isozymes are differently regulated catalytically, transcriptionally and post-transcriptionally, but they share the same catalytic activities.

COX-1 gene is considered a “housekeeping gene” and the protein is highly expressed in platelets where it is responsible for the generation of thromboxane A₂ (TXA₂), which promotes platelet activation and aggregation, vasoconstriction and proliferation of vascular smooth muscle

cells^[31,33]. In addition, COX-1 is highly expressed in gastric epithelial cells where it plays an important role in cytoprotection through the generation of prostanoids, such as prostaglandin E2 (PGE2)^[31,33]. In contrast, COX-2 gene, a primary response one with many regulatory sites^[34], is constitutively expressed in some tissues in physiological conditions, such as the endothelium, kidney and brain, and in pathological conditions, such as in cancer^[35]. In cancer cells, the major prostanoid produced through COX-2 is PGE2, which plays important roles in modulating motility, proliferation and resistance to apoptosis^[36,37].

Unlike other NSAIDs, aspirin is able to produce an irreversible inactivation of COX isozymes through the acetylation of a specific serine moiety (Ser529 of COX-1 and Ser516 of COX-2)^[38]. Acetylation of the allosteric subunit of COX-1 by aspirin causes an irreversible inhibition of COX activity and, in turn, of the generation of PGG₂ from AA. Acetylated COX-2 is not able to form PGG₂ but it generates 15R-hydroxyeicosapentaenoic acid (15R-HETE) from AA^[39]. However, there is no convincing evidence that these lipid mediators triggered by aspirin are generated *in vivo* in humans.

ASPIRIN PHARMACOLOGY

Aspirin has a short half-life when administered *in vivo* and it is rapidly inactivated by plasma and tissue esterases into salicylic acid, which is a weak inhibitor of COXs (in the millimolar range)^[40-42]. The inhibitory effects of aspirin have been found to be > 100-fold more potent in inhibiting platelet COX-1 than monocyte COX-2^[17-20]. Aspirin at low doses (75-100 mg daily) is able to cause nearly complete inhibition of the capacity of platelet COX-1 to generate TXA₂^[43,44]. Due to irreversible inhibition of COX-1 and the limited capacity of platelets for *de novo* protein synthesis^[45], the profound inhibitory effect of platelet function by aspirin persists throughout the dose interval (*i.e.*, 24 h).

The major part of the inhibitory effect of platelet COX-1 by the oral administration of low-dose aspirin occurs in the presystemic circulation where the drug reaches higher concentrations^[46,47]. The impact of low-dose aspirin, administered once daily, on COX-2 activity *in vivo* is marginal. In summary, the pharmacokinetics and pharmacodynamics of low-dose aspirin support the fact that the drug acts mainly by modifying platelet function as a consequence of COX-1 inhibition. At higher doses, aspirin may affect COX-2 in a dose-dependent fashion.

COX-DEPENDENT MECHANISMS FOR ANTITUMOR EFFECTS

Randomised clinical trials have shown that once daily LDA provides a chemopreventive effect against atherothrombosis^[24] and CRC^[13,14]. This finding suggests that enhanced platelet activation is involved in the development of these two pathological conditions. In fact, these aspirin doses and dosing intervals are consistent with a

selective inhibitory effect of aspirin on platelet COX-1 activity and on TXA₂-dependent platelet function.

Transcriptional upregulation of the COX-2 gene has been observed in nearly half of human colorectal adenomas and 80%-90% of CRC, probably related to the disturbed function of the APC gene. However, COX-1 gene and protein expression are not affected^[48-52] and the role of this enzyme in CRC carcinogenesis remains unclear. In colonic mucosa, COX-2 is localized predominantly in tumor tissue, including epithelial cells, mononuclear cells, endothelial and stromal cells, but not in nearby normal tissue. Upregulation of COX-2 is associated with increased cell adhesion, phenotypic changes, resistance to apoptosis and tumor angiogenesis^[53-57]. COX-2 expression does not always correlate with survival and/or with Duke's stage of the disease^[58-60]. This suggests a role of upregulated COX-2 for the initial stages of colon carcinogenesis but not for clinical outcome at advanced stages. The best studied consequence of upregulated COX-2 in CRC is enhanced prostaglandin production^[48]. Prostaglandin levels in CRC tissue are 3-4 fold higher than in healthy tissue in the vicinity, with PGE2 being the predominant product^[61]. PGE2 inhibits apoptosis and stimulates tumor growth and angiogenesis via stimulation of b-catenin/T-cell factor dependent transcription^[62]. In addition, PGE2 acts as an immunosuppressant in patients with CRC^[53,63]. The clearest clinical evidence for COX-2 as a pharmacological target for the chemopreventive action of aspirin was the finding that aspirin reduced the risk of CRC exclusively in individuals with elevated COX-2 expression but not in those without^[64]. This was associated with a reduction in mortality^[65]. Although these findings were from observational studies, they confirmed experimental data that prostaglandins and non-prostaglandin COX-2 products are central to the pathogenesis of CRC. The vast majority of published experimental studies have reported beneficial antitumor effects for aspirin, celecoxib and non-aspirin NSAIDs in a variety of experimental models^[65-67]. These data strongly suggest a central role of COX-2 in CRC and its inhibition is an effective chemopreventive measure (Figure 2).

The generation of TXA₂, a major product of platelet COX-1 which promotes platelet aggregation and vasoconstriction^[68], represents another important mechanism by which platelets can affect tumorigenesis. One study has shown that enhanced TXA₂ generation into murine colon-26 adenocarcinoma cell line (C26) stimulated tumor angiogenesis, tumor growth *in vivo*^[69] and promoted the interaction between metastasizing tumor cells and the host hemostatic system^[69], thus suggesting a role of TXA₂ in promoting angiogenesis and the development of tumor metastasis^[70].

In one study, the authors demonstrated PGE2 inhibition in rectal biopsies performed 1 mo after treatment with three different doses of aspirin (81, 325 and 650 mg) versus placebo^[71]. Unexpectedly, the 81 mg daily aspirin dose suppressed PGE2 levels to the same extent as the 650 mg dose. In another study, treatment with 81 mg of aspirin per day for 3 mo reduced mucosal PGE2

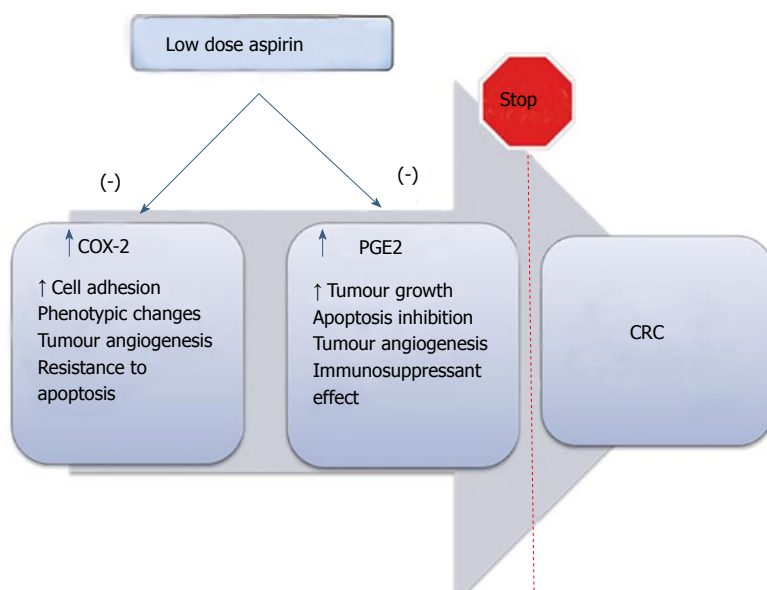


Figure 2 Cyclooxygenase-dependent mechanisms for antitumoral effects of low dose aspirin. CRC: Colorectal cancer; COX: Cyclooxygenase; PGE2: Prostaglandin E2.

and transforming growth factor- α expression in apparently normal rectal mucosa of individuals with a history of adenomatous polyps^[72]. Further studies using more appropriate methodologies are required to definitively clarify whether LDA affects COX-1 activity in the gastrointestinal tract.

Some investigators have proposed that both COX-1 and COX-2 pathways are involved in intestinal tumorigenesis and that they operate sequentially. This is strongly supported by the findings of experimental animal studies in which the loss of either *COX-1* or *COX-2* genes blocks intestinal polyposis in mouse models of FAP by about 90%^[66,67].

COX-INDEPENDENT MECHANISMS OF ANTITUMOR EFFECTS

Evidence from different lines of research indicates that COX-2 independent mechanisms may also affect apoptosis and cell proliferation in CRC and are sensitive to both aspirin and non-aspirin NSAIDs. Nearly but not all human colon cancer cells express COX-2 and produce prostaglandins^[53,73]. Today, several COX-independent mechanisms of aspirin have been reported that might contribute to its chemopreventive effects in tumorigenesis^[73]. Most of these effects have been found *in vitro* using supra-therapeutic concentrations of aspirin which cannot be obtained in systemic circulation with low doses of the drug. However, no convincing evidence has been obtained to demonstrate that these mechanisms are operative *in vivo*, particularly with low doses of aspirin which have been associated with chemopreventive benefits in randomised clinical trials. In any case, currently available evidence clearly points to the existence of further cellular targets of NSAIDs, in addition to COX-2 inhibition, which may contribute to their antitumor effects. Further studies are needed to completely understand the mechanisms involved.

CONCLUSION

A large body of clinical evidence supports the protective action of aspirin as a chemopreventive agent for different types of cancer, in particular CRC^[6]. Also, increasing indirect evidence has led to the hypothesis that the antiplatelet effect of aspirin is a central mechanism for its antitumor effect^[34,41]. The finding of an apparent maximum chemopreventive efficacy against cancer and atherothrombosis by low-dose aspirin lends support to this hypothesis^[6]. At low doses every 24 h, aspirin acts as a complete and persistent inhibitor of COX-1 in platelets (in pre-systemic circulation)^[47], while causing a limited and rapidly reversible inhibitory effect on COX-2 and/or COX-1 expressed in nucleated cells^[39]. Despite uncertainty about the precise mechanisms that underlie aspirin's anticancer benefit, the evidence supporting its effectiveness for the prevention of CRC is substantial; daily aspirin for at least 5 years has been shown to reduce the 20-year risk of CRC by 32% and 20-year mortality by 43%^[13]. Therefore, the potential benefit of aspirin in both CVD and prevention of cancer at multiple sites may favor its use for broader chronic disease prevention. It is likely that the benefits in terms of morbidity and mortality will outweigh concerns about gastrointestinal bleeding, which is rarely life threatening, and cerebral bleeding, which is extremely uncommon. Health authorities should consider the possibility of extending recommendations on the routine use of aspirin, taking into account its beneficial effects in cardiovascular disease and cancer prevention.

Extensive translational medical research is required to confirm the hypothesis of platelet-mediated colon tumorigenesis. Importantly, these studies will need to address the current uncertainty concerning the optimal aspirin dose, the dosing regimen for cancer prevention, the possible contribution of individual genetic cancer susceptibility to aspirin response^[74] and also the target

population most likely to benefit from daily aspirin use.

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- 16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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Contents

Quarterly Volume 5 Number 2 May 6, 2014

EDITORIAL

- 50 Colonoscopy preparation-induced disorders in renal function and electrolytes
Florentin M, Liamis G, Elisaf MS
- 55 Endoscopic ultrasonography: Challenges and opportunities in the developing world
Ahmed F

REVIEW

- 57 Histamine₂-receptor antagonists: Rapid development of tachyphylaxis with repeat dosing
McRorie JW, Kirby JA, Miner PB
- 63 Use of thiopurines in inflammatory bowel disease: Safety issues
Konidari A, El Matary W
- 77 Understanding and treating refractory constipation
Bassotti G, Blandizzi C
- 86 Esophageal motility abnormalities in gastroesophageal reflux disease
Martinucci I, de Bortoli N, Giacchino M, Bodini G, Marabotto E, Marchi S, Savarino V, Savarino E

META-ANALYSIS

- 97 EVS vs TIPS shunt for gastric variceal bleeding in patients with cirrhosis: A meta-analysis
Bai M, Qi XS, Yang ZP, Wu KC, Fan DM, Han GH

Contents

World Journal of Gastrointestinal Pharmacology and Therapeutics

Volume 5 Number 2 May 6, 2014

APPENDIX

I-V Instructions to authors

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Colonoscopy preparation-induced disorders in renal function and electrolytes

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Abstract

Colonoscopy and flexible sigmoidoscopy are commonly used mainly for colon cancer screening and detection, but also in several other situations such as inflammatory bowel disease (for diagnosis and follow up) and gastrointestinal hemorrhage. Bowel cleansing preparations mainly include polyethylene glycol and oral sodium phosphate solutions, with the later being most frequently used due to better toleration from patients. Despite their favourable safety profile these agents have been associated with renal function deterioration and electrolyte disorders, some of which were serious or even fatal. The present paper discusses the complications associated with colonoscopy preparation agents.

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Key words: Oral sodium phosphate; Hyperphosphatemia; Hypocalcemia; Phosphate nephropathy

Core tip: Despite the important diagnostic and clinical utility of colonoscopy, serious electrolyte disorders and impaired kidney function have been observed with the agents used for bowel cleansing. The aim of this paper

is to present these complications and discuss their prevention and management.

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Colonoscopy is widely used worldwide particularly for colon cancer screening and detection. The use of bowel cleansing agents one or two days prior the procedure improves its diagnostic utility. Various preparations have been developed throughout the years, but few of them are currently being used, specifically polyethylene glycol and oral sodium phosphate (OSP) solutions, because of their ease of use and safety profile^[1]. As minimal volume of oral intake is required with OSP, this is associated with less patient discomfort, greater compliance and improved colonic cleansing compared with polyethylene glycol^[2]. However, serious electrolyte disorders and impaired kidney function have been observed with these agents and particularly with OSP. The aim of this paper is to present these complications and discuss their prevention and management. Disturbances in electrolyte levels with less frequently used bowel cleansing agents are also mentioned.

KIDNEY FUNCTION IMPAIRMENT

Two distinct patterns of kidney injury induced by sodium phosphate preparations (phosphate nephropathy) have been described; early symptomatic and late insidious^[3]. In the former patients present acutely with symptoms of hypocalcemia (e.g., abdominal pain, vomiting, muscle cramping, dizziness, lethargy, confusion, seizures, tetany, cardiovascular collapse) within hours to a few days after sodium phosphate administration, either p.o.s or as en-

ema^[4,5]. Acute renal failure along with marked hyperphosphatemia and hypocalcemia are the hallmarks of this clinical entity. The second pattern of kidney injury has an insidious onset and becomes obvious a few days to several weeks or months after sodium phosphate ingestion^[6]. The administration of laxatives and sodium phosphate enemas generally increases phosphorus and decreases calcium concentration due to rapid phosphate absorption and binding to serum calcium, respectively^[4,7-9]. Interestingly, in a study including 100 adults without predisposing conditions for kidney injury or electrolyte disorders, hyperphosphatemia occurred in 87% of them the day after colonic cleansing^[10]. Phosphate concentration correlated inversely with glomerular filtration rate (GFR), total body water and weight^[10]. Indeed, well-hydrated adults with normal renal function usually tolerate this amount of phosphate and infrequently develop symptomatic hyperphosphatemia and hypocalcemia^[6,11].

Kidney function improved or even returned to baseline within one month in most of the patients with the early symptomatic type of renal injury; however, chronic kidney disease and death have also been reported^[3]. The pathological diagnosis remains unknown as no biopsies were performed in these cases. A possible explanation for kidney function deterioration is that excessive hyperphosphatemia is directly tubulotoxic^[3].

Predisposing factors for renal injury and hyperphosphatemia are old age, female sex, impaired renal function (which diminishes phosphate clearance), diabetes mellitus, dehydration, decreased bowel motility or vitamin D supplementation which lead to increased intestinal absorption of phosphorus, repeated OSP dosing, hypertension, treatment with inhibitors of renin-angiotensin-aldosterone-system (RAAS) or diuretics and hyperparathyroidism^[10,12]. The greater increase in phosphorus levels in the elderly may be related to the subclinical loss of renal function and/or the altered absorption due to increased intestinal transit time^[13]. Importantly, if the recommended dose of 60 g (90 mL) of OSP is surpassed or the interval between doses is < 5 h, severe hyperphosphatemia may develop^[4,8].

Treatment aims at phosphate absorption reduction by oral phosphate binders, increased phosphate clearance and correction of dehydration and subsequently hypocalcemia. Whether calcium supplement should be used is a matter of debate, as renal calcium-phosphate precipitation may further deteriorate renal function^[4]. The rapid correction of hypocalcemia and hyperphosphatemia seems to be able to prevent chronic kidney injury and return renal function to baseline.

The second type of phosphate nephropathy is frequently discovered incidentally at follow-up or due to the occurrence of nonspecific symptoms, such as malaise, nausea or vomiting. In fact, the true incidence of this entity remains unknown as it often goes under recognized. Affected patients present with gradual onset renal insufficiency and low-grade proteinuria, usually < 1 g/d. Phosphorus and calcium levels are normal or near normal at the time of diagnosis^[3].

Phosphate nephropathy after OSP administration seems to be a direct consequence of the acute phosphate load and subsequent increase in serum phosphorus level. The decrease in plasma calcium associated with OSP-induced hyperphosphatemia is the result of calcium binding to the elevated phosphorus, leading to the tubular deposition of calcium phosphate^[4]. Calcium-phosphate product (CPP) is generally regarded as an indicator of the risk of calcium phosphate precipitation in the kidney. The normal range for CPP is 21-46. A mean peak of 71.28 is transiently reached after OSP administration^[14].

It has been suggested that the tubular fluid in the loop of Henle becomes supersaturated in calcium and phosphate ions after OSP administration, resulting in the production of a solid-phase material, probably immature apatite^[15]. The calcium phosphate crystals potentially stimulate inflammatory reactions and cellular damage^[16,17].

These assumptions appear to be confirmed histopathologically. In patients with the late-onset phosphate nephropathy the histologic hallmark is nephrocalcinosis. This is characterized by abundant renal parenchymal deposits of calcium phosphate in distal tubules and collecting ducts along with chronic tubulointerstitial injury; in contrast, glomeruli and blood vessels remain almost intact^[3]. Interestingly, acute tubular cell injury has been observed in tubules both with and without calcium phosphate deposition^[3].

We should note that despite the normal baseline renal function, patients frequently have risk factors for kidney function deterioration and/or electrolyte abnormalities. In fact, nephrocalcinosis is frequently encountered in patients with a propensity for hypercalcemia, such as hyperparathyroidism, increased bone turnover, excess intake of vitamin D or calcium, hypercalcemia of malignancy, sarcoidosis and distal renal tubular acidosis^[9]. Furthermore, when urine is concentrated, the urinary level of CPP rises exponentially, increasing the risk of calcification. Therefore, excessive fluid loss without sufficient hydration predisposes to phosphate nephropathy after OSP administration.

The largest case series comes from Markowitz *et al* who presented 21 cases of biopsy-proven phosphate nephropathy associated with OSP administration prior colonoscopy. The baseline creatinine of the patients was 1.0 mg/dL and reached 3.9 mg/dL at a median of one month after the procedure^[9]. Serum calcium was within normal limits, while phosphorus was increased in at least a third of patients (data was available for 16 out of 21 patients), in some of whom excessively so (*e.g.*, 10.2 mg/dL)^[9]. In the follow-up period (mean 16.7 mo) 4 patients developed end-stage renal disease requiring renal replacement therapy, one of whom subsequently underwent renal transplantation. In 16 of the remaining 17 patients an improvement in renal function was observed at the end of follow-up, with a mean serum creatinine of 2.4 mg/dL. We should note, though, that only 4 patients reached a serum creatinine < 2.0 mg/dL and none returned to baseline^[9]. The aforementioned histologic changes of nephrocalcinosis were observed in all biopsies^[9].

ELECTROLYTE DISORDERS (OTHER THAN HYPERPHOSPHATEMIA)

Hypocalcemia

A decrease in calcium concentration of 0.3-0.8 mg/dL occurs in all patients after OSP administration due to the subsequent hyperphosphatemia^[5]. Elderly patients are particularly prone to develop hypocalcemia due to impaired kidney function, vitamin D deficiency and reduced intestinal absorption^[18]. Calcium levels are usually normalized within 24 h^[19].

Hypokalemia

A frequent electrolyte disorder associated with OSP use is hypokalemia, with an incidence of 20%-30%^[2], which, importantly, is doubled (56%) in hospitalized patients over 65 years old^[18]. Potassium losses are gastrointestinal and renal. The former are due to sodium and potassium exchange across the colonic epithelium and/or diarrhea; the latter may be accompanied by metabolic acidosis because of concurrent bicarbonate loss^[5,20]. Potassium is excreted by the kidneys as a consequence of hyperphosphaturia-induced luminal electronegativity^[21] and volume contraction-induced secondary aldosteronism^[4,22]. We should note that the combination of hypokalemia and hypocalcemia increase the risk of QT interval prolongation. Furthermore, in the presence of cardiac disease and/or arrhythmia ventricular tachycardia may be triggered by OSP-induced hypokalemia^[23].

Hypernatremia

Oral ingestion of isotonic bowel cleansing agents does not by itself affect plasma sodium concentration^[14], but in patients with impaired renal handling of water or in old patients with decreased thirst^[24], the risk of hypernatremia exists. In fact, mild, asymptomatic hypernatremia is quite common after OSP use, especially in the elderly. It occurs due to intestinal absorption of sodium from OSP (the standard dose of OSP contains 434 mEq of sodium), dehydration and colonic shifting between sodium and potassium^[4,22]. Severe hypernatremia (in the range of 166 mEq/L) and acute kidney injury was demonstrated in a patient with Alzheimer's and Parkinson's diseases who took 45 mL of OSP, probably because of insufficient fluid intake^[25]. Hypernatremia may also complicate the use of the osmotic cathartics lactulose and sorbitol which are used in the treatment of hepatic encephalopathy and drug intoxications, respectively^[26,27]. In these cases, water is lost in excess of sodium plus potassium (*i.e.*, hypotonic losses), thus leading to increased plasma sodium.

Hyponatremia

Hyponatremia after colonoscopy preparation may occur as a consequence of intravascular volume depletion (mainly from diarrhea) and subsequent excessive thirst, increased water intake together with the bowel preparation agent, renal disease or syndrome of inappropriate antidiuretic hormone release (SIADH)^[28,29]. ADH release

during bowel preparation may be triggered from nausea, pain or anxiety^[30]. Hyponatremia after OSP has been sporadically observed due to excessive free water intake^[31]. Importantly, the affected patients had other risk factors for hyponatremia, *e.g.*, treatment with thiazide diuretics, RAAS inhibitors or antidepressants^[31]. In contrast, polyethylene glycol administration has been associated with serious cases of hyponatremia. Specifically, a 73-year-old woman who had ingested polyethylene glycol experienced gastrointestinal disturbances and tonic-clonic seizures which were attributed to hyponatremia and reversed with lorazepam and correction of sodium levels^[30]. This patient was currently treated with a selective serotonin re-uptake inhibitor (SSRI) and thyroxine for hypothyroidism, but thyroid stimulating (TSH) levels were slightly elevated. Both SSRIs and hypothyroidism are causes of hyponatremia, while nausea is a major stimuli for ADH release. We should stress, though, that hyponatremia has been reported in patients with moderate to severe hypothyroidism. Thus, the imperfect treatment of hypothyroidism does not seem to have affected sodium levels in this patient. Of note, age is another contributing factor for SIADH. In fact, in 60% of 50 hospitalized patients no reason other than age was identified as a cause of SIADH in one study^[32]. Overall, polyethylene glycol induced severe hyponatremia in a background of SSRI use and old age in this case.

Two other patients who experienced nausea, abdominal distension and diarrhea and consumed large amounts of fluids after polyethylene glycol administration, exhibited symptomatic hyponatremia (unconsciousness, seizures)^[33]. The first one was currently taking thiazides and the second one had end stage renal disease and was receiving regular hemodialysis.

Sodium picosulfate/magnesium citrate (Picolax) has also been associated with hyponatremia and indeed in patients without risk factors for sodium concentration abnormalities^[34,35].

Hypomagnesemia

Lower intestinal tract fluids contain up to 15 mEq/L of magnesium; therefore, in cases of protracted diarrhea which frequently happens during colonoscopy preparation, magnesium depletion may occur. Indeed, laxative abuse, even if these contain magnesium, has been associated with hypomagnesemia due to excessive intestinal magnesium loss^[36,37].

Hypermagnesemia

Picolax has been associated with hypermagnesemia, which was attributed to the magnesium load delivered within the agent^[38-40]. This electrolyte disorder does not seem to have serious clinical consequences^[38,39]. Other cleansing agents that do not contain magnesium do not confer an increased risk for hypermagnesemia.

PREVENTION OF ADVERSE EFFECTS

Several precautions may prevent from phosphate ne-

phropathy and electrolyte disturbances in patients undergoing colonoscopy. The OSP dose may be minimized and the interval between OSP doses may be lengthened; these strategies do not diminish the efficacy of the procedure^[41,42]. Of major importance is the sufficient fluid administration to avoid volume depletion and its deleterious consequences. It is also prudent to perform biochemistry tests, including renal function and electrolyte levels, in order to treat any disorders before administering OSP and proceeding to colonoscopy. In cases of significant renal dysfunction and/or electrolyte disorders, the use of OSP should probably be reconsidered. Some authors have suggested the use of alternative agents, at least in the place of the second OSP dose, which seems to be the main culprit for phosphate nephropathy development^[5].

CONCLUSION

Despite the valuable clinical utility of colonoscopy, its preparation possesses excess risk for kidney impairment and electrolyte disorders, particularly in certain patient populations. Taking into account that these adverse effects may be permanent and sometimes life-threatening, it would be prudent to take simple precautions in patients at risk or even avoid the procedure if the associated risk of serious adverse events is considered higher than its benefit.

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Endoscopic ultrasonography: Challenges and opportunities in the developing world

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Abstract

Endoscopic ultrasonography (EUS) has become a vital diagnostic modality for the evaluation of mediastinal lymphadenopathy, pancreatic cysts and masses, anorectal pathology, subepithelial gastrointestinal lesions, and for the staging of many gastrointestinal and pulmonary malignancies. Establishing a EUS program in a developing country presents many challenges. Doing so in Pakistan has led to the identification of the following challenges: initial investment, ongoing costs (particularly fine needle aspiration needle costs), awareness and cytopathology. Endoscopic ultrasonography has revolutionized aspects of the practice of gastroenterology and oncology in the West. This technique is becoming increasingly available in the developing world, where it poses unique challenges to its practice. These challenges include those relating to service initiation and maintenance costs, physician awareness, and on-site cytopathology access. If these issues are anticipated and addressed in ways appropriate to local circumstances, obstacles to the institution of EUS programs can be overcome.

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Key words: Endoscopic ultrasound; Gastroenterology; Cancer; Cytopathology; Developing world

Core tip: Endoscopic ultrasonography has revolutionized aspects of the practice of gastroenterology and oncology in the West. This technique is becoming increasingly available in the developing world, where it poses unique challenges to its practice. These challenges include those relating to service initiation and maintenance costs, physician awareness, and on-site cytopathology access. If these issues are anticipated and addressed in ways appropriate to local circumstances, obstacles to the institution of EUS programs can be overcome.

Ahmed F. Endoscopic ultrasonography: Challenges and opportunities in the developing world. *World J Gastrointest Pharmacol Ther* 2014; 5(2): 55-56 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v5/i2/55.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v5.i2.55>

ENDOSCOPIC ULTRASONOGRAPHY

Endoscopic ultrasonography (EUS) has become a vital diagnostic modality for the evaluation of mediastinal lymphadenopathy, pancreatic cysts and masses, anorectal pathology, subepithelial gastrointestinal lesions, and for the staging of many gastrointestinal and pulmonary malignancies^[1]. Establishing a EUS program in a developing country presents many challenges. Doing so in Pakistan has led to the identification of the following challenges.

Initial investment

The initial investment costs for EUS are greater than that for other endoscopic procedures. A complete EUS service ideally would include a linear echoendoscope, a radial echoendoscope, miniprobe and the ultrasound unit. Such a complete setup would be prohibitively expensive. One option to lower costs would be to buy used instead of new equipment. That, of course, comes with its own

limitations. A better option is to start small and purchase the absolutely essential elements to allow therapeutic procedures and maintain the option of expanding the setup as the service grows and generates funds. Such an approach would require a processor and linear echoendoscope initially. This approach would allow starting the service with the capability to perform fine needle aspiration (FNA).

Ongoing costs

We have found that the largest proportion of the cost of an individual EUS FNA procedure is the FNA needle. Although there are some centers in the developing world that sterilize and reuse these disposable needles, there is no published data on the safety and feasibility of such a practice. From our perspective, the development of cheaper EUS FNA needles should be a priority.

Equipment service

When purchasing new equipment to establish a EUS program, it is vitally important to ensure that one also obtains a good and reliable service contract. We encountered a problem when our EUS processor developed a problem and had to be sent to Singapore. The repair process took more than nine months. Any momentum our program had built by then was lost in those ensuing nine months.

Awareness

The introduction of new procedural techniques requires education of existing practitioners as to its indications and uses. In particular, oncologists, gastroenterologists, pulmonologists, surgeons and infectious disease specialists, as the major referring specialties for EUS procedures, need to be taught about endosonography and its potential relevance to their practices. Some resistance may be inevitable but once successful cases are demonstrated, acceptance can be anticipated. This teaching can take the form of CME sessions, seminars and grand rounds lectures.

Cytopathology

A singular important aspect of endosonography is the ability to do FNA of lesions. Specimens obtained by EUS FNA need to be examined by an experienced cytopathologist to assess sample adequacy as well as cytological diagnosis. Studies have shown that optimal results of EUS FNA are obtained when the cytopathologist is present in the EUS procedure room and able to assess the samples as they are obtained^[2-4]. We have had difficulty in obtaining support from our cytopathologist in being in the procedure room for real-time sample analysis.

Endoscopic ultrasonography has revolutionized aspects of the practice of gastroenterology and oncology in the West. This technique is becoming increasingly available in the developing world, where it poses unique challenges to its practice. These challenges include those relating to service initiation and maintenance costs, physician awareness, and on-site cytopathology access. If these issues are anticipated and addressed in ways appropriate to local circumstances, obstacles to the institution of EUS programs can be overcome.

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Histamine₂-receptor antagonists: Rapid development of tachyphylaxis with repeat dosing

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Abstract

Histamine₂-receptor antagonists (H₂RAs) are available over-the-counter (OTC) for the treatment and prevention of heartburn, but more than occasional, single-dose use can lead to rapid development of tachyphylaxis. The aim of this review is to assess the published evidence regarding the development of tachyphylaxis with repeat usage of H₂RAs. PubMed and SCOPUS were searched across all years to identify clinical studies that examined the development of tachyphylaxis with repeated dosing of H₂RAs. Although a single (first) dose of an H₂RA can be effective for controlling gastric acid and preventing or relieving food-related heartburn, numerous studies confirm that tachyphylaxis, also known as tolerance, is consistently detected at the first time point assessed after the first dose, including the second day and/or second dose. Even if symptom relief is achieved with an H₂RA, it may be due to desensitization of the esophagus to acid exposure, potentially providing symptom relief without significantly decreasing esophageal acid exposure. When recommending OTC drugs for treatment of frequent heartburn, clinicians should be aware of the potential for rapid development of

tachyphylaxis in patients who use H₂RAs for 2 or more consecutive days. Even if symptom relief is achieved, it may be due to desensitization of the esophagus to acid by the H₂RA, potentially providing symptom relief without significantly decreasing esophageal acid exposure. Other strategies, such as an OTC proton pump inhibitor, may be needed to optimize management of frequent heartburn.

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Key words: Histamine₂-receptor antagonist; Tachyphylaxis; Tolerance; Gastric pH

Core tip: Histamine₂-receptor antagonists (H₂RAs) are available over-the-counter (OTC) for the treatment and prevention of heartburn, but recommendations for use should be limited to those with infrequent heartburn. A single dose of an H₂RA can be effective for controlling gastric acid and preventing or relieving heartburn, but tolerance (tachyphylaxis) develops rapidly, and is evident by the second day/second dose. Even if symptom relief is achieved, it may be due to desensitization of the esophagus to acid by the H₂RA, potentially providing symptom relief without significantly decreasing esophageal acid exposure. For frequent heartburn (≥ 2 d/wk), an OTC proton pump inhibitor should be considered.

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INTRODUCTION

Heartburn is a common complaint^[1-4]. In a recent review

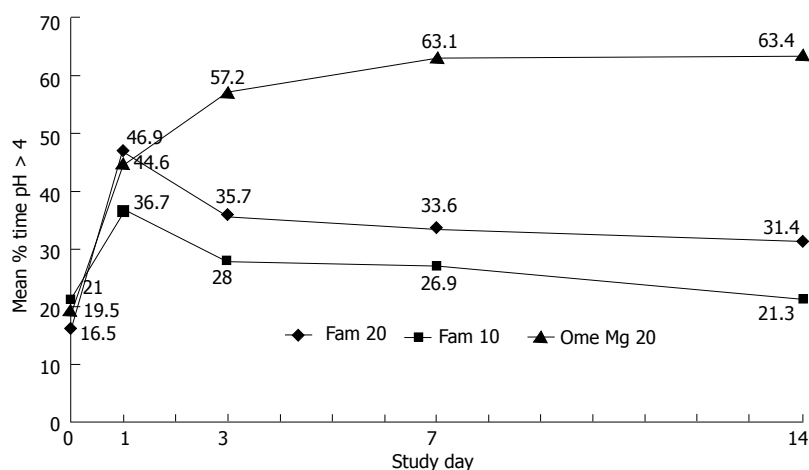


Figure 1 Famotidine vs omeprazole for 14-d gastric acid control. Mean percentage of time gastric pH > 4 across 14 d of dosing in subjects with frequent heartburn^[14]. Famotidine (Fam) 10 mg or 20 mg was dosed twice a day. Omeprazole (Ome Mg 20) was dosed once a day (omeprazole-magnesium 20.6 mg). Gastric pH was assessed for 24-h on day 0 (baseline) and dosing days 1, 3, 7 and 14. Both famotidine doses showed a rapid decline in gastric acid control by dosing day 3, followed by a more gradual decline across 14-d of dosing. Omeprazole showed similar gastric acid control to famotidine 20 mg on dosing day 1, with an increase in gastric acid control over the first several days, followed by a sustained effect across 14-d of dosing (reprinted with permission^[14]).

of 29 published studies, the range of prevalence for experiencing heartburn at least once per week was estimated at 18%-28% in North America, 9%-26% in Europe, 3%-8% in East Asia, 9%-33% in the Middle East, 12% in Australia and 23% in South America^[5]. More than 50 million Americans experience frequent heartburn, defined as occurring at least 2 d a week^[2]. The majority of adults with frequent heartburn have consulted a healthcare provider^[6], but they primarily rely on over-the-counter (OTC) heartburn remedies to manage their symptoms. Therefore, it is important that healthcare providers understand the benefits and limitations of different heartburn remedies.

The mainstay of pharmacologic therapy for frequent heartburn is acid suppressive therapy^[7]. All OTC heartburn therapies may provide a benefit when taken as directed, but there are important differences among the available therapies. Antacids act to rapidly but transiently neutralize acid that has refluxed into the esophagus^[8]. Histamine₂-receptor antagonists (H₂RAs) slow gastric acid production by competitively and reversibly binding to one of the pathways for stimulation of acid production, the H₂ receptors on parietal cells^[9,10]. H₂RAs act relatively quickly, with gastric pH rising within 30 min of a single dose, and have a longer duration of action (up to 10 h) than antacids^[9-11]. Proton pump inhibitors (PPIs) suppress acid production by binding to and inhibiting the H⁺/K⁺ ATPase enzyme system ("proton pump", the final step in acid production) at the secretory surface of the gastric parietal cell^[11,12]. PPIs inhibit gastric acid secretion by all known stimuli and are more potent anti-secretory agents than H₂RAs^[11-14]. Proton pump inhibitors have a slower onset of action (1-2 h), but exhibit comparable 24-h acid control to H₂RAs on dosing day 1, and reach peak effect after several days, providing sustained acid suppression throughout the 14 d course of therapy (Figure 1)^[14-16].

Owing to their pharmacodynamic profiles, both antacids and OTC H₂RAs are indicated for on-demand therapy when relief of an existing episode of heartburn is desired^[8-10,17]. An OTC H₂RA can also be useful for preventing symptoms that are associated with eating food or drinking beverages that cause heartburn^[9,10]. Both ant-

acids and H₂RAs are indicated for a maximum of 14 d of therapy, after which consumers with persisting symptoms should seek advice from a physician^[9,10,17]. In contrast, the OTC PPIs are indicated to treat frequent heartburn (occurring 2 or more days weekly), and are indicated for once daily use every day for 14 d, with a repeat 14-d course every 4 mo^[12,13]. As with antacid and OTC H₂RAs, consumers with symptoms persisting beyond 14 d are encouraged to consult a physician. Any patient presenting with "alarm" symptoms (*e.g.*, dysphagia, odynophagia, bleeding, weight loss, anemia)^[6] may have a more serious condition and should be referred to a physician for further evaluation. With the availability of OTC acid-suppressive therapies, it is important for health professionals to appreciate the strengths and weaknesses of these agents in order to help guide effective consumer-directed management of heartburn.

RESEARCH

We conducted a comprehensive search of PubMed and SCOPUS scientific databases across all years to identify clinical studies that examined the development of tachyphylaxis with repeated dosing of H₂RAs. The terms "histamine receptor antagonist", "H₂RA", "tachyphylaxis", "tolerance", "intra-gastric pH", "gastric acid" and "heartburn" were used to identify clinical studies. Additionally, the reference section of each identified publication was explored for publications not identified in the PubMed and SCOPUS searches. Published clinical studies that assessed intra-gastric pH and/or gastric acid production following multiple days of dosing of an H₂RA were included, and data regarding H₂RA dose, timing of dosing, sample size, gastric acid production and/or intra-gastric pH were extracted and examined.

RESULTS

Fifteen publications, reporting a total of 18 clinical studies, were identified for this review, and the details of these studies are summarized in Table 1. With repeated doses of an H₂RA, tachyphylaxis was consistently observed by the first time period tested after the initial dose, includ-

Table 1 Intra-gastric pH studies demonstrating histamine₂-receptor antagonists¹ tachyphylaxis

Ref.	Design	Treatment regimen	Dosing duration	n	Days pH recorded	Tachyphylaxis observed
Wilder-Smith <i>et al</i> ^[38] , 1990	OL	Famotidine 40 mg QD	28 d	14	1, 14, 28	Day 14
		Ranitidine 300 mg QID	7 d	10	1, 7, 28	Day 7
	R, PC, DB, crossover	Ranitidine 300 mg QHS	21 d			
		Ranitidine 300 mg TID	14 d	14	1, 14	Day 14
Smith <i>et al</i> ^[39] , 1990	R, PC, DB, crossover	Ranitidine 300 mg QHS				Day 14
		Famotidine 40 mg QD	28 d	13	0, 1, 14, 28	Day 14
	R, PC, crossover	Ranitidine 300 mg QID	7 d	13	1, 8	Day 8
		Ranitidine 300 mg QHS	21 d			Day 8
Nwokolo <i>et al</i> ^[24] , 1990	R, OL	Cimetidine 800 mg QD	29 d	48	0, 1, 15, 29	Day 15
		Nizatidine 300 mg QD				Day 15
		Famotidine 40 mg QD				Day 15
		Ranitidine 150 mg BID				Day 15
		Ranitidine 150 mg QD				Day 15
		Ranitidine 300 mg QD				Day 15
Nwokolo <i>et al</i> ^[40] , 1991	R, DB, PC	Ranitidine 150 mg QHS	5 mo	17	1, 29, 57, 85, 113, 141	Day 29
		Ranitidine 150 mg prn				Day 29
Wilder-Smith <i>et al</i> ^[23] , 1992	R, PC, blinded, crossover	Ranitidine IV 0.25 mg/kg per hour	6 d	10	1, 6	Day 6
		Ranitidine IV by pH-feedback, up to 800 mg/24 h				Day 6
		Ranitidine 300 mg PO QID				Day 6
Merki <i>et al</i> ^[31] , 1993	R, PC, crossover	Ranitidine IV ≤ 600 mg/24 h, pre/post 300 mg QD for 9 d	9 d	11	1, 9	Day 9
Merki <i>et al</i> ^[30] , 1994	R, DB, crossover	Ranitidine IV, ≤ 576 mg/24 h	3 d	12	1, 3	Day 3
		Omeprazole IV, ≤ 288 mg/24 h				Not observed
Hurlimann <i>et al</i> ^[22] , 1994	R, DB, parallel	Ranitidine 150 mg QID	14 d	28	1, 2, 7, 14	Day 2
		Omeprazole 40 mg QD				Not observed
Lachman <i>et al</i> ^[41] , 2000	OL	Ranitidine 150 mg QID	5 d	28	1, 5	Day 5
Komazawa <i>et al</i> ^[20] , 2003	R, crossover	Ranitidine 150 mg BID	14 d	7	1, 14	Day 14
		Famotidine 150 mg BID				Day 14
Hsu <i>et al</i> ^[18] , 2004	R, OL	Cimetidine IV 1200 mg/24 h	5 d	80	1, 2, 3, 4, 5	Day 2
		Ranitidine IV 200 mg/24 h				Day 2
		Omeprazole IV 40 mg BID				Not observed
		Famotidine 10 mg BID	14 d	31	1, 3, 7, 14	Day 3
Miner <i>et al</i> ^[14] , 2006	R, PC, crossover	Famotidine 20 mg QD				Day 3
		Omeprazole 20 mg QD				Not observed
Shimatani <i>et al</i> ^[25] , 2007	R, OL, crossover	Famotidine 20 mg BID	15 d	8	1, 8, 15	Day 8
		Omeprazole 10 mg QD			1, 8, 15	Not observed
Fändriks <i>et al</i> ^[16] , 2007	R, crossover	Famotidine 10 mg	8 d	8	1, 8	Day 8
		Omeprazole 20 mg				Not observed
Ono <i>et al</i> ^[15] , 2008	R, crossover	Ranitidine 150 mg BID	10 d	7	-1, 1-7, 11-12	Day 2
		Rabeprazole 20 mg QD			(no dose 8-10)	Not observed

¹Includes only histamine₂-receptor antagonists marketed in the United States. All doses were administered orally unless specified otherwise. BID: Twice daily; HS: Bedtime; IV: Intravenous; OL: Open-label; PC: Placebo-controlled; PRN: As needed; QD: Daily; QID: Four times daily; QHS: Every bedtime; R: Randomized.

ing the second day/second dose^[14-16,18-32]. This effect was reported over a wide range of doses of H₂RAs, including both prescription and OTC doses, and occurred regardless of route of administration (oral and IV). This phenomenon has been observed under both fasting and fed conditions, and in various populations, including healthy subjects, patients with gastro-esophageal reflux disease, and post-surgical patients. While the mechanism for H₂RA tachyphylaxis remains speculative, it may involve the up-regulation of parietal cell receptors for other mediators of acid secretion (*i.e.*, acetylcholine, gastrin), the sensitization of H₂ receptors, the impairment of inhibitory neurohormonal control of acid secretion, and/or an alteration in receptor turnover after chronic competitive inhibition^[19-21].

In a study assessing the gastric acid control of OTC doses of an H₂RA, 31 healthy adults with frequent heartburn (occurring ≥ 2 d/wk) underwent 24-h gastric pH monitoring over a 14-d period while taking famotidine 10 mg twice daily, famotidine 20 mg twice daily, or omeprazole 20 mg once daily, in a double-blind, 3-period crossover fashion^[14]. As depicted in Figure 1, the mean percentage of time gastric pH > 4 was at its highest level on dosing day 1 for both doses of famotidine, then decreased on subsequent days of continued dosing, consistent with H₂RA tachyphylaxis reported previously with prescription doses^[20,22-24]. A similar loss of H₂RA antisecretory effect has been reported in other studies examining OTC doses of famotidine (versus omeprazole; Table 1)^[16,25]. In contrast to H₂RAs, PPIs do not exhibit

tachyphylaxis, but rather show similar efficacy to H₂RAs on dosing day 1, then increase in effectiveness over several days before reaching a sustained plateau effect for the duration of dosing (Figure 1)^[14-16,18,22,25-29].

Tachyphylaxis to H₂RAs does not appear to be progressive, as studies have typically demonstrated no further reduction in antisecretory effect after the initial loss of potency is detected^[21-23]. In one of the longest studies assessing H₂RA tolerance, Nwokolo *et al*^[24] demonstrated no further reduction in acid inhibitory efficacy from 1 to 5 mo of dosing in 17 healthy patients taking ranitidine 150 mg nightly. However, once tachyphylaxis to an H₂RA has developed, increasing the dose does not appear to be effective in overcoming the loss of anti-secretory effect^[29,30]. Merki *et al*^[31] found that the tolerance observed with ranitidine infusion could not be overcome by individual pH-regulated titrated doses of ranitidine of more than 500 mg/24 h, even after three days of treatment.

The physiologic effect of tachyphylaxis has been found to persist for 3 d after H₂RA dosing is discontinued. In one study, ranitidine was dosed twice daily for 7 d, dosing was stopped for 3 d, and then dosing was resumed for 3 d^[15]. Tachyphylaxis was evident by day 2 of dosing in the first period, and the physiological effects of tachyphylaxis were still evident (decreased response to the H₂RA) when dosing was re-initiated after the 3-d hiatus^[15]. This study showed that it took longer than 3 d to recover the beneficial effects of an H₂RA once tachyphylaxis had occurred, supporting that only occasional, isolated doses of an H₂RA provide the maximum benefit of the drug.

CLINICAL IMPLICATIONS OF H₂RA TACHYPHYLAXIS

Although prospective data evaluating the clinical relevance of H₂RA tachyphylaxis for patients with frequent heartburn are limited, decreasing gastric acid production is the accepted mechanism of action for H₂RAs, studies have demonstrated a link between gastric acid suppression and heartburn relief^[32,33], and the percentage of time that the gastric pH is > 4.0 over a 24-h period is frequently used as a surrogate marker for the clinical efficacy of acid suppressive therapies^[14,34,35]. If tachyphylaxis is a class effect for H₂RAs that significantly decreases acid control starting the second day/second dose, why do some frequent heartburn sufferers appear to achieve symptom control with daily dosing? A 2004 study sought to determine if daily doses of ranitidine decreased esophageal sensitivity to chemical and/or mechanical stimulation^[36]. Eighteen patients who experienced functional heartburn (normal esophageal pH) received oral ranitidine 150 mg bid or placebo for 7 consecutive days (double-blind, randomized, crossover study) and underwent mechanical (Barostat balloon distention) and chemical (Bernstein acid infusion) stimulation on study day 1 (90 min post-dose) and study day 7. After a single dose of ranitidine 150 mg, time to pain with esophageal acid

infusion was increased by 29% ($P < 0.05$) and heartburn pain was decreased by 20% (VAS score, $P < 0.06$) and 23% (Likert score, $P < 0.02$) compared with placebo. After 1 wk of ranitidine dosing, subjects still exhibited decreased sensitivity to esophageal acid exposure. In contrast, mechanical (balloon distention) sensory parameters were not altered by ranitidine^[36]. These data show that an H₂RA can significantly decrease esophageal sensitivity to acid exposure, potentially providing symptom relief without significantly decreasing esophageal acid exposure.

Taken together, these data support that patients with frequent heartburn may be better managed by daily use of an OTC PPI, rather than repeated doses of H₂RAs. Two well-controlled clinical studies showed that an OTC dose of omeprazole was superior to OTC doses of ranitidine for the management of frequent heartburn^[28,37]. In a randomized controlled trial in 144 patients with endoscopically verified erosive esophagitis, Sandmark *et al*^[37] found that symptoms had resolved in 51% of patients treated with omeprazole 20 mg daily by the end of the first week of treatment compared with 27% of patients treated with ranitidine 150 mg twice daily ($P = 0.009$). Similar results were found in a larger controlled study in 677 patients with heartburn and either no or mild erosive esophagitis^[28]. Patients were randomized to one of three treatment regimens, and omeprazole was found to be superior to ranitidine, with 55%, 40%, and 26% of patients symptom-free who were treated with omeprazole 20 mg, omeprazole 10 mg, or ranitidine 150 mg, respectively ($P < 0.001$)^[28].

CONCLUSION

While a single dose of an H₂RA can be effective for controlling gastric acid and preventing or relieving isolated heartburn episodes, repeat dosing for more frequent heartburn may lead to the rapid development and sustained effects of tachyphylaxis. Even if symptom relief is achieved with multiple doses of an H₂RA, it may be due to desensitization of the esophagus to acid, potentially providing symptom relief without significantly decreasing esophageal acid exposure. Other OTC strategies, such as a PPI, may be needed to optimize management of frequent heartburn.

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Use of thiopurines in inflammatory bowel disease: Safety issues

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Abstract

Thiopurines are widely used for maintenance treatment of inflammatory bowel disease. Inter-individual variability in clinical response to thiopurines may be attributed to several factors including genetic polymorphisms, severity and chronicity of disease, comorbidities, duration of administration, compliance issues and use of concomitant medication, environmental factors and clinician and patient preferences. The purpose of this review is to summarise the current evidence on thiopurine safety and toxicity, to describe adverse drug events and emphasise the significance of drug interactions, and to discuss the relative safety of thiopurine use in adults, elderly patients, children and pregnant women. Thiopurines are safe to use and well tolerated, however dose adjustment or discontinuation of treatment must be considered in cases of non-response, poor compliance or toxicity. Drug safety, clinical response to treatment and short to long term risks and benefits must be balanced throughout treatment duration for different categories of patients. Treatment should be individu-

alised and stratified according to patient requirements. Enzymatic testing prior to treatment commencement is advised. Surveillance with regular clinic follow-up and monitoring of laboratory markers is important. Data on long term efficacy, safety of thiopurine use and interaction with other disease modifying drugs are lacking, especially in paediatric inflammatory bowel disease. High quality, collaborative clinical research is required so as to inform clinical practice in the future.

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Key words: Thiopurines; Azathioprine; Mercaptopurine; Inflammatory bowel disease; Adult; Children; Therapeutic drug monitoring

Core tip: This review summarises the safety issues around thiopurine use in adult and paediatric inflammatory bowel disease. Adverse drug effects, toxicity and malignancy risks, interactions with concomitant medications, clinical and laboratory drug surveillance and value of pharmacogenetics in therapeutic drug monitoring are discussed.

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INTRODUCTION

Thiopurines are commonly used for maintenance of clinical remission in inflammatory bowel disease (IBD)^[1,2]. They include azathioprine, mercaptopurine (MP) and thioguanine (TG). The onset of their action may vary from 4 to 16 wk^[3-5]. Thiopurine induce apoptosis of antigen specific T cells following repetitive encounters with

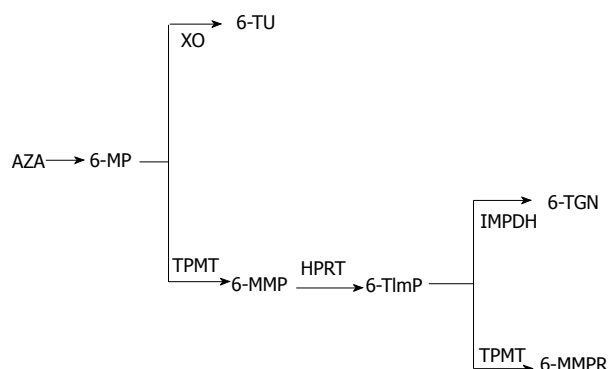


Figure 1 Metabolism of azathioprine and mercaptopurine. AZA: Azathioprine; MP: Mercaptopurine; XO: Xanthine oxidase; 6-TU: 6-thiouric acid; TPMT: Thiopurine methyltransferase; 6-MMP: 6-methylmercaptopurine; HPRT: Hypoxanthine phosphoribosyltransferase; 6-TIMP: 6-thioinosino-5' monophosphate; IMPDH: Inosine monophosphate dehydrogenase; 6-MMPR: 6-methylmercaptopurine ribonucleotides; 6-TGN: 6-thioguanine nucleotides.

the antigen over a prolonged period of time^[6]. Continued medium to long term use is recommended^[7], however there is lack of evidence about optimal treatment duration so as to sustain the therapeutic efficacy in both Crohn's disease (CD) and ulcerative colitis (UC)^[8-11].

Safety issues and risk/benefit analysis must be considered prior to and during treatment with thiopurines, therefore regular follow up for detection of poor response, dose adjustment or treatment modification is necessary. Surveillance is essential for prompt identification of adverse drug events, thiopurine toxicity, interactions with concomitant medication and loss of response over time. Factors including genetic differences, age, disease duration and severity, comorbidities and the environment may influence treatment efficacy and safety^[12-14].

MECHANISM OF ACTION OF THIOPURINES

Thiopurines are structural purine analogues which, when orally administered, are absorbed across the gastrointestinal tract epithelium and carried by the portal venous system to the liver before entering systemic circulation. Trials of intravenous thiopurine infusions have been reported^[15,16]. Azathioprine is a pro-drug with good oral bioavailability and long duration of action. In the liver, azathioprine undergoes enzymatic^[17,18] and non-enzymatic reduction in the presence of glutathione and releases MP and 6-TG. There is a paucity of evidence on the use of thioguanine^[19], but its use has been associated with severe irreversible hepatotoxicity and therefore is not currently recommended outside a clinical trial setting^[20]. Small scale retrospective cohort studies by van Asseldonk *et al*^[21] and Herrlinger *et al*^[22] advocate short term efficacy of thioguanine for maintenance of remission; however the risk of hepatotoxicity outweighs potential clinical benefits. MP is converted to thioinosine monophosphate (tIMP) by hypoxanthine guanine phosphoribosyltransferase (HGPRT); tIMP is either converted to inactive inosine-triphosphate

(ITP by ITPase) or 6TImP (6 thioinosino-5' monophosphate), by thiopurine methyltransferase (TPMT), which inhibits nucleic acid synthesis. Through this cytotoxic effect on dividing cells, thiopurines inhibit clonal proliferation during the induction phase of the adaptive immune response; as a consequence, antibody and cell mediated immune responses of the effector phase are also suppressed^[23]. 6-TG is ribosylated and phosphorylated by HGPRT and irreversibly inhibits 6-thioguanine nucleotides (6-TGN) production. Various metabolites are enzymatically produced during this process: mainly active metabolites are 6-TGN, which mediate the pharmacologic effect of thiopurines, and 6-methylmercaptopurine ribonucleotides (6-MMPR) which are formed by TPMT and principally mediate thiopurine induced hepatotoxicity (Figure 1).

The immunosuppressive effect of thiopurines has been reported to be exerted by alternative mechanisms; for instance by gene expression suppression of inflammatory genes such as $\alpha 4$ -integrin, tumour necrosis factor (TNF) ligand superfamily member 10 and TNF receptor superfamily member 7^[24]. Other proposed in vitro mechanism by Tiede *et al*^[25] is through inhibition of GTPase Rac1 activation by azathioprine metabolite 6-thioguanine triphosphate (6-Thio-GTP) in CD4⁺ human T lymphocytes^[26].

THERAPEUTIC EFFECTIVENESS OF THIOPURINES

Recent Cochrane reviews have highlighted that azathioprine is beneficial for maintenance but not induction of remission in active CD and UC^[10,27]. Thiopurines exert indirect steroid sparing effect due to their effectiveness in sustaining prolonged clinical remission^[10,28,29], especially when used in moderate to severe IBD^[30]. Reduced rates of first laparotomy in adults with CD and positive effect in the avoidance of advanced colorectal cancer have been reported^[31,32]. No clear evidence on surgery sparing benefit has been demonstrated in a population based study which investigated the effect of immunosuppressive drugs and surgery rates over time^[33]. Thiopurine use has been shown to improve quality of life in adult patients with IBD^[34,35]. Children also tolerate thiopurines and can achieve prolonged remission^[36,37].

A meta-analysis by French *et al* investigated relapse rates following withdrawal of azathioprine in CD and reported lack of strong evidence in support of continuous thiopurine treatment beyond 18 mo for maintenance of remission in CD^[38]. Fraser *et al* in a 30 years review reported sustained efficacy for five years in adult patients with CD and UC^[8]. A randomised controlled trial by Hawthorne *et al*^[39] reported that early withdrawal of azathioprine in UC patients in clinical remission for at least six months, resulted in significantly higher relapse rate, when compared to patients on minimum of two years continuous treatment. Thiopurines are therefore safe, effective medicines with a pivotal role in the treatment of

Table 1 Reported incidence of thiopurine adverse drug effects

ADE	Incidence
GI symptoms ^[155]	8%
Hepatotoxicity ^[179,180]	3%-15%
NRH ^[51]	0.6%-1.28%
Pancreatitis ^[155]	4%
Leucopenia ^[180]	2%-15%
Myelotoxicity ^[155]	4%
Lymphoma ^[155]	0.1%
Infections ^[180]	0.3%-7.4%
Intolerance ^[57,155]	17%

ADE: Adverse drug effects; GI: Gastrointestinal; NRH: Nodular regenerative hyperplasia.

IBD^[40].

THIOPURINE ADVERSE DRUG REACTIONS: IMPLICATIONS FOR SAFETY

Adverse drug reactions have historically been divided in idiosyncratic and intrinsic (dose dependent). The first type is probably immune mediated, unpredictable and can occur within few weeks of treatment commencement. Reactions include intolerance and hypersensitivity manifestations, such as malaise, dizziness, vomiting, diarrhoea, fever, myalgia, arthralgia, rash and hypotension^[41]. Rare idiosyncratic reactions include renal impairment^[42], pneumonitis^[43-45], and pancreatitis^[46].

Thiopurine-induced liver dysfunction secondary to methylated intermediate metabolites can manifest as elevated liver enzymes, hepatitis, cholestatic jaundice or hepatic veno-occlusive disease. Liver impairment following thiopurine administration has been divided into three categories: hypersensitivity, idiosyncratic cholestatic reactions, and nodular regenerative hyperplasia (NRH). Characteristic nodules of hypertrophied hepatocytes with adjacent areas of atrophied hepatocytes arise following thiopurine induced sinusoidal endothelial injury or obliterative portal venopathy^[47,48]. Dose-dependent toxicity is possible in cases of NRH^[20,49]. The cumulative incidence of NRH in IBD patients treated with thiopurines is approximately 0.6% and 1.28% at 5 and 10 years respectively^[50]; patients on higher dose of thiopurines have increased risk of NRH^[51]. NRH however can occur in thiopurine-naïve patients with IBD (reported incidence 6%) and IBD may be an independent predisposing risk factor^[51]. Pathogenesis of NRH is obscure. Reported risk factors for NRH, other than thiopurine dose in patients with IBD, are male gender, older age, stricturing disease and small bowel resection^[50,52,53]. Mild liver impairment presenting with raised liver function tests, which represents the majority of cases, may resolve with or without dose reduction^[20].

Bone marrow toxicity manifesting as myelosuppression and/or aplasia is the most severe haematological adverse drug reaction and leads to discontinuation of treatment^[54]. Haematological toxicity presenting as leucopenia

is presumed to be avoided with reduced doses^[55], however there are studies which do not support that this type of toxicity is dose related^[56,57]. Patients with UC treated with immunomodulators have demonstrated a higher incidence of infections than patients not on thiopurines^[58], due to impaired immune response. In particular, patients with inflammatory bowel disease are at increased risk of acquiring opportunistic infections (OR = 3.1; 95%CI: 1.7-5.5)^[59], for instance cytomegalovirus (CMV) infection (especially pneumonitis or enteritis), which may cause aggravation of underlying disease and failure of immunosuppressive treatment^[60]. Pneumocystis jiroveci pneumonitis is an opportunistic infection with severe morbidity. Antibiotic prophylaxis should be considered on a case by case basis, especially in patients with advanced age, increased disease severity and extensive disease^[61]. Parasitic or other fungal infections are extremely rare in IBD^[62].

Due to thiopurine induced immunosuppression, 3-5 yearly pneumococcal and annual prophylactic influenza vaccinations with trivalent inactivated vaccine are recommended^[62]. International consensus guidance recommends varicella, tuberculosis, Hepatitis B, Hepatitis C, HIV screening and prophylactic vaccination (not BCG) prior to treatment with immunomodulator^[62,63].

The incidence of adverse drug events are summarised in Table 1.

SAFETY OF THIOPURINES IN ELDERLY PATIENTS

In the elderly population, there is good evidence of functional alterations in cells from the innate and adaptive immune systems resulting in a state of dysregulated immune function and increased susceptibility to infection^[64-66]. There are data to demonstrate that mainly bacterial infections (urinary tract infections and community acquired pneumonia), and few viral infections, such as influenza, are more prevalent and severe in the elderly patients with IBD than in younger adults^[66].

Elderly patients (> 65 years of age) on immunosuppressants are at increased risk of developing malignancies^[67], when compared to younger adults and children. Past or co-existing comorbidities, such as previous cancer, predispose them to additional malignancy risk, for example lymphoma^[68].

In elderly patients therefore, the benefit of long term (over 5 years) thiopurine use may not outweigh the risks^[69]; disease chronicity and severity may of course exert a confounding effect in observed outcomes in this population. Adverse drug events such as NRH have been more frequently reported in older age^[51].

SAFETY OF THIOPURINES IN CHILDREN

Variation in disease management is common in paediatric IBD due to lack of high quality randomised controlled trials in children. The ongoing development of service networks will accelerate collaborative standardised re-

search in children with IBD for generation of high quality evidence and improvement of care^[70].

The thiopurines have been rarely implicated in lymphoproliferative disorders in childhood IBD. The relative risk is 3-4 folds increased, however the absolute risk is very low^[71].

In paediatric IBD, early life onset of disease translates into longstanding disease activity requiring life-long medication^[72]. Paediatric-onset UC has a different phenotype than adult-onset disease with more extensive (pan colitis) and more aggressive disease course. Special consideration in the decision making process about treatment in children must be given to growth, puberty and bone density accrual. A large number of children are at risk for steroid-dependency, therefore steroid sparing strategies with early use of immunomodulators such as thiopurines are recommended in high-risk patients^[73]. On the other hand, the safety profile of immunosuppressive therapy in children stipulates a more conservative approach, with early treatment intensification applied in patients with severe or refractory disease^[12]. Punati *et al.*^[30] published a prospective multicentre observational study where early thiopurine use was associated with reduced corticosteroid exposure and possibly fewer hospitalizations per patient. Similarly, Riello *et al.*^[37], in a retrospective study of 105 children treated with thiopurines, reported that the majority of patients who were in steroid-free remission by 12 mo, remained in prolonged remission.

SAFETY OF THIOPURINES DURING PREGNANCY

Use of thiopurines is not an absolute contraindication throughout pregnancy; however data on their safety profile during pregnancy is insufficient. The human placenta is believed to act as a barrier; a human placental perfusion model has been used to demonstrate the binding of the drug to placental tissue. Maternal pharmacokinetic parameters could restrict the fetal exposure to drug metabolite^[74]. Fetal 6-TGN levels correlated with maternal 6-TGN in a prospective study of 28 pregnant women on thiopurines; maternal thiopurine metabolism was affected during pregnancy. 60% of the neonates were noted to be anaemic at birth^[75]. Casanova *et al.*^[76] conducted a retrospective multicentre study with 571 pregnant women and found no increase in adverse outcomes for pregnant women and newborns following exposure to thiopurine. There is no evidence whether IBD or medical therapy [5-aminosalicylates (5-ASA), thiopurines, corticosteroids] during pregnancy increase the risk of major congenital anomalies in the off-springs; this was recently shown in a retrospective case control study of women with IBD ($n = 1703$) and women without the disease ($n = 38481$)^[77]. Akbari *et al.*^[78] identified the risk of preterm delivery in a recent meta-analysis which reviewed the effects of thiopurines on birth outcomes of female and male patients with IBD; despite this, exposure to thiopurine at the time of conception was not associated with increased risk of

congenital abnormalities. The development and immune function of children exposed to thiopurines in utero has not been affected until the age of six years^[79].

RISK OF MALIGNANCY AND THIOPURINES: IMPLICATIONS FOR SAFETY

Thiopurines can increase the incidence of malignancies by different plausible mechanisms, such as by incorporating 'rogue' thiopurine nucleotides in the DNA or by rendering DNA highly sensitive to ultraviolet radiation, thereby promoting mutagenesis^[80-82].

Four to six fold increased risk of hematologic malignancies^[83] has been observed in patients treated with azathioprine; however no causality has been established to date. Risk increases gradually over successive years of therapy and discontinuation of thiopurine therapy reduces the risk^[84]. The absolute risk of lymphoma however is low; balancing the potential risk of lymphoma against the risk of undertreatment IBD should inform decision making in the medical management of IBD^[85].

Latent or primary opportunistic EBV infection during immunosuppressive therapy may result in post-transplant like lymphoproliferative disease or haemophagocytic lymphohistiocytosis^[86,87]; the latter has also been reported following CMV infection^[88]. 5% of EBV negative peripheral T cell lymphomas reported in IBD patients are non-Hodgkin's hepatosplenic T cell lymphomas (HSTCL) with poor prognosis^[89,90]. A systematic review on medication, therapy duration and patient age in reported cases of HSTCL concluded that most patients were male, younger than 35 years old, and had received at least 2 years of combined treatment with anti-TNF agent and thiopurine^[91] or anti-TNF monotherapy^[92].

Relative increase in non-melanoma skin cancer was shown in a large retrospective cohort study of over 50000 adult patients with IBD, conducted by Long *et al.*^[93]; thiopurine treatment of a minimum of three months has been associated with increased risk of non-melanoma skin cancers compared to controls. Melanoma skin cancer may increase by 37% according to a meta-analysis investigating the risk of melanoma in a cohort of 172837 patients with IBD; however no specific increase in risk has been associated with thiopurine treatment *per se*^[94].

Since the beginning of this century, there is a controversy in the medical literature with regards to increased risk of gastrointestinal neoplasia in patients with IBD.

A meta-analysis by Eaden *et al.* in 2001 investigated the colorectal cancer risk in UC, and reported a risk of 3% (95%CI: 2.2-3.8) at 10 years, 5.9% (95%CI: 4.3-7.4) at 20 years, and 8.7% (95%CI: 6.4-10.9) at 30 years after diagnosis. A non-significant increase in the risk of colorectal cancer (CRC) by decade of disease was also reported. In 2005, a more recent meta-analysis of population based studies by Jess *et al.* however reported a 2.4 (95%CI: 2.1-2.7) fold increase in CRC risk in patients with UC^[95].

Canavan *et al.*^[96] performed a meta-analysis to ascertain the combined relative risk of gastrointestinal malignancies and reported increased relative risk in CD. The most recent meta-analysis by Lutgens *et al.*^[97] has updated CRC risk in both ulcerative and Crohn's colitis, by investigating time trends, and identifying high-risk modifiers; it has been concluded that the risk of CRC is increased in patients with IBD but not as high as previously reported and that the risk of CRC is significantly higher in patients with longer disease duration, extensive disease, and IBD diagnosis at young age^[97].

Thiopurine treatment did not decrease the risk of colorectal neoplasia in UC ($n = 315$ patients)^[98]. Beaugerie *et al.*^[99] nevertheless conducted a large prospective cohort study with 20000 patients and found that thiopurine treatment significantly lowered the multivariate adjusted hazard ratio for colorectal neoplasia (HR = 0.28, 95%CI: 0.1-0.9; $P = 0.03$). A recent meta-analysis by Gong *et al.*^[100] which addressed the same issue concluded that thiopurines exerted a chemo prophylactic effect and a tendency of reducing advanced colorectal neoplasms in IBD, however due to the heterogeneity of included studies, the authors suggested that the results should be interpreted with caution.

Young female smokers with concomitant 5-aminosalicylic acid and thiopurine exposure^[101] are at increased risk of cervical cancer; it is still unclear whether the disease itself or whether the treatment may predispose to cervical dysplasia^[102]. Evidence to date is inconclusive; no clear increase in relative risk secondary to the use of thiopurines has been shown in the majority of relevant studies, with the exception of a population based case control study by Singh *et al.*^[103], which reported increased risk for cervical dysplasia or cancer in patients on both steroids and immunomodulators. Lees *et al.*^[104] conducted a large case control study where women with IBD were not shown to have increased rates of abnormal cervical smears unless they smoked; this finding was not affected by immunosuppressant therapy or disease phenotype. Cervical cancer surveillance and HPV vaccination is currently recommended^[102].

Treatment with immunosuppressive drugs had no major impact on the increased risk of developing new or recurrent cancer in a prospective cohort of 405 IBD patients with pre-existing history of cancer^[105]. Pasternak *et al.*^[106] conducted a retrospective cohort study and reported that azathioprine treatment in IBD was associated with marginally increased risk for lymphoma and urinary tract cancer, therefore reported an overall increased risk of malignancy with azathioprine treatment (RR = 1.41; 95%CI: 1.15-1.74) interestingly though, previous use of azathioprine or increasing cumulative received doses did not increase the cancer risk. Finally, a meta-analysis of Masunaga *et al.*^[107] investigated whether long-term administration of immunosuppressants in patients with IBD increased the risk of malignancy and concluded that treatment did not increase the overall cancer risk in patients with IBD; drugs other than thiopurines, such as cyclosporine, methotrexate, tacrolimus were included in the analysis.

CLINICAL AND BIOCHEMICAL SURVEILLANCE FOR ENHANCED DRUG SAFETY: THERAPEUTIC DRUG MONITORING

Clinical surveillance and patient follow up are required for identification of adverse drug events throughout the duration of thiopurine treatment; this can be enhanced by regular monitoring of laboratory indices, such as peripheral blood counts, pancreatic and liver function tests^[108]. There is a lack of consensus on the usefulness of active thiopurine metabolite monitoring as surrogate markers of thiopurine efficacy and toxicity. Their use is not widely recommended or adopted^[109] and largely depends on local practices and individual clinician preferences.

Higgs *et al.*^[110] published a meta-analysis about the increased risk of myelosuppression in patients with intermediate TPMT activity and concluded that in spite of controversial findings between studies, "higher 6-TGN levels were generally associated with clinical remission". Gonzalez-Lama conducted a prospective multicentre cohort study which did not support determination of TPMT activity or 6-TGN concentrations for prediction of treatment outcome; no clinically useful serum metabolites threshold value was identified for dose adjustment purpose^[111]. Poor correlation between 6-TGN levels and thiopurine dose has also been reported by other studies^[4,56,112,113], however metabolites levels may contribute to earlier and safer dose tailoring, especially in patients with poor clinical response or non-compliance^[114-117].

Measurement of active thiopurine metabolite levels in addition to regular full blood count monitoring, especially over the first 1-2 mo after treatment commencement, and regularly thereafter has been proposed^[118-120]. 6-MMPR levels and liver enzymes may be used in combination as surrogate markers of hepatotoxicity; a 6-MMPR cut-off value $> 5700 \text{ pmol}/8 \times 10^8 \text{ RBC}$ has been recommended as indication of liver dysfunction^[117,121-123]. There is currently no clear consensus on optimal frequency of blood monitoring^[124].

DRUG INTERACTIONS: IMPLICATIONS FOR THIOPURINE SAFETY PROFILE

Allopurinol

Allopurinol is known to reduce thiopurine-induced hepatotoxicity^[125] and may have a synergistic therapeutic effect to patients who preferentially produce 6-MMPR, rather than 6-TGN^[126-128]. The underlying mechanism remains enigmatic. Different pathways have been proposed: (1) allopurinol inhibition of both xanthine dehydrogenase and TPMT and promotion of 6-TGN production at the expense of 6-MMPR^[129]; (2) TPMT inhibition by allopurinol's active metabolite oxypurinol^[130]; and (3) increased HGPRT activity and subsequent 6-TGN increase^[131]. Co-administration of allopurinol has been reported to al-

low thiopurine dose reduction by up to 75%^[23]; this may enhance efficacy in azathioprine refractory patients and avoid high doses with potential dose dependent adverse events; conversely combined therapy could potentially induce toxicity in azathioprine-naïve patients due to the synergistic effect of these two medicines. Combination therapy with allopurinol is therefore currently not widely used, due to the lack of adequate high grade evidence from double blinded randomised controlled trials assessing the risk-benefit ratios of dual therapy, especially in paediatrics.

5-aminosalicylates

5-ASA have been reported to increase 6-TGN and decrease the production of 6-MMPR in two prospective adult studies^[132,133]. de Boer *et al*^[134] conducted a prospective multicentre pharmacokinetic study and reported a significant dose-dependent increase in 6-TGN levels in cases of 5-ASA co-administration. It was concluded that patients refractory to standard thiopurine therapy may benefit from the co-administration of 5-ASA. A systematic review by Andrews *et al* addressed the clinical outcomes following concomitant 5ASA and thiopurines administration; it was unclear whether combination therapy improved outcomes of disease control, drug toxicity or compliance, but concurrent therapy could decrease colorectal risk at “acceptable cost”^[135]. An increased risk of myelotoxicity has been noted in children with IBD treated with combination therapy^[136]. Nguyen *et al*^[137] in a study of 71 children with IBD, reported more frequently observed lymphopenia and elevated 6-TGN concentrations, without increase in remission rate in patients on combined treatment. A favourable clinical outcome has been described by Tajiri *et al*^[56] in paediatric UC, however a high rate (40%) of myelosuppression was noted. The clinical benefit of combination treatment therefore needs to be further researched and careful weighted against toxicity risk^[132].

Anti-tumour necrosis factor alpha agents

New disease modifying drugs such as infliximab, adalimumab have been increasingly used in adult and paediatric gastroenterology. Infliximab is the first anti-TNF alpha agent ever introduced in the treatment of IBD and therefore has been more widely researched to date. It is a partially humanised monoclonal antibody against tumour necrosis factor alpha (TNF- α), a cytokine mediator of inflammation. Historically anti-TNF alpha drugs were principally introduced as rescue medical therapy in patients with treatment refractory, severe or extensive disease^[138].

A top-down versus a “bottom-up” approach to treatment is the epicentre of attention within the international community of gastroenterologists; early introduction versus rescue use of anti-TNF agents, with or without concomitant thiopurine for prompt treatment intensification and avoidance of disease complications is the dilemma in current clinical practice^[139,140].

Combination therapy with infliximab and azathioprine

has been reported more favourable than monotherapy for induction and maintenance of steroid-free remission, and for avoidance of postoperative recurrence^[140,141]. Higher serum trough concentrations of infliximab, lower anti-infliximab antibodies and better clinical outcomes may occur more frequently in patients receiving combination therapy with azathioprine^[139,142]. Colombel *et al*^[139] specifically reported that combination may be superior to azathioprine or infliximab alone, not only for induction and maintenance of steroid free remission, but also for increased mucosal healing rates in adult patients with moderate to severe CD. The described effect was sustained at one-year follow up. The beneficial effects of combination therapy on mucosal healing, steroid-free remission and sustained increase in quality of life have been reported in both UC^[143] and CD^[144]. Sokol *et al*^[145] conducted a prospective cohort study and reported favourable six month outcomes with regards to disease activity, infliximab dose and need of other anti-TNF alpha agents such as adalimumab.

On the contrary, Lichtenstein *et al*^[146] pooled data from multicentre prospective randomised controlled trials in adult patients with IBD and concluded that concomitant use of immunomodulators-principally thiopurines but also methotrexate in isolated cases with CD did not significantly increase efficacy or alter thiopurine safety; infusion reactions were about 50% less in combined therapy than in infliximab monotherapy.

Van Assche *et al*^[147] conducted an open label randomised controlled trial to evaluate the influence of anti-TNF alpha discontinuation after patients had been in remission for at least six months with combined therapy; no clear benefit of continuing combined therapy beyond six months from clinical remission was demonstrated.

Combination of anti-TNF agents with thiopurines may therefore enhance immunosuppression, however the risk of related adverse drug reactions and toxicity is also enhanced^[148]. Combination therapy may also be associated with higher relative risk of opportunistic infections in UC, but no significantly increased absolute risk of serious infections has been observed^[58,149].

Limited evidence exists on drug interactions between azathioprine and adalimumab. The latter has recently obtained approval by the European Medicines Agency and the United States Food and Drug Administration for use in adult patients with moderate-to-severe, active, refractory UC, who are intolerant to corticosteroids and thiopurines^[150]. Adalimumab is effective in inducing and maintaining remission in patients with active, moderate-to-severe, luminal or perianal CD, or patients with previous loss of response or intolerance to infliximab^[151-153]. A recent prospective case series of twelve adult patients with CD reported that 6-TGN and 6-MMPR were not influenced by concomitant administration of adalimumab during a 12 wk follow up period^[154]. The same study reported no change in TPMT, ITPase or HGPRT enzyme activity after 4 wk of combined treatment. More research is required into the efficacy and safety of combination treatment.

THIOPURINE TOXICITY: IS ALTERNATIVE THIOPURINE USE SAFE?

Chaparro *et al* has recently reported the findings of a large prospective nationwide cohort study from Spain, where 67% of 1026 patients had to discontinue thiopurine treatment due to adverse drug events such as nausea, arthralgia, alopecia, abdominal pain, liver and pancreatic toxicity, infection, leucopenia, myelotoxicity. 37% percent of them were restarted on the same thiopurine. 40% had recurrent side effects; 4% following treatment with the same thiopurine and 36% after introduction of an alternative thiopurine^[155]. Interestingly even in patients with severe complications such as hepatotoxicity, thiopurine re-introduction was tolerated in 74% of cases; abdominal pain recurred in 18% of cases, nausea and arthralgia recurrence close to 50% was noted. 85% of patients demonstrated recurrence of pancreatic toxicity, recurrence rates of infection and bone marrow failure were 80% and 65% respectively. Overall over half of the patients tolerated re-introduction of thiopurine treatment following drug induced side effects.

Ledder *et al*^[146] published a small case series of four adult patients with successful introduction of mercaptopurine following azathioprine induced pancreatitis.

A recent meta-analysis on alternative use of mercaptopurine in adult patients with IBD who suffered azathioprine-induced toxicity ($n = 455$ patients), concluded that favourable outcomes had been observed in two-thirds of patients where trial of mercaptopurine was implemented^[57]. In detail, 62% with gastrointestinal toxicity, 81% with hepatotoxicity and 36% with flu-like illness had been able to tolerate mercaptopurine. Trial of mercaptopurine is therefore advised in cases of azathioprine intolerance, except in patients with severe pancreatitis or bone marrow aplasia. Among patients who discontinued mercaptopurine for further adverse effects, 59% experienced the same adverse effect as they had with azathioprine.

Further studies are required so as to quantify the hepatotoxicity risk associated with thioguanine as an alternative therapy for IBD treatment; metabolism of this non-conventional thiopurine does not generate 6-methyl mercaptopurine^[156].

VALUE OF PHARMACOGENETICS IN THIOPURINE SAFETY MONITORING

Observed genetic polymorphisms have been reported to play pivotal role in the occurrence of adverse drug reactions for various drugs, including thiopurine^[157,158], warfarin^[159], antiepileptic^[160] and anti-retroviral drugs^[161]. Inter-individual variability in drug response reflects differences in genetic polymorphisms which, to some extent, may be responsible for variation in drug metabolism^[116,162].

TPMT is an extensively researched example of the clinical applicability of pharmacogenetics; pre-treatment testing is currently implemented; common variant alleles such as TPMT*2, TPMT*3A, TPMT*3C, TPMT*8 may

give rise to decreased enzyme production; heterozygous or homozygous TPMT deficient patients require decreased dose upon treatment commencement, or thiopurine avoidance respectively. Weinshilboum and Sladek first reported that approximately 0.3% Caucasians have complete deficiency, approximately 10% have low or intermediate activity and about 90% have high activity^[163]. Numerous studies have since verified the differences in TPMT levels and activity between different ethnic group^[164-166].

Pre-treatment determination of TPMT genotype and phenotype may be useful for prediction of thiopurine toxicity; TPMT testing is however not universally implemented by gastroenterologist^[167-169], because evidence on its predictive value for thiopurine toxicity in IBD is still unclear; Colombel *et al*^[170] has reported that the majority of adult patients with myelotoxicity had normal TPMT genotype. The controversy in the medical literature may exist because of the non-absolute concordance between genotype and phenotype (TPMT activity status)^[167,171], and the diversity in the laboratory methods (enzymatic, radiochemical, high performance liquid chromatography assays) used for quantification of TPMT activity in red blood cells^[172]. A systematic review by Booth *et al*^[173] reported that in patients with intermediate and low enzymatic activity, genotyping sensitivity to identify patients with low enzymatic activity ranged from 70.33% to 86.15% (lower-bound 95%CI: 54.52%-70.88%; upper-bound 95%CI: 78.50%-96.33%) and was therefore imprecise. Despite this finding, variant genotype and low TPMT activity were reported to be strongly associated with haematological toxicity.

Additional polymorphic genes implicated in thiopurine metabolism are under investigation for possible effect on effectiveness and safety. These are the ITPase^[174], the guanine monophosphate synthetase (GMPS mediates tIMP conversion to tGMP) and the glutathione S transferases (GST which catalyses MP production from azathioprine)^[175,176].

Smith *et al*^[177] reported that single nucleotide polymorphism (SNP) of aldehyde oxidase (AOX1) c.3404A > G may predict lack of response ($P = 0.035$, OR = 2.54, 95%CI: 1.06-6.13); when combined with TPMT activity, this information allowed stratification of a patient's chance of response to azathioprine, ranging from 86% in patients where both markers were favourable to 33% where both were unfavourable ($P < 0.0001$)^[177].

A common SNP, associated with a dramatically reduced ABCC4 function, has been identified in approximately 14%-18% of the Japanese population. In these patients, the OR of carrying the ABCC4 variant and having leucopenia following thiopurine introduction has been reported to be 3.30 (95%CI: 1.03-10.57; $P = 0.036$)^[178].

In the future, the pharmacogenetic approach may enhance pre-treatment safety and prompt dose adjustment^[179].

CONCLUSION

Safety of thiopurine treatment in IBD stipulates fine tun-

ing between therapeutic efficacy, intolerance and toxicity. This balance must be achieved on a long term basis. Combination therapy with new disease modifying drugs has further modified the safety profile of thiopurines; the importance of such interactions has yet to be confirmed in large studies across all age groups. There is a need for a standardised approach in therapeutic drug monitoring. Further research into disease pathogenesis and pharmacokinetic/pharmacodynamic pathways may identify potentially useful biomarkers for thiopurine safety monitoring. High quality, prospective and collaborative clinical research will establish a robust evidence foundation which will safely inform future clinical practice.

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Understanding and treating refractory constipation

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Abstract

Chronic constipation is a frequently encountered disorder in clinical practice. Most constipated patients benefit from standard medical approaches. However, current therapies may fail in a proportion of patients. These patients deserve better evaluation and thorough investigations before their labeling as refractory to treatment. Indeed, several cases of apparent refractoriness are actually due to misconceptions about constipation, poor basal evaluation (inability to recognize secondary causes of constipation, use of constipating drugs) or inadequate therapeutic regimens. After a careful re-evaluation that takes into account the above factors, a certain percentage of patients can be defined as being actually resistant to first-line medical treatments. These subjects should firstly undergo specific diagnostic examination to ascertain the subtype of constipation. The subsequent therapeutic approach should be then tailored according to their underlying dysfunction. Slow transit patients could benefit from a more robust medical treatment, based on stimulant laxatives (or their combination with osmotic laxatives, particularly over the short-term), enterokinetics (such

as prucalopride) or secretagogues (such as lubiprostone or linaclotide). Patients complaining of obstructed defecation are less likely to show a response to medical treatment and might benefit from biofeedback, when available. When all medical treatments prove to be unsatisfactory, other approaches may be attempted in selected patients (sacral neuromodulation, local injection of botulinum toxin, antegrade continence enemas), although with largely unpredictable outcomes. A further although irreversible step is surgery (subtotal colectomy with ileorectal anastomosis or stapled transanal rectal resection), which may confer some benefit to a few patients with refractoriness to medical treatments.

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Key words: Chronic constipation; Laxatives; Medical treatment; Refractory constipation; Surgical treatment

Core tip: The majority of patients affected by chronic constipation can be managed by conventional therapeutic approaches. However, a subset of constipated patients displays a condition of actual refractoriness to standard medical treatment, even after careful clinical re-evaluation. These patients require more in-depth diagnostic evaluations to ascertain the underlying pathophysiological mechanisms, as well as more intensive, targeted and tailored therapeutic approaches, which may rely on the use of newly released drugs (enterokinetics, enteric secretagogues), rehabilitation (biofeedback), invasive measures (sacral neuromodulation, local injection of botulinum toxin, antegrade continence enemas) and surgical procedures (subtotal colectomy with ileorectal anastomosis or stapled transanal rectal resection).

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INTRODUCTION

Chronic constipation is one of the most frequent complaints faced by physicians during their daily activity^[1] since a relatively large number of subjects in the general population (ranging from about 9% to more than 20%, depending on the geographical area) is or believe to be affected by constipation^[2]. The costs for medical care are high for patients complaining of constipation, from childhood to adulthood^[3]. Constipation is highly prevalent among female subjects^[4] and it has been demonstrated that women with constipation have significantly higher medical care utilization and expenditures compared with women without constipation^[5].

At present, constipated patients can be managed by a variety of medical therapeutic options that yield satisfying results in most cases^[6,7]. However, a subset of constipated patients fails to benefit from conventional (and sometimes even intensive) treatments^[8]. Although these subjects are often regarded as being resistant to therapy, their refractoriness may not be actual in nature but rather results from several factors related to the patient, the physician, or to their false beliefs and misunderstandings, as discussed in detail below. However, after careful re-evaluation, some patients are found to hold a condition of true refractory constipation, which is often a therapeutic challenge and deserves different and more tailored therapeutic approaches, up to demolitive surgical procedures.

The pharmacotherapy of refractory constipation is currently regarded as a challenging area, where the paucity of supportive clinical evidence and the persistence of unmet medical needs demand urgent attention in terms of focused clinical research and consensus by experts. Based on these considerations, the present article intends to review current information on the different approaches to the therapeutic management of refractory constipation, with the primary purpose of fostering the debate on this issue and generating new ideas for future clinical research on the employment of old and new drugs.

GENERAL CONSIDERATIONS

As anticipated above, to date, refractory constipation is suspected when a patient, fulfilling the standard diagnostic criteria for functional constipation^[9] and lacking any alarm feature for organic conditions, fails to improve upon intake of a high-fiber diet and laxatives, usually polyethylene glycol (PEG) or other osmotic agents^[10], the former being superior to lactulose in improving stool frequency, stool consistency and abdominal pain^[11].

When facing a constipated patient complaining of resistance to the above therapeutic approaches, there are several issues which deserve careful consideration before labeling the patient as refractory to standard treatment and going on with further diagnostic evaluations and/or

therapeutic interventions.

Reliability of information and patient compliance

This point could appear tautological in nature but, based on clinical experience, a certain number of patients are labeled as being “refractory” to medical treatment merely because of misunderstandings with the prescribing physician (*i.e.*, poor or altogether complete lack of communication, lack of acceptance of chronicity of the condition, unwillingness to use drugs long-term, scarce understanding of dose regimens, *etc.*) or as a result of misconceptions on the actual nature and relevance of constipation^[12].

Patient expectations

It frequently happens that patients initially classified as refractory to treatment at a more accurate medical interview disclose that they discontinued drug intake after a very few days of therapy owing to the lack of effect onset. In these cases, it is common to find that these patients had not had explained to them that the basic treatment of constipation (*i.e.*, high-fiber diet, PEG or other osmotic agents) may require several days or weeks prior to achieving the effect onset or full effectiveness.

Poor basal evaluation

Patients with suspected refractoriness to medical treatment should be accurately re-evaluated for secondary forms of constipation, with particular regard for those associated with the use of drugs, a condition which can be unraveled only after repeated enquiries, focusing on specific drug classes. This issue is of particular relevance since some forms of drug-induced constipation (*e.g.*, that secondary to the use of opioid analgesics) can be managed by specific therapeutic approaches^[13], whereas other (*e.g.*, that secondary to the use of antidepressants) can influence colonic motility to such a degree of severity^[14,15] that they may require discontinuation of the offending drug or a switch to different drugs. Another sensitive and easy to miss condition, requiring a strong patient-physician relationship owing to the peculiarity of the issue, is a previous history (often only disclosed after several interviews) of physical or sexual abuse, found mainly in patients with symptoms of obstructed defecation (OD)^[16].

Once ascertained that none of the above conditions can be called into play, the patient usually undergoes further diagnostic evaluations in an attempt to highlight specific pathophysiological mechanisms which might drive focused therapeutic interventions^[17]. For this purpose, a further diagnostic step must include the evaluation of intestinal transit time^[18], anorectal manometry^[19] (complemented by the rectal balloon expulsion test^[20]) and defecography^[21]. Upper gastrointestinal (which might limit or preclude surgical procedures)^[22,23] and colonic manometry (possibly with pharmacological testing in patients regarded as eligible for surgery, see below)^[24,25] might also be performed.

These investigations usually allow allocation of con-

stipated patients into two major subgroups, comprising those with slow transit constipation (STC) and those with OD, even although it is not uncommon that some patients display both features at the same time^[6].

The hallmark of patients with STC is delayed colonic transit, a condition which can be documented by a delayed distribution of radiopaque markers (or radionuclides) throughout the visceral lumen^[26] and is characterized by a severe impairment of colonic motor activity that, in some instances, can be almost absent or progress up to a true picture of colonic inertia^[27].

In patients with OD, the main pathophysiological features are basically related to rectoanal dysfunction, including the inability to relax or the paradoxical contraction of the pelvic floor while attempting to defecate^[28], the lack of rectal motor activity^[29], and an abnormal rectal sensitivity^[30], although anatomical abnormalities (particularly rectocele and rectal intussusceptions) can also play a role in this setting^[31].

A condition of apparent refractoriness to drug therapy in these two subgroups of patients may thus be underpinned by different pathophysiological grounds that may deserve different medical and/or non-medical (surgical, behavioral) approaches.

THERAPEUTIC MANAGEMENT OF REFRACTORY PATIENTS: PHARMACOLOGICAL APPROACH

In patients with true unresponsiveness to first-line osmotic laxatives, a combination approach can be used, introducing stimulant laxatives such as bisacodyl and sodium picosulfate. These agents are able to elicit bowel propulsion^[32], as well as to exert antiabsorptive and secretory effects on the enteric mucosa, and appear to be quite safe even in the long-term^[6]. Initially, these drugs should be employed as rescue agents when patients do not defecate after two-three days while using osmotic agents^[33], although patients should be encouraged to persist for longer periods on osmotic laxatives before adding a stimulant agent. Of note, controlled clinical studies on stimulant laxatives have been published only in recent years and they have documented both the effectiveness of these agents and their favorable impact on disease-related quality of life, even in the medium-term^[34-36]. Moreover, the supposed damaging actions of stimulant laxatives on enteric neural structures have not been confirmed by means of modern techniques^[37]. Nevertheless, data on the long-term use of stimulant laxatives, either alone or in combination with osmotic laxatives, are lacking. Moreover, even laxative combinations may not be sufficient to achieve a satisfactory and steady resolution of constipation. Therefore, pharmacological research in this area has moved towards drugs that might be able to increase or restore the propulsive activity of the large bowel, in both the short- and long-term.

In this context, a promising drug, tegaserod, endowed with enteric prokinetic effects resulting from its

partial agonistic activity on 5-HT₄ serotonin receptors, was withdrawn from the drug market due to concerns about possible adverse cardiovascular effects^[38] and the research in this area shifted towards the development of effective drugs devoid of cardiovascular toxicity. Along this line, prucalopride, a thoroughly studied prokinetic drug with particular regard for its cardiovascular safety, has been recently introduced in Europe for treatment of constipated women not responding to conventional first-line regimens^[39]. This compound is a potent and selective 5-HT₄ receptor full agonist endowed with enterokinetic properties^[40], able to accelerate the gastrointestinal and colonic transit in constipated patients without abnormal rectal evacuatory dysfunction^[41], probably as a result of an increase of high-amplitude propulsive contractions^[42]. Controlled studies in patients (mostly women) unresponsive to standard medical regimens have shown that this drug (at the dose of 2 mg/d in adults and 1 mg/d in the elderly) can be effective in relieving constipation both in the short- and long-term^[43-47], even in patients from non-Western countries^[48]. Of note, although in clinical trials prucalopride appeared to be less effective in patients with symptoms of OD^[49], a recent study conducted under real-life conditions showed its efficacy even in this setting (with similar percentages to those reported in clinical trials)^[50], suggesting that this drug can be regarded as an additional therapeutic tool for refractory patients to provide them with an additional chance to manage their complaints^[51]. Moreover, a recent report showing that prucalopride can be as effective as PEG in resolving constipation^[52] allows the hypothesis that these two drugs, known to act in different ways and not burdened by serious adverse effects, might be (as already observed empirically in our routine clinical experience) combined advantageously to achieve positive therapeutic results in refractory patients.

With the recent introduction of enteric secretagogues, other therapeutic tools have been made available. The first drug of this class to be approved (currently in USA, but not in Europe) was lubiprostone, a fatty acid structurally related to prostaglandin E₁ which acts primarily by activating apical ClC-2 chloride channels in enteric epithelial cells^[53]. This compound has been shown to improve constipation (at a dose of 24 µg twice a day)^[54-57], even in the long-term^[58]. More recently, linaclotide, a guanylate cyclase-C agonist, has been approved in some European countries for treatment of patients with chronic constipation^[59]. The activation of guanylate cyclase-C by linaclotide results in an increase in both intracellular and extracellular levels of cyclic guanosine monophosphate, which then stimulates chloride and bicarbonate secretion from enteric epithelial cells into the bowel lumen, leading to an increment of luminal fluids and transit acceleration^[59]. This drug (at a dose of 145 µg once daily) has been found to be effective in the short-term for treatment of chronic constipation^[60-62], as also stressed in a recent meta-analysis^[63]. Since both lubiprostone and linaclotide display different mechanisms of action compared to laxatives or prokinetics, it is likely that their combination with other drugs (for instance, stimulant laxatives

or prucalopride) might help to improve symptoms in patients with refractory constipation. This represents an important medical need which should be addressed by means of specific clinical trials exploring the suitability of drug combinations.

Other pharmacological options

There is evidence from both controlled and non-controlled clinical trials that colchicine, an old drug still available for the treatment of gout at the dose of 0.6 mg three times per day, can be effective (at least in the short-term) to manage constipated patients, including those refractory to other therapeutic approaches^[64-66]. Unfortunately, colchicine has a narrow therapeutic index which is associated with underestimated toxicity and frequent and serious adverse effects^[67] that confines its use to extremely selected cases in whom no other options, including surgery (see below), are viable.

The inhibitor of ileal bile acid transporter A3309, after having displayed some benefit in a small pilot clinical study^[68], was also shown to be effective in accelerating colonic transit^[69] and in treating constipated patients at a daily dose of 10 mg in phase 2 studies^[70]. Based on these preliminary experiences, further studies are clearly needed to confirm both the efficacy and safety profile of A3309 in chronic refractory constipation.

An interesting small phase 2 study has suggested that the cholinesterase inhibitor pyridostigmine (60-120 mg three times per day), available in the pharmaceutical market for many years, is able to accelerate colonic transit and improve symptoms in constipated patients with type 2 diabetes mellitus^[71]. Since this drug is widely available and (in contrast to newer drugs) relatively inexpensive, further studies are warranted to explore its suitability in idiopathic chronic constipation.

THERAPEUTIC MANAGEMENT OF REFRACTORY PATIENTS: OTHER THERAPEUTIC APPROACHES

In patients with primary defects involving defecatory disorders, particularly OD, currently available medical regimens (although often used as first-line approaches) can be disappointing. Indeed, these patients have been shown to benefit more from behavioral and retraining techniques^[72-74], particularly biofeedback^[75,76], than drug therapies. Although the efficacy of biofeedback has been advocated for a long time^[77], controlled trials have only in recent years shown its efficacy in the treatment of constipation associated with OD^[78-83], even in elderly patients^[84], although some authors have reported a limited efficacy in the long-term when compared to botulinum toxin^[85]. Interestingly, there seems to be no difference among the various available biofeedback techniques in terms of efficacy^[86]. However, notwithstanding the good results achieved in the treatment of OD with better therapeutic performances than those of laxatives, biofeedback still appears to have a quite limited role in routine clinical

practice, likely because of the scarce appreciation of the benefits achievable with pelvic floor retraining and the limited availability of experienced trainers. Electrogalvanic stimulation, although effective in individual OD patients^[87,88], has not been formally studied in controlled trials.

In selected OD patients, local injections of botulinum toxin have been attempted with a certain success, although the evidence remains very scarce and is based on uncontrolled studies^[89,90]. Owing to such limitations, this approach cannot be proposed as a standard treatment but should be restricted to patients unresponsive to any other available medical therapy before considering a surgical approach or be employed only for research purposes.

When considering surgical strategies, one should always keep in mind that surgery is usually an irreversible option and that the surgical approach *per se* may introduce or add further damage to an already malfunctioning intestine. Thus, the ideal surgical approach should be aimed at achieving the best results with the minimum of invasiveness. Accordingly, the following paragraphs address the surgical options currently employed for the management of refractory constipated patients.

There is limited experience in adults with antegrade continence enemas (the so-called Malone procedure), which is a relatively invasive surgical procedure proven to be quite successful in children^[91] that seems to work in approximately 50% of patients undergoing this procedure^[92,93]. The theoretical advantage associated with this technique is that, should the colonic function be recovered, it would be possible to restore the intestinal continuity without a need for resections. However, in current practice this goal can be rarely, if ever, pursued.

A more draconian approach, which can be considered in refractory STC patients when pelvic floor dysfunction and relevant upper gastrointestinal motor abnormalities have been excluded^[22,94,95], is subtotal colectomy with ileorectal anastomosis^[96]. Provided that the above criteria are fulfilled, this technique offers interesting long-term benefits to patients^[97]. Conversely, the results are poorer when such criteria are not duly fulfilled^[98]. Of course, postoperative complications (*i.e.*, small bowel obstruction, wound infection, anastomotic leakage) may occur as with any surgical intervention, the most frequent being small bowel obstruction^[99]. However, most of these complications can be managed in a conservative manner and they usually do not require repeated surgical interventions.

In recent years there has been a renewed interest in treating refractory OD patients by a new surgical approach, designated as stapled transanal rectal resection^[100,101], conceived for correcting rectal intussusceptions and large rectoceles (usually at least 5 cm in diameter). Although this procedure may confer benefits to some patients over the short-term period, its efficacy is not superior to that achievable with osmotic laxatives^[102], it is burdened by several complications (*e.g.*, pain, bleeding, bowel perforation, fistulas, pelvic sepsis, peritonitis)^[103,104] and the results over the long-term period appear to be disappointing even in "ideal" patients^[105].

The interesting (although collateral) aspect of surgical procedures is the availability of full-thickness colonic or rectal tissue specimens, which are very useful for pathological investigations^[106,107]. Indeed, morphological analyses have the potential of disclosing useful information for understanding the pathophysiological bases of severe constipation^[108,109].

Another approach that can be attempted in patients with refractory constipation deals with sacral nerve stimulation (or sacral neuromodulation). This technique is based on the physiological principle that the presence of bioelectrical activity in one neural pathway can modulate a pre-existing activity in another pathway through synaptic interactions. It is carried out by percutaneous placement of an electrode in the third sacral foramen and implantation of a stimulating device under the skin in the buttocks^[110]. Although it may be effective in individual patients, the overall efficacy of sacral nerve stimulation is limited and unpredictable^[111], with positive results reported in 40%-100% of cases^[112]. Therefore, it requires additional well conducted prospective studies to assess its exact role and safety in the management of constipated patients as there is a significant underreporting of the incidence of untoward events associated with this technique^[113].

CONCLUSION

To date, the refractoriness of constipation to medical treatments is still a significant issue. Current literature on this topic suggests that a number of pharmacological or non-pharmacological therapeutic options can be offered to patients with true constipation refractory to first-line conventional treatments. However, for the majority of such options, the available supportive evidence is scanty and the clinical outcomes are often not satisfactory. A number of different factors are likely to contribute to these deficiencies, particularly: anecdotal or heterogeneous findings in a limited number of patients; data stemming from randomized clinical trials which may not meet the needs of real clinical life and are usually not suitable for designing tailored interventions based on the pathophysiology of individual patients; and scarcity or lack of information on the possible efficacy and, most importantly, safety of long-term treatments. Besides these arguments, both the literature and daily clinical practice clearly point out that, in a proportion of patients, the refractoriness of their constipation cannot be overcome even with second-line pharmacological interventions and that these subjects become candidates for third-line, more aggressive and/or demolitive, non-pharmacological/surgical therapeutic options which do not ensure favorable outcomes while posing relevant safety issues. Urgent and intensive clinical research efforts are therefore needed to address and resolve these problems. Besides attempts of identifying and developing novel drugs endowed with innovative mechanisms of actions, consistent efforts should be focused on the implementation of improved treatment regimens based on currently available old and

new drugs to pursue optimized benefit/risk ratios and long-term maintenance of constipation relief. In this context, attention should be paid to drug combination and/or alternating administration regimens which, while taking advantage of different mechanisms of action, would prevent excessive dose increments of the individual drugs and/or the loss of therapeutic effectiveness on long-term exposure as a possible consequence of tolerance. In particular, based on preliminary evidence and experience in daily clinical practice, combinations of enterokinetics with laxatives (either osmotic or stimulant), enterokinetics with secretagogues, different secretagogues, and even non-pharmacological interventions with enterokinetics or secretagogues might be worthy of validation by clinical research and subsequent consensus agreement by expert panels.

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Esophageal motility abnormalities in gastroesophageal reflux disease

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motility abnormalities. In particular, using novel techniques, such as high resolution manometry with or without concurrent intraluminal impedance monitoring, transient lower esophageal sphincter (LES) relaxations, hypotensive LES, ineffective esophageal peristalsis and bolus transit abnormalities have been better defined and strongly implicated in gastroesophageal reflux disease development. Overall, recent findings suggest that esophageal motility abnormalities are increasingly prevalent with increasing severity of reflux disease, from non-erosive reflux disease to erosive reflux disease and Barrett's esophagus. Characterizing esophageal dysmotility among different subgroups of patients with reflux disease may represent a fundamental approach to properly diagnose these patients and, thus, to set up the best therapeutic management. Currently, surgery represents the only reliable way to restore the esophagogastric junction integrity and to reduce transient LES relaxations that are considered to be the predominant mechanism by which gastric contents can enter the esophagus. On that ground, more in depth future studies assessing the pathogenetic role of dysmotility in patients with reflux disease are warranted.

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Abstract

Esophageal motility abnormalities are among the main factors implicated in the pathogenesis of gastroesophageal reflux disease. The recent introduction in clinical and research practice of novel esophageal testing has markedly improved our understanding of the mechanisms contributing to the development of gastroesophageal reflux disease, allowing a better management of patients with this disorder. In this context, the present article intends to provide an overview of the current literature about esophageal motility dysfunctions in patients with gastroesophageal reflux disease. Esophageal manometry, by recording intraluminal pressure, represents the gold standard to diagnose esophageal

Key words: Gastroesophageal reflux disease; High-resolution manometry; Ineffective esophageal motility; Esophagogastric junction; Transient lower esophageal sphincter relaxations

Core tip: Esophageal motility abnormalities are among the main factors implicated in the pathogenesis of gastroesophageal reflux disease. In particular, transient lower esophageal sphincter (LES) relaxations, hypotensive LES, ineffective esophageal peristalsis and bolus transit abnormalities have been strongly implicated in gastroesophageal reflux disease development. Moreover, recent findings suggest that these abnormalities are increasingly prevalent with increasing severity of

reflux disease. Currently, surgery represents the only reliable way to restore the esophagogastric junction integrity and to reduce transient LES relaxations. On that ground, more in depth future studies assessing the pathogenetic role of dysmotility in patients with reflux disease are warranted.

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INTRODUCTION

Gastroesophageal reflux disease (GERD) develops when the reflux of gastric contents into the esophagus leads to troublesome symptoms, such as heartburn and regurgitation, with or without mucosal damage and/or complications^[1]. Population-based studies indicate that GERD is a highly prevalent condition in Western countries^[2]. However, these patients are markedly heterogeneous in terms of clinical features, pathophysiological mechanisms and response to acid suppression, including Barrett's esophagus (BE), erosive esophagitis (ERD) and non-erosive reflux disease (NERD)^[3-8]. Of note, the pattern of esophageal motility has been shown to differ in the various subclasses of GERD patients^[4].

The pathogenesis of GERD is multifactorial, involving transient lower esophageal sphincter (LES) relaxations (TLESRs) as well as other LES pressure abnormalities (*i.e.*, hypotensive LES)^[9]. Moreover, other factors contributing to the pathophysiology of GERD include impairment of the esophagogastric junction (EGJ) (*i.e.*, hiatal hernia), ineffective esophageal acid and bolus clearance, delayed gastric emptying and impaired mucosal defensive factors^[9,10].

The recent advent of new technologies, such as combined impedance-manometry and high-resolution manometry (HRM), has represented a major advance in defining and characterizing esophageal motility abnormalities in GERD patients^[11,12].

The present article intends to appraise and critically discuss the current literature about esophageal motility dysfunctions in patients with GERD.

MOTILITY ABNORMALITIES IN GERD

The anti-reflux barrier, consisting of LES, crural diaphragm (CD), angle of His and normal thorax-abdomen pressure gradient, prevents reflux of gastric contents into the esophagus, whereas esophageal peristalsis helps to clear the refluxate and reduce exposure to noxious components of gastric juice. Consequently, impairment of both EGJ, including LES and CD, and/or esophageal clearance may favor GERD^[10]. The main motility ab-

normalities contributing to the occurrence of refluxes in GERD are impairment of the EGJ (*i.e.*, TLESRs, hypotensive LES, anatomic distortion of the EGJ) and ineffective esophageal motility (IEM).

Impairment of the EGJ

In this context, TLESRs are considered to be the predominant mechanism by which gastric contents can enter the esophagus^[13]. According to Spechler and Castell criteria, using conventional manometry testing, TLESRs were defined as periods, lasting 10-60 s, of spontaneous (non preceded by a swallow) LES relaxation^[14]. In patients with reflux, up to 75% of reflux events occur during TLESRs, but the proportion of reflux episodes that can be attributed to TLESRs decreases inversely with respect to the severity of GERD, probably due to the increasing prevalence of hypotensive LES in patients with severe GERD^[15-19]. In addition, TLESRs appear to play a less important role for reflux episodes in patients with hiatal hernia than in patients without hiatal hernia^[15]. Hypotensive LES is diagnosed when LES basal pressure is less than 10 mmHg and represents another major determinant for the EGJ incompetence^[20]. Of note, the mean resting LES pressure was found to be significantly lower in patients with ERD than in those with NERD^[21,22] and patients with BE and ERD, thus a higher severity of GERD usually has the highest frequency of hypotensive LES^[16,17]. Anatomic degradation of the EGJ was also observed to play a role in the genesis of reflux events. Pandolfino *et al*^[23] observed that the opening characteristics of the relaxed EGJ during low-pressure distention were different between GERD patients and normal subjects, that is a wider opening in GERD patients than in normal controls. Such a difference could not be explained entirely by presence of an axial hiatus hernia or by alterations in LES pressure. Thus, mechanical degradation of the EGJ other than hiatal hernia distinguishes GERD patients from normal subjects (*i.e.*, competence of the CD, integrity of the phrenoesophageal ligament, alterations in the muscular wall of the LES)^[23].

Ineffective esophageal motility

Impaired esophageal clearance can be caused by IEM, such as ineffective peristalsis or failed peristalsis^[24,25]. In 2001, using conventional manometry, IEM was defined as distal esophageal hypocontractility in $\geq 30\%$ of wet swallows, characterized either as contraction amplitude < 30 mmHg in the distal esophagus, 3 and 8 cm above the LES, or as peristaltic waves that are not propagated to the distal esophagus, or absent peristalsis^[14]. Hypocontractility is considered the most prevalent esophageal motor disorder in GERD^[26,27] and this concept has been further supported by different studies showing that esophageal peristaltic dysfunction was increasingly prevalent with more severe GERD presentation^[16,19,26,28]. Of note, in a group of patients with respiratory symptoms associated with reflux, IEM was found in 53% of asthmatics, 41% of chronic coughers and 31% of those with laryngitis^[29].

Table 1 Manometric criteria for esophageal motility associated with gastroesophageal reflux disease

TLESRs: periods (lasting more than 10–60 s) of spontaneous LES relaxation characterized by: (i) absence of swallowing for 4 s before to 2 s after the onset of LES relaxation, (ii) relaxation rate of ≥ 1 mmHg/s (iii) time from onset to complete relaxation of ≤ 10 s (iv) nadir pressure of ≤ 2 mmHg LES relaxations associated with a swallow and fulfilling the above mentioned criteria (ii), (iii) and (iv) that lasted more than 10 s are considered as TLESR Esophagogastric junction: Type 1: no separation between the LES and the crural diaphragm Type 2: minimal separation (> 1 and < 2 cm) making for a double-peaked pressure profile that is not yet indicative of hiatal hernia Type 3: more than 2 cm separation between the LES and the crural diaphragm at inspiration so that two high-pressure zones can be clearly identified 3a: respiratory inversion point distal to the LES 3b: respiratory inversion point proximal to the LES Weak peristalsis with large (a) and small peristaltic defects (b): (i) Mean integrated relaxation pressure < 15 mmHg and $> 20\%$ swallows with large breaks in the 20 mmHg isobaric contour (> 5 cm in length) (ii) Mean integrated relaxation pressure < 15 mmHg and $> 30\%$ swallows with small breaks in the 20 mmHg isobaric contour (2–5 cm in length)
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LES: Lower esophageal sphincter; TLESRs: Transient lower esophageal sphincter relaxations.

Recently, studies carried out in patients with scleroderma, who are characterized by failed or absent peristalsis and low basal LES pressure, observed that these patients are frequently affected by GERD and its complications^[30–32], thus substantiating the relevant role of esophageal clearance for the development of GERD.

As reported in the next section, HRM, with or without concurrent intraluminal impedance monitoring, allows a more complete definition of peristalsis that is, at least in part, highlighted in the Chicago Classification^[33].

ADVANCES IN THE DIAGNOSIS OF GERD MOTILITY ABNORMALITIES

Esophageal manometry, by recording intraluminal pressure, represents the gold standard to diagnose esophageal motility abnormalities. Until recently, stationary esophageal manometry was performed using an intraluminal catheter with four to eight water-perfused channels connected to external pressure sensors, including a sleeve sensor to measure continuously the maximum LES pressure^[34,35].

Although the diagnosis of esophageal motility disorders is based on manometric findings, it is worth noting that endoscopy represents an important diagnostic tool to identify specific causes of motility disorders (*i.e.*, diverticulum, mechanical obstruction, eosinophilic esophagitis)^[36–38].

High-resolution manometry

HRM is a recently developed technique using multiple (up to thirty-six) closely spaced pressure sensors to measure intraluminal pressure changes of the entire esophagus during swallowing^[39]. Indeed, a dynamic representation of pressure variations is recorded from the upper esophageal sphincter to the lower one, providing an esophageal pressure topography study^[40]. Of note, a new practical classification of esophageal motor disorders, the Chicago Classification^[33], has been developed using several esophageal pressure topography metrics, constructed

from HRM data, able to thoroughly characterise tests swallows^[41,42]. Moreover, HRM-based studies improved both EGJ and TLESRs assessment and underlined their role as main mechanisms involved in the development of reflux events^[43,44].

As already known, GERD is primarily a motility disorder in which TLESRs plays a crucial role^[45] and, in this regard, recent evidence has highlighted that HRM is reproducible and more sensitive than stationary manometry to detect TLESRs associated with refluxes (Figure 1A), also providing a better interobserver agreement^[46–47]. HRM data from patients with esophageal symptoms (dysphagia, chest pain and heartburn/regurgitation), compared to asymptomatic volunteers, revealed that measuring the duration of the esophageal low pressure zone, in the area of transition from striated to smooth muscle, as defined by the time delay between the proximal and distal esophageal contraction waves, might be a meaningful variable in GERD and dysphagia^[48]. To date, using HRM with esophageal pressure topography, the definition of TLESRs has been further detailed (Table 1)^[43,49,50].

Regarding the EGJ assessment, HRM provides a dynamic representation of the EGJ high-pressure zone, making it possible to isolate the extrinsic CD contraction from expiratory LES pressure^[51]. On this basis, Pandolfino *et al.*^[51] reported that reduced inspiratory EGJ pressure augmentation, an indicator of impaired CD function, was a common finding in GERD patients and a better predictor of GERD prevalence than either LES pressure or LES-CD separation (the HRM signature of hiatus hernia). By means of HRM monitoring, it is possible to document the presence of hiatal hernia measuring simultaneously the separation between the intrinsic LES and CD^[52]. Bredenoord *et al.*^[15] showed that, in patients with a small hiatal hernia, temporal reduction of the hernia occurs frequently. During spatial separation of the diaphragm and LES, reflux events occurred more often than during reduction of the hernia^[15]. Overall, the defects of the EGJ can be evaluated in more detail by means of HRM monitoring and, thus, future studies will better clarify the role of EGJ morphology in GERD de-

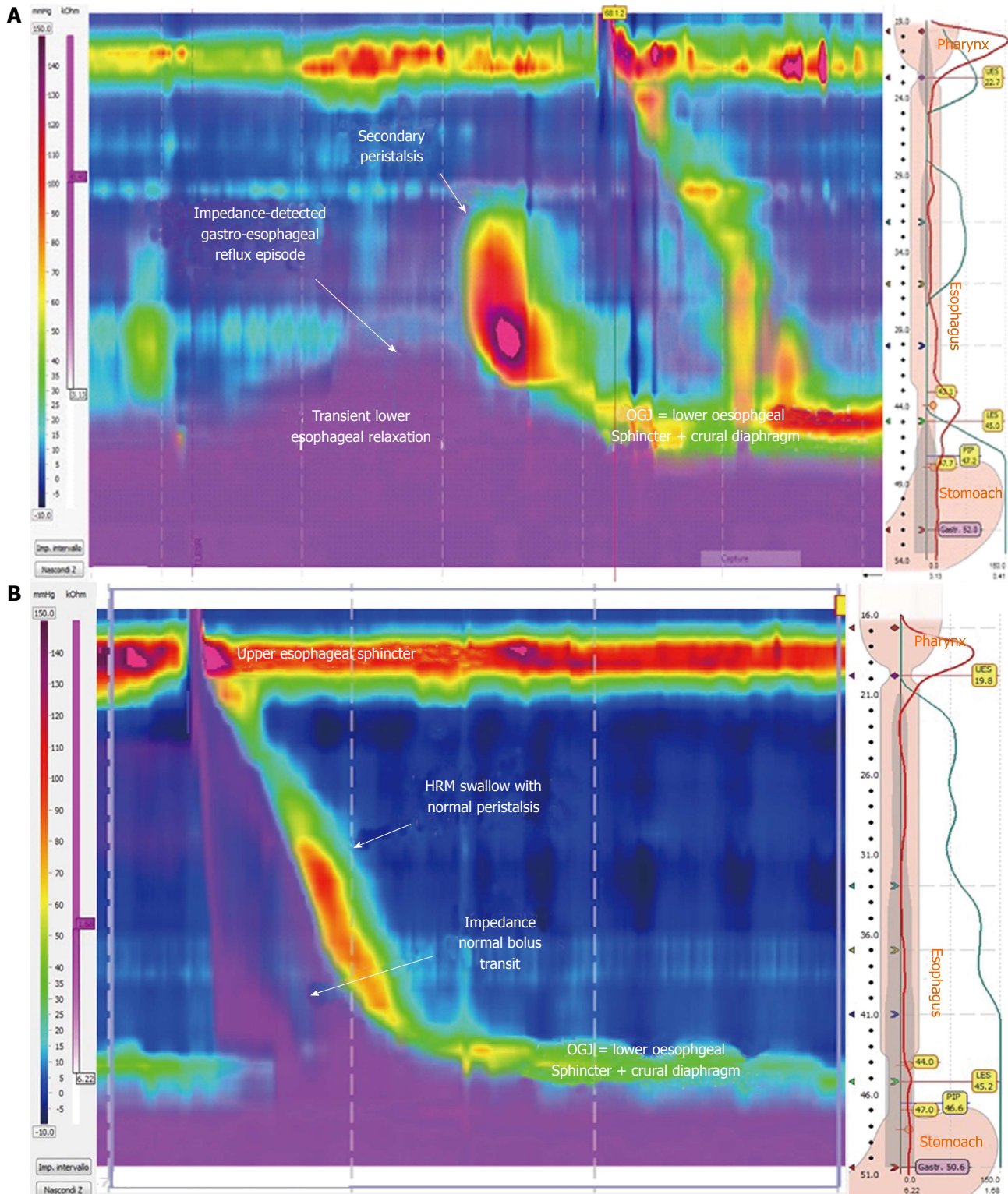


Figure 1 Portion of high-resolution impedance manometry tracing. A: Showing an example of transient lower esophageal sphincter relaxation and impedance-detected gastroesophageal reflux event with secondary peristaltic wave; B: Showing an example of complete bolus transit during a peristaltic swallow.

velopment and progression (Table 1).

As for the esophageal peristalsis, a recent HRM study aimed at developing a classification of weak peristalsis in esophageal pressure topography, showed that large (> 5 cm) and small (2-5 cm) pressure troughs in the 20 mm Hg isobaric contour of peristalsis, but not failed peristal-

sis, occurred more frequently in patients with unexplained non-obstructive dysphagia than in control subjects^[53].

In addition, it has been demonstrated that HRM predicts bolus movement more accurately than conventional manometry and identifies clinically relevant esophageal dysfunction not detected by other investigations, includ-

ing conventional manometry^[54].

Combined impedance-manometry

The application of combined impedance-manometry technology provides relevant additional data regarding esophageal motility compared to conventional manometry^[55], particularly to identify abnormal bolus transport and clearance during swallows, and to investigate the relationships between bolus transit and LES relaxation^[12] (Figure 1B).

After instillation of an acid bolus in healthy subjects, in which Sildenafil provoked a graded impairment in esophageal motility without affecting saliva secretion, simultaneous manometry, pH and impedance study showed that, during upright acid reflux, volume clearance was slightly prolonged only with severe IEM (> 80% abnormal peristaltic sequences)^[24]. In the supine position, severe IEM significantly prolonged chemical and volume clearance^[24]. In keeping with these results, Fornari *et al.*^[25] observed that mild IEM did not affect esophageal clearance in GERD patients. Only severe IEM was associated with prolonged clearance and acid exposure, particularly in supine periods^[25]. Recently, we demonstrated that an increasing degree of esophageal mucosal damage in GERD patients (ERD and BE) was associated with a progressively more severe deflection of esophageal function, expressed by an increased frequency of IEM and bolus transit abnormalities, the latter ones also occurring in cases of a normal motility pattern^[16].

A new study with combined HRM and impedance monitoring reported that the frequency of TLESRs did not differ between patients with NERD and healthy controls. In patients with NERD, TLESRs were associated more often with reflux episodes than in controls and this association increased when only liquid and mixed refluxes were considered^[56]. If confirmed in larger series, it might be hypothesized that factors involved in the occurrence of reflux in NERD patients during TLESRs are different from those in healthy subjects and that the volume of refluxate in patients is higher than that in healthy subjects^[56].

Finally, the importance of concomitant manometry and impedance monitoring in order to distinguish rumination syndrome from GERD in patients with predominant regurgitation has been highlighted^[57].

DIFFERENCES IN MOTILITY ABNORMALITIES AMONG GERD SUBGROUPS

As mentioned above, several studies reported that motor abnormalities are increasingly prevalent with the severity of GERD, from NERD, ERD to BE^[19,21-22,26]. On the other hand, there are studies in contrast with these findings, showing no significant difference in terms of hypotensive LES and IEM between NERD and ERD^[58-59]. However, it should be pointed out that, to assess any difference in motility dysfunction among GERD subgroups,

a careful classification is warranted. In particular, the inclusion of patients with reflux symptoms and negative upper endoscopy as NERD patients is often made without any attempt of distinguishing functional heartburn (as defined by Rome III criteria) from NERD by means of pathophysiological investigations. To date, NERD is identified as a condition in which typical reflux symptoms appear in patients with negative endoscopy and presence of troublesome reflux-associated symptoms (to acid, weakly acidic or non-acid reflux)^[5], whereas functional heartburn is a disorder characterized by symptoms of heartburn not related to GERD, as diagnosed by means of 24-h impedance-pH testing, and must be distinguished from NERD^[60-64].

In this context, Frazzoni *et al.*^[18] showed that the mean LES pressure was significantly lower in ERD and NERD patients than in controls and functional heartburn patients and that the mean distal esophageal wave amplitude was lower in patients with ERD than in patients with NERD, functional heartburn and controls. In addition, the authors found that the prevalence of hiatal hernia was significantly higher in ERD and NERD than in functional heartburn and controls^[18]. In line with these results, we reported that GERD patients have a greater prevalence of abnormally low LES pressure, IEM and hiatal hernia compared with patients with functional heartburn and healthy controls. In particular, we observed that IEM gradually increased from controls and functional heartburn to NERD and from ERD to BE patients^[16]. By the application of concurrent manometry and impedance study, it has been shown that ERD is characterized by longer esophageal bolus transit and fewer complete bolus transit than NERD and healthy controls. The authors hypothesized that the noted differences in esophageal bolus transit might reflect a continuum of dysfunction secondary to increasing esophageal mucosal damage^[65]. On the other hand, a recent HRM study reported a peristaltic dysfunction in 56% of NERD and 76% of ERD patients, with no significant difference between the two groups^[66]. Nevertheless, the authors observed that, using HRM, the frequency of esophageal motility abnormalities in GERD was higher than that reported in a previous study performed with conventional manometry^[66].

It is also worth noting the possibility that reflux-induced esophageal contractions can be responsible for symptom perception. Indeed, Pehlivanov *et al.*^[67], while studying 12 unclassified subjects with heartburn by means of 24-h pH-metry, combined with synchronized pressure recording and high-frequency intraluminal ultrasound imaging of the esophagus, highlighted a close correlation between heartburn episodes, associated with acid reflux or not, and abnormally long durations of longitudinal muscle contractions. Afterwards, in 10 unclassified subjects complaining of chronic heartburn, Bhalla *et al.*^[68] observed that an increase in symptom sensitivity, elicited by acid infusion, occurred in concomitance with a perturbation of esophageal contractility, as revealed by a greater increase in contraction amplitude, contraction duration, muscle thickness and incidence of sustained esophageal contrac-

tions during the second acid infusion, in comparison with the first one. On the other hand, Thoua *et al.*^[69] showed that acid infusions, although inducing greater acid sensitivity in NERD patients compared to ERD patients and controls, did not induce any changes in the amplitude of esophageal contractions in any of the studied patient groups.

Overall, further studies with advanced techniques such as HRM and proper physiological classification of patients with reflux symptoms are needed in order to better assess the prevalence and pathophysiological role of esophageal motility abnormalities in GERD patients.

TREATMENT PERSPECTIVES

Studies using impedance-pH monitoring have demonstrated that 30%-40% of GERD patients have persistent symptoms despite proton pump inhibitor therapy, mostly related to non-acid reflux events^[70,71]. In keeping with this assumption and considering that TLESRs represent the main pathogenetic mechanism of all types of reflux events, controlling the occurrence of TLESRs is considered to be a relevant therapeutic goal in GERD management. However, the actual therapeutic options are limited because there is no specific pharmacological intervention that reliably restores LES function. Drugs interfering with TLESRs, called reflux inhibitors, have been developed, including: γ -aminobutyric acid (GABA)_B-receptor agonists (baclofen, lesogaberan, arbaclofen placarbil) and antagonists to the metabotropic glutamate receptor 5 (ADX10059). Despite the promising initial results of these compounds^[72-74], their clinical use has been compromised by various side effects or small therapeutic gain^[75-77].

The use of prokinetic drugs (*i.e.*, metoclopramide, bethanecol, domperidone) is supposed to accelerate gastric emptying, increase LES tone and hasten esophageal clearance. However, the currently available prokinetics are poorly effective in treating GERD-related dysmotility^[78,79] and compounded by side effects^[80,81].

Considering the lack of specific effective drugs on reflux-related esophageal dysmotility, dietary and lifestyle interventions are strongly suggested. Particularly, weight loss, head of bed elevation and left lateral decubitus position provided an improvement in GERD measures^[82]. Reduction in calorie density and a low-fat diet has positive effects respectively on acid exposure after a meal and on the frequency of reflux symptoms^[83]. In addition, chewing gum for half an hour after meals could reduce acidic postprandial esophageal reflux, likely stimulating salivation and swallowing, and thus improving both volume and chemical clearance^[84].

Currently, surgery represents the only reliable way to treat esophageal motility abnormalities. Indeed, fundoplication is able to reduce both the volume of proximal stomach and the rate of TLESRs^[85-88]. Particularly, the percentage of TLESRs associated with reflux was found to be decreased after fundoplication^[85,89]. In addition, surgery is able to restore the eventual EGJ disruption acting

on a hiatal hernia, CD defects and gastroesophageal high pressure zone^[89]. Pandolfino *et al.*^[90] showed that anti-reflux surgery decreases the distensibility of the EGJ to a level similar to normal subjects. Recently, the LOTUS trial, a 5-year randomized, open, parallel-group trial, showed that the remission rate did not differ between optimized esomeprazole therapy (92%) and standardized laparoscopic anti-reflux surgery (85%) in GERD patients (Log-Rank $P = 0.048$)^[91]. However, at 5 years, acid regurgitation was more prevalent in the esomeprazole group than in the anti-reflux surgery group; whereas dysphagia, bloating and flatulence were more prevalent in the anti-reflux surgery group^[91]. Moreover, both treatments were well tolerated, with similar safety profiles^[91]. Overall, the positive effects of anti-reflux surgery have been clearly demonstrated, not only on reflux symptoms^[91-93] but also on dysphagia^[94-96]. However, in this context, performing manometry to exclude severe motility abnormalities during the preoperative assessment of patients being considered for anti-reflux surgery is warranted^[35]. As to the best surgical procedure to adopt, a systematic review and meta-analyses comparing the effects of laparoscopic Nissen (360°) and Toupet (270°) fundoplication for GERD have assessed that both procedures are equally effective with respect to the percentage of patients with recurrent pathological acid exposure (RR = 1.26, $P = 0.29$) and subjective reflux recurrence (RR = 1.11, $P = 0.61$)^[92]. In contrast, the Nissen procedure was associated with higher prevalence of postoperative dysphagia (13.5% *vs* 8.6%, $P = 0.02$)^[92]. On the other hand, another meta-analysis reported that the Toupet procedure was more likely to result in surgery-related complications during the early post-operative period ($P = 0.04$)^[97].

In the last decade, a variety of endoscopic techniques (*i.e.*, radiofrequency ablation, suturing devices) have been proposed as less invasive and costly alternatives to laparoscopic surgery, even although many methods are no longer available primarily because of unacceptable side effects, modest or lack of long-term efficacy, cost, time invested and lack of reversibility^[98-100]. To our best knowledge, currently available anti-reflux endoscopic devices include the Stretta procedure (Mederi Therapeutics, Inc, Greenwich, CT), the EsophyX transoral incisionless fundoplication (EndoGastro Solutions, Redmond, WA) and the Endoscopic Plicator (former distributor NDO Surgical, Inc., Mansfield, MA, United States; current product of Ethicon Endosurgery, Somerville, NJ, United States). The Stretta procedure is an example of a thermal ablation technique for reflux control that has been supposed to be effective in decreasing esophageal acid sensitivity without significant effect on esophageal acid exposure^[101-103]. However, a recent review article showed that, in selected GERD patients, Stretta reduces esophageal acid exposure, decreases the frequency of TLESRs, increases patient satisfaction, decreases medication use and improves quality of life^[104]. In GERD patients with a small or no hiatal hernia, transoral incisionless fundoplication has been shown to reduce the number of postprandial TLESRs, the number of TLESRs associated

with reflux and EGJ distensibility. This resulted in a reduction of the number and proximal extent of reflux episodes and improvement of acid exposure in the upright position after 6 mo^[105]. In addition, previous studies have shown that this procedure permits an increase in LES length and resting pressure and is able to normalize esophageal acid exposure in GERD patients, improving reflux symptoms and quality of life, and reducing PPI use^[106]. Endoscopic treatment by full-thickness plication using the Endoscopic Plicator has been shown to significantly reduce GERD symptoms, PPI use and distal esophageal acid exposure in both prospective open-label and randomized controlled trials^[107,108]. Recently, a novel Plicator device able to place multiple sutures has been applied, showing that this technique is safe and effective in reducing GERD symptoms, medication use and esophageal acid exposure at 12 mo follow-up without clinical significant side-effects^[109,110]. Overall, considering the feasibility and safety profiles that are similar to those of anti-reflux surgery, endoscopic techniques appear to be procedures of interest for GERD; however, they are not as effective as surgery for returning acid exposure to normal, healing esophagitis and resolution of symptoms^[111]. Thus, widespread use of these techniques cannot be recommended yet and further controlled prospective trials are urgently required.

Finally, an alternative surgical method to augment LES function has been assessed with a magnetic ring device that is laparoscopically placed around the external diameter of the distal esophagus, the LINX reflux management system, with encouraging results in eliminating reflux symptoms and normalizing esophageal acid exposure^[112]. As with the endoscopic techniques, the LINX device is not able to treat the anatomical abnormality of a large hiatus hernia. In conclusion, further controlled prospective studies with long-term follow-up comparing the LINX device to standard pharmacological and surgical alternatives are needed.

CONCLUSION

The pathogenesis of GERD is multifactorial, including esophageal motility abnormalities of which the most important are TLESRs, hypotensive LES and IEM. High-resolution manometry, with or without concurrent intraluminal impedance monitoring, providing an esophageal pressure topography study of the esophagus, allows a more complete definition of esophageal peristalsis. Moreover, HRM-based studies improved both EGJ and TLESRs assessment as a pathophysiological mechanism for the occurrence of reflux events. Particularly, the combination of esophageal manometry and intraluminal impedance measurement allows assessment of abnormal bolus transport and clearance during swallows and investigation of the relationships between bolus transit and LES relaxation. On that ground, more in depth future studies assessing the pathogenetic role of dysmotility in GERD patients are warranted.

To date, the majority of studies in this field report

that these esophageal motility abnormalities are increasingly prevalent with increasing severity of GERD, thus from NERD, to ERD and BE patients. A more comprehensive diagnosis and characterization of reflux patients with esophageal dysmotility is nowadays possible and this additional information may be of help in identifying patients who could potentially benefit more from surgery due to restoration of OGJ integrity or reduction of TLESRs.

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EVS vs TIPS shunt for gastric variceal bleeding in patients with cirrhosis: A meta-analysis

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Abstract

AIM: To evaluate the clinical effects of transjugular intrahepatic portosystemic shunt (TIPS) vs endoscopic variceal sclerotherapy (EVS) in the management of gastric variceal (GV) bleeding in terms of variceal rebleeding, hepatic encephalopathy (HE), and survival by meta-analysis.

METHODS: Medline, Embase, and CNKI were searched. Studies compared TIPS with EVS in treating GV bleeding were identified and included according to our predefined inclusion criteria. Data were extracted independently by two of our authors. Studies with prospective randomized design were considered to be of high quality. Hazard ratios (HRs) or odd ratios (ORs) were calculated using a fixed-effects model when there was no inter-trial heterogeneity. Oppositely, a random-effects model was employed.

RESULTS: Three studies with 220 patients who had at least one episode of GV bleeding were included in the present meta-analysis. The proportions of patients with viral cirrhosis and alcoholic cirrhosis were 39% (range 0%-78%) and 36% (range 12% to 41%), respectively. The pooled incidence of variceal rebleeding in the TIPS group was significantly lower than that in the EVS group (HR = 0.3, 0.35, 95%CI: 0.17-0.71, $P = 0.004$). However, the risk of the development of any degree of HE was significantly increased in the TIPS group (OR = 15.97, 95%CI: 3.61-70.68). The pooled HR of survival was 1.26 (95%CI: 0.76-2.09, $P = 0.36$). No inter-trial heterogeneity was observed among these analyses.

CONCLUSION: The improved effect of TIPS in the prevention of GV rebleeding is associated with an increased risk of HE. There is no survival difference between the TIPS and EVS groups. Further studies are needed to evaluate the survival benefit of TIPS in cirrhotic patients with GV bleeding.

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Key words: Gastric variceal bleeding; Transjugular intrahepatic portosystemic shunt; Endoscopic variceal sclerotherapy; Cirrhosis; Hepatic encephalopathy

Core tip: This meta-analysis provides evidence-based guidance regarding the effect of transjugular intrahepatic portosystemic shunt and endoscopic variceal sclerotherapy on rebleeding, hepatic encephalopathy, and survival. The results suggest that transjugular intrahepatic portosystemic shunt is more effective than endoscopic variceal sclerotherapy in the prevention of gastric variceal rebleeding, but is associated with an increased risk of hepatic encephalopathy.

Bai M, Qi XS, Yang ZP, Wu KC, Fan DM, Han GH. EVS vs TIPS shunt for gastric variceal bleeding in patients with cir-

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INTRODUCTION

Variceal hemorrhage occurs in 25%-35% of patients with cirrhosis. Gastric varices (GV) are the origin in about 27% of variceal bleeders^[1,2]. In comparison with esophageal varices (EV), the bleeding from GV is more severe. Furthermore, patients with GV bleeding are usually associated with higher mortality than those with EV bleeding^[2,3].

Endoscopic variceal sclerotherapy (EVS) has been proved to be effective in the treatment of EV bleeding^[4-6]. But, EVS is less successful in the treatment of GV bleeding, probably because of its size, location, and high-volume blood flow^[3,7]. Compared to patients with EV, GV patients who accepted EVS treatment were associated with a higher rebleeding rate (23%-90%) and more side effects such as fever and retrosternal and abdominal pain^[8,9]. Additionally, Lo *et al*^[10] accomplished a randomized trial and proved that EVS was more effective than endoscopic band ligation in the management of GV bleeding. Recently, transjugular intrahepatic portosystemic shunt (TIPS) became more and more popular in the management of complications related to portal hypertension. TIPS was recommended as the second-line therapy for the treatment of acute variceal bleeding and variceal rebleeding in the American Association for the Study of Liver Diseases (AASLD) practice guideline and British Society of Gastroenterology guidelines for the management of variceal hemorrhage in cirrhotic patients^[6,11,12].

Currently, both EVS and TIPS were employed for the treatment of GV bleeding in cirrhotic patients. Several studies have evaluated the effect of these two therapies in the management of GV bleeding. Results of these studies were controversial. For example, Mahadeva *et al*^[13] demonstrated a significantly lower rebleeding rate in patients who underwent the TIPS procedure, but Procaccini *et al*^[7] reported similar rebleeding rates between the EVS and TIPS groups^[7,13]. Hence, a meta-analysis of the data derived from the published literature would be necessary. The purpose of this study is to provide an evidence-based guidance regarding the effect of EVS and TIPS on variceal rebleeding, HE, and survival by meta-analysis.

MATERIALS AND METHODS

Selection of studies

We searched Medline (1950 to October 2012, limited in English), Embase (1974 to October 2012), and CNKI (China National Knowledge Infrastructure, <http://www.edu.cnki.net>, 1979 to October 2012) using “transjugular intrahepatic portosystemic shunt”, “gastric varices”, and “endoscopic sclerotherapy” as the key words. We

also reviewed the citations of the identified articles and manually searched the abstracts from the meetings of the European Association for the Study of Liver Disease, the American Association for the Study of Liver Disease, and the World Congress of Gastroenterology.

Studies included in our meta-analysis met the following criteria: (1) participants were adult patients (> 18 years) with at least one episode of GV bleeding; (2) interventions were TIPS *vs* EVS; and (3) studies reported at least one of the following outcomes: variceal rebleeding, HE, and survival. Study searches and screenings were conducted independently by two of our authors (Bai M, Qi XS). Discrepancies were solved by consensus.

Data extraction

Data were extracted independently by two of our authors (Bai M, Qi XS) using a predefined form. Disagreements were resolved by discussions.

Study quality assessment

The methodological qualities of the included trials were assessed independently by two investigators (Bai M and Han GH). Studies with prospective randomized design were considered to be of high quality.

Definitions

GV included GV1 (varices continuous with EV and extending along the lesser curve for about 2-5 cm below the gastroesophageal junction), GV2 (varices, often long and tortuous, extending from the esophagus below the gastroesophageal junction toward the fundus), type 1 isolated GV (IGV1, varices located in the fundus that often are tortuous and complex in shape), and type 2 isolated GV (IGV2, ectopic varices in the antrum, corpus, and around the pylorus)^[3].

Variceal rebleeding included rebleeding from all kinds of varices caused by portal hypertension, most commonly EV and GV. GV rebleeding was distinguished from EV rebleeding on the basis of whether active GV bleeding or erosive spots on the GV were observed during endoscopic examination. HE was defined as any episode of clinically overt encephalopathy and was graded according to the West Haven criteria^[14].

Statistical analysis

The statistical package RevMan version 5.0 (The Nordic Cochrane Centre, Copenhagen, Denmark) provided by the Cochrane Collaboration was used for all analyses. According to the PRISMA statement, the hazard ratios (HRs) are used for time-to-event outcomes^[15]. At first, the observed minus expected difference (O-E) and variance (V) were tried to be calculated directly from the detailed data (observed events and expected events or hazard rates on research and control arm, O-E on research arm and log-rank V) or indirectly from the estimated data (HR and confidence intervals, HR and events in each arm, HR and total events, log-rank test result and the number of events in the two groups)^[16,17]. A negative

O-E implied that the outcome favoured TIPS. And then, the pooled HR was estimated according to the O-E and V. For dichotomous endpoints which were not reported as time-to-event outcomes, such as HE in the present study, pooled odds ratios (ORs) were calculated by the Mantel-Haenszel model. Significance and 95%CI were provided for the combined HRs and ORs.

According to Higgins *et al.*^[18], the χ^2 *Q*-test ($P < 0.10$ was considered to represent significant statistical heterogeneity) and the I^2 statistic were applied to investigate inter-trial heterogeneity. A fixed-effect model was employed for the analyses with no significant inter-trial heterogeneity and a random-effect model was employed for those with significant inter-trial heterogeneity. Both the test for publication bias and meta-regression were not performed because less than ten studies were included in the present meta-analysis.

RESULTS

Study selection

According to our search strategy, 286 articles were identified. Most ($n = 265$) of these articles were excluded after abstract reading (Figure 1). Of the remaining 17 articles, 14 were excluded after full-text reading for the following reasons: EVS was not compared with TIPS ($n = 7$); both patients with EV and GV bleeding were included ($n = 8$); TIPS was compared with therapies other than EVS ($n = 3$).

Study characteristics

Table 1 shows the main characteristics of the three included studies^[7,13,19]. All of the three studies were published in full-text. TIPS procedures were performed with bare stents in two studies^[13,19], and partially with covered stents in the remaining one (Table 1)^[7,13,19].

Quality assessment

Only the study by Lo *et al.*^[19] was a prospective randomized trial, and was considered to be of high quality. However, the two groups in their study were not comparable in the number of previous variceal bleeding episodes. The rest two trials were retrospective studies and were considered as low-quality studies according to our quality assessment criteria. However, these two studies have study groups which were comparable in base-line data.

Patient characteristics

The three studies included 220 patients altogether. Almost 35% and 82% of the patients in the study by Mahadeva *et al.*^[13] and Lo *et al.*^[19] had GV1, respectively^[13,19]. Three patients in the study by Lo *et al.*^[19] had IGV. The study by Procaccini *et al.*^[7] did not classify GV according to its location. The pooled proportions of patients with viral cirrhosis and alcoholic cirrhosis were 39% (range 0%-78%) and 36% (range 12%-41%), respectively. Most of the patients in the study by Mahadeva *et al.*^[13] were Child-Pugh C (44%) and more than half of the patients in the study by Lo *et al.*^[19] were Child-Pugh B (54%, Table 2).

Outcomes

TIPS shunt stenosis, final patency rate of TIPS shunt, number of endoscopic therapy per patient, patient cross-over to TIPS, incidences of variceal rebleeding and HE, and survival were assessed in all of the three studies. The incidences of TIPS shunt stenosis were lower than 25% during the follow-up in the TIPS group in all of the included studies. On average, at least 2-3 EVSs were needed to achieve variceal obliteration for the patients in the EVS group.

Both the study by Mahadeva *et al.*^[13] and the study by Lo *et al.*^[19] demonstrated lower incidences of clinical failure in the TIPS group than in the EVS group. However, the difference between groups was not statistically significant^[13,19]. Additionally, 2, 1, and 7 patients in the ET group crossed over to the TIPS group in the study by Mahadeva *et al.*^[13], the study by Lo *et al.*^[19] and the study by Procaccini *et al.*^[7], respectively (Table 3).

The risk of variceal rebleeding was significantly lower in the TIPS group in two of the included studies^[13,19]. The pooled risk of variceal rebleeding in the TIPS group was significantly lower (Figure 2A, HR = 0.35, 95%CI: 0.17-0.71, $P = 0.004$) than that in the EVS group. Inter-trial heterogeneity of this analysis was not significant ($P = 0.17$, $I^2 = 46\%$). The study by Procaccini *et al.*^[7] was not included in this analysis, because it had insufficient information for the calculation of O-E and V.

Two of the three studies reported the probabilities of patients with GV rebleeding^[13,19]. In the study by Lo *et al.*^[19], more patients with GV rebleeding were observed in the EVS group (Log-Rank test, $P = 0.01$)^[19]. However, the study by Procaccini *et al.*^[7] demonstrated similar incidences of GV rebleeding in the TIPS and EVS groups (Fisher's exact test, 72 h: $P = 0.71$, 3 mo: $P = 0.25$, 1 year: $P = 0.16$)^[7]. For GV rebleeding, the combination of these results could not be performed because of the unfeasibility of the detailed data of this outcome.

A higher risk of HE was reported in the patients who underwent TIPS procedure in all of the included studies^[7,13,19]. Only the difference between groups in the study by Mahadeva *et al.*^[13] was not significant, probably because only the patients with severe HE were counted. The pooled risk of developing any degree of HE was higher in the TIPS group (Figure 2B, OR = 15.97, 95%CI: 3.61-70.68, $P < 0.001$). Two studies with 177 patients were involved in the estimation of the pooled OR of HE^[7,19]. No significant heterogeneity was observed among the involved studies ($I^2 = 0\%$, $P = 0.76$).

In all of the three included studies, patients with TIPS and EVS did not demonstrate a significant difference in survival. The heterogeneity of this analysis was not statistically significant ($I^2 = 0\%$, $P = 0.59$), and the pooled HR of survival was 1.26 (Figure 2C, 95%CI: 0.76-2.09, $P = 0.36$).

DISCUSSION

According to this meta-analysis of three controlled trials which compared EVS and TIPS in the treatment of GV

Table 1 Characteristic of studies included

Ref.	Type of stent	Type of EVS	Randomized	Prospective study	Consecutive patients	Comparable base-line data	Definition of variceal rebleeding	Definition of post-TIPS HE	Lost (TIPS/EVS)
Mahadeva <i>et al</i> ^[13]	Bare	Cyanoacrylate	No	No	No	Yes	NR	NR	0/0
Lo <i>et al</i> ^[19]	Bare	Cyanoacrylate	Yes	Yes	Yes	No	Rebleeding from GV or EV was defined as the presence of hematemesis or melena with the bleeding source being endoscopically proven to originate from GV or EV, respectively	Patients with altered consciousness levels and elevated arterial ammonia levels requiring hospitalization were recorded as having the complication of hepatic encephalopathy	1/6
Procaccini <i>et al</i> ^[7]	29 with covered stent, 15 with bare stent	Cyanoacrylate	No	No	NR	Yes	Rebleeding was defined by a significant decrease in hemoglobin or hematocrit requiring packed red cell transfusion or evidence of hematemesis or melena with hemodynamic insufficiency	NR	NR/NR

EV: Esophageal varices; GV: Gastric varices; NR: Not reported; EVS: Endoscopic variceal sclerotherapy; TIPS: Transjugular intrahepatic portosystemic shunt.

Table 2 Characteristic of patients included

Ref.	Patients screened	Patients included Total/TIPS/EVS	Age TIPS/EVS	Gender TIPS/EVS male (female)	Child-Pugh classes TIPS/EVS			Etiology			GV anatomy TIPS/EVS		
					A	B	C	Viral TIPS/EVS	Alcohol TIPS/EVS	Others TIPS/EVS	GOV1	GOV2	IGV
Mahadeva <i>et al</i> ^[13]	NR	43/20/23	52/55	13 (7)/15 (8)	4/4	6/8	10/9	0/0	12/14	8/9	13/15	7/8	0/0
Lo <i>et al</i> ^[19]	460	72/35/37	55/52	25 (10)/28 (9)	9/12	20/19	6/6	30/26	4/8	1/3	19/17	14/19	2/1
Procaccini <i>et al</i> ^[7]	NR	105/44/61	52/54.5	26 (18)/43 (18)	NR	NR	NR	14/16	17/24	13/21	NR	NR	NR

NR: Not reported; EVS: Endoscopic variceal sclerotherapy; TIPS: Transjugular intrahepatic portosystemic shunt.

Table 3 Endpoints of included studies *n* (%)

Ref.	Successful stent placement	Shunt stenosis	Final patency	EVS per patient mean $\pm$ SD	Patients crossing over to TIPS	Clinical failure TIPS/EVS	Variceal rebleeding TIPS/EVS	HE TIPS/EVS	Refractory HE TIPS/EVS	Death TIPS/EVS (%)	Follow-up time (mo) TIPS/EVS (mean)
Mahadeva <i>et al</i> ^[13]	18/20 (90)	2/20 (10)	16/20 (80)	3 $\pm$ 1.5	2 (9)	2 (10)/4 (17)	4 (20)/8 (35)	2 ¹ (10)/0 (0)	NR	1 (5)/2 (9)	6/6
Lo <i>et al</i> ^[19]	35/35 (100)	8/35 (23)	27/35 (77)	3.3 $\pm$ 1.4	1 (3)	1 (3)/3 (8)	7 (20)/19 (51)	9 (26)/1 (3)	NR	16 (46)/12 (32)	33/32
Procaccini <i>et al</i> ^[7]	NR	6/42 (14)	36/42 (86)	2 $\pm$ median	7 (11)	NR	UA	11 (25)/1 (2)	NR	14 (32)/17 (28)	47.8/73.9

¹Patients with severe HE. NR: Not reported; UA: The accurate data is unavailable; EVS: Endoscopic variceal sclerotherapy; TIPS: Transjugular intrahepatic portosystemic shunt; HE: Hepatic encephalopathy.

bleeding due to portal hypertension in cirrhotic patients, TIPS is more effective in the prevention of GV rebleeding. Two of the three included studies reported a significant reduction of variceal rebleeding rate in the TIPS group, and the rest one demonstrated similar efficacy between the two therapies^[7,13,19]. Probably, the cause of the distinct results is that Procaccini *et al*^[7] used a different method to present their results. They removed the deaths from the analysis, which led to a unusually lower rate of

GV rebleeding at 1 year than that at 3 mo^[7]. Consequently, we could not get the accurate number of patients with GV rebleeding. Therefore, this study was not pooled in the estimation of GV rebleeding risk.

The advantages of TIPS in the prevention of GV rebleeding were implied in previous studies. Two observational studies proved that EVS was more effective in the control of EV rebleeding than GV rebleeding^[12,20]. The effect of TIPS in the prevention of GV rebleeding

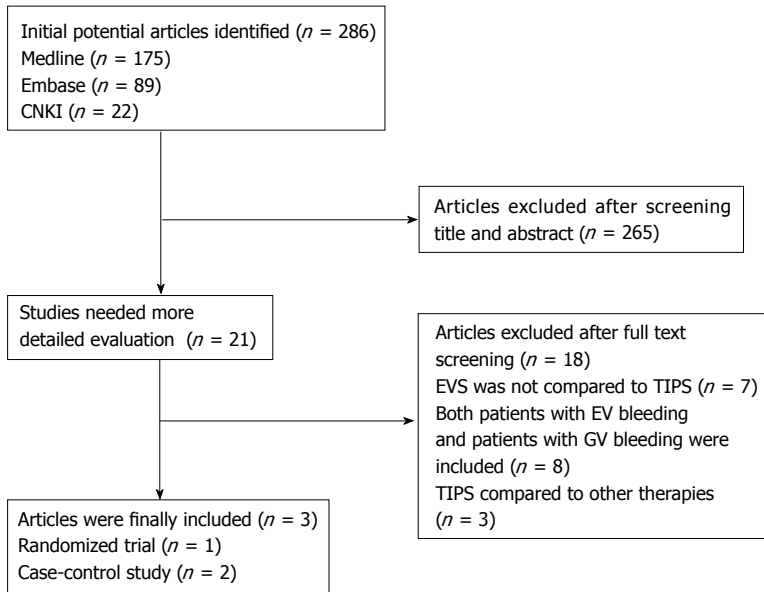
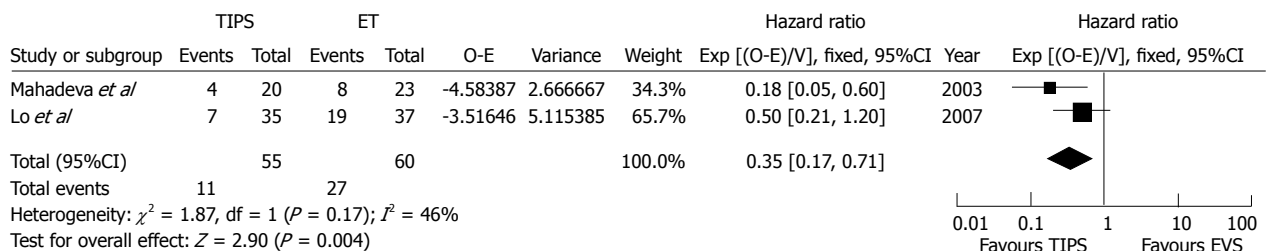
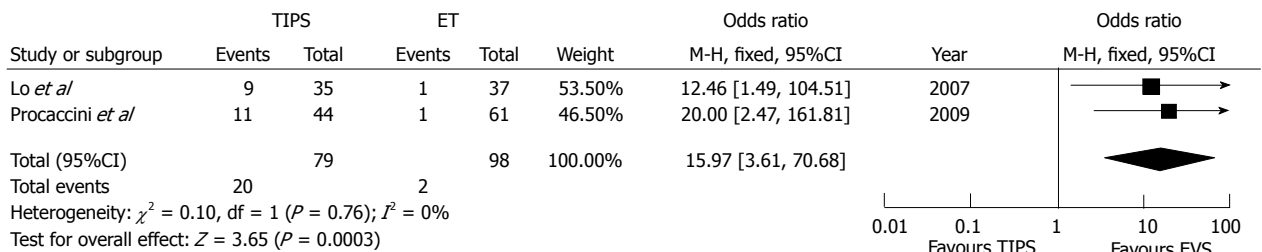
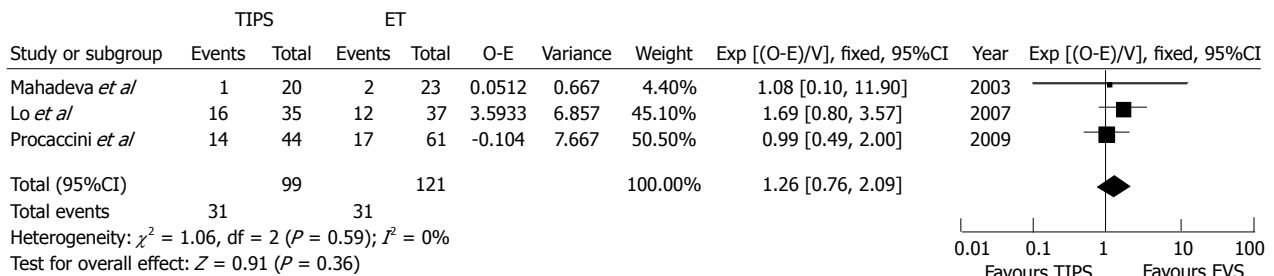
**A** Variceal rebleeding**B** Hepatic encephalopathy**C** Survival

Figure 2 Forest plots of variceal rebleeding (A), hepatic encephalopathy (B), and survival (C). Open squares represent the HRs (or ORs) per each trial and the size of the squares is proportional to the weight of trials. The error bars denote the 95%CI of the HRs (or ORs). HRs (or ORs) on the left equivalence line suggest benefit with TIPS, whereas those on the right demonstrate benefit with EVS. HE: Hepatic encephalopathy; HR: Hazard ratio; OR: Odds ratio; TIPS: Transjugular intrahepatic portosystemic shunt; EVS: Endoscopic variceal sclerotherapy.

was similar to its effect in the prevention of EV rebleeding^[21]. In the patients with previous EV bleeding, several randomized trials have confirmed that TIPS was better

than EVS in the reduction of variceal rebleeding^[22-24]. Accordingly, TIPS may perform better in the management of GV rebleeding than EVS.

All of the three studies demonstrated higher incidences of HE in the patients who received TIPS than the patients treated by EVS. In the study by Mahadeva *et al.*^[13], the number of patients with severe HE but not overt HE was demonstrated. Contrarily, the other two studies did not show the number of patients with severe HE^[7,19]. Hence, the meta-analysis of HE just included the later two studies. A significantly higher incidence of HE in the patients treated by TIPS is suggested by the pooled OR. The increased portal vein blood bypassing the liver is considered as one of the most reasonable causes of the increased HE risk after TIPS procedure. According to the study by Watanabe *et al.*^[25], the patients with GV were more frequently associated with large gastro-renal shunts and chronic HE when compared with the patients with EV. Riggio *et al.*^[26] proved that cirrhotic patients with recurrent or persistent HE were more commonly associated with large spontaneous portosystemic shunts. According to the results of these studies and our thirteen years of experience in performing TIPS procedure, the embolization of the large spontaneous shunt during a TIPS procedure could most likely reduce hepatofugal blood flow and then reduce the post-TIPS HE risk.

The improvement of TIPS in the prevention of GV rebleeding did not improve patient survival, which was most likely due to the expense of the increased risk of HE. Garcia-Pagan and his colleagues proved that TIPS could improve the survival of the patients with acute variceal bleeding and high treatment-failure risk^[27]. With a little regret, this study did not include patients who had variceal bleeding from GV and did not estimate the effect of TIPS and endoscopic therapy in the subgroup of patients with GV. Thus, further studies are needed to evaluate whether TIPS could improve the survival of high-risk patients with acute GV bleeding.

Because most of the patients accepted bare-stent TIPS procedure in the three included studies, 10%-23% of them had shunt stenosis during the follow-up^[7,13,19]. Over the last decade, the use of polytetrafluoroethylene-covered stents improved the shunt patency rate^[28-30]. A recent meta-analysis demonstrated that the use of covered-stents could even reduce the post-TIPS HE risk^[31]. With this improvement, the role of TIPS in the treatment of GV probably would be up-graded in the near future.

Several limitations were accompanied with this meta-analysis. First, only one of the included studies was a randomized controlled trial. Even the offered baseline data of the two groups were comparable in the two nonrandomized studies, the use of randomization could balance the known and unknown confounding factors^[32]. Second, different endpoints were used for the estimation of one outcome, which suggested that it was inappropriate to pooled them together. For example, the study by Mahadeva *et al.*^[13] used the number of patients with severe HE but the remaining two studies used the number of patients with overt HE to demonstrate the risk of post-intervention HE. The third limitation was that only one study provided raw data by the type of GV and none

of the three studies provided raw data by Child-Pugh class^[19]. Therefore, subgroup analyses according to the type of GV and degree of liver function were precluded.

In conclusion, the improvement of TIPS in the prevention of GV rebleeding is associated with an increased HE risk. TIPS procedure did not improve survival of patients with GV bleeding. The role of covered-stent TIPS in the management of patients with GV bleeding should be evaluated in the near future.

COMMENTS

Background

Gastric varices (GV) are the origin in about 27% variceal bleeders with hepatic cirrhosis. Patients with GV bleeding are usually associated with higher mortality than those with esophageal variceal bleeding. Both endoscopic variceal sclerotherapy (EVS) and transjugular intrahepatic portosystemic shunt (TIPS) were employed for the treatment of GV bleeding. Several studies have evaluated the effect of EVS vs TIPS on GV bleeding, and the results of these studies were controversial.

Research frontiers

In the area of GV bleeding treatment, the research hotspot is how to reduce GV rebleeding rate and improve patient survival and life quality.

Innovations and breakthroughs

The present meta-analysis found out that patients with GV bleeding who underwent TIPS procedure had lower incidence of variceal rebleeding than those who underwent EVS treatment. However, TIPS procedure increased the HE risk and did not improve the patient survival.

Applications

The results of the present meta-analysis suggest that the use of TIPS procedure for the treatment of GV bleeding could reduce the risk of rebleeding. Further studies are needed to evaluate the effect of TIPS procedure on survival in patients with cirrhosis and GV bleeding.

Terminology

GV included GV1 (varices continuous with EV and extending along the lesser curve for about 2–5 cm below the gastroesophageal junction), GV2 (varices, often long and tortuous, extending from the esophagus below the gastroesophageal junction toward the fundus), type 1 isolated GV (IGV1, varices located in the fundus that often are tortuous and complex in shape), and type 2 isolated GV (IGV2, ectopic varices in the antrum, corpus, and around the pylorus).

Peer review

It is important to evaluate the effect of TIPS vs EVS in the treatment of patients with GV bleeding. A few studies on this topic showed controversial results. This meta-analysis demonstrates interesting results which are helpful for clinical practice and further clinical research. This paper was well written and is very suitable for publication.

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Contents

Quarterly Volume 5 Number 3 August 6, 2014

REVIEW

- 105 Diagnosis and treatment of gastroesophageal reflux disease
Badillo R, Francis D
- 113 Use of methotrexate in inflammatory bowel disease in 2014: A User's Guide
Swaminath A, Taunk R, Lawlor G
- 122 Visceral hypersensitivity and electromechanical dysfunction as therapeutic targets in pediatric functional dyspepsia
Rosen JM, Cocjin JT, Schurman JV, Colombo JM, Friesen CA
- 139 Approach to *Helicobacter pylori* infection in geriatric population
Cizginer S, Ordulu Z, Kadayifci A
- 148 Pharmacological therapy of feed intolerance in the critically ill
Nguyen NQ
- 156 Renal dysfunction in patients with cirrhosis: Where do we stand?
Pipili C, Cholongitas E

MINIREVIEWS

- 169 Inflammatory bowel diseases: Current problems and future tasks
Actis GC, Pellicano R, Rosina F
- 175 Prevention of hepatocellular carcinoma in patients with chronic hepatitis B
Fernández-Rodríguez CM, Gutiérrez-García ML

CASE CONTROL STUDY

- 183 Ischemic heart disease, factor predisposing to Barrett's adenocarcinoma: A case control study
Tsibouris P, Hendrickse MT, Mavrogianni P, Isaacs PET

CASE REPORT

- 191 Statins and their role in acute pancreatitis: Case report and literature review
Etienne D, Reda Y
- 196 Rhabdomyolysis after midazolam administration in a cirrhotic patient treated with atorvastatin
Gigante A, Di Lazzaro G, Gasperini ML, Barbano B, Liberatori M, Sardo L, Di Mario F, Giorgi A, Rossi-Fanelli F, Amoroso A

Contents

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APPENDIX

I-V Instructions to authors

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Diagnosis and treatment of gastroesophageal reflux disease

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Core tip: Given the high prevalence of gastroesophageal reflux disease (GERD) and the various complications which can result from inadequate treatment, it is important for practitioners to have a proper understanding of the current approach to its diagnosis and management. Diagnostic tools including various methods of pH testing are discussed. Furthermore, it is important to understand the indications and contraindications to anti-reflux surgery in order to optimize our patient's surgical outcomes. Management of GERD in the obese patient may involve bariatric surgery and this is also further discussed.

Abstract

Gastroesophageal reflux disease (GERD) is a common disease with a prevalence as high as 10%-20% in the western world. The disease can manifest in various symptoms which can be grouped into typical, atypical and extra-esophageal symptoms. Those with the highest specificity for GERD are acid regurgitation and heartburn. In the absence of alarm symptoms, these symptoms can allow one to make a presumptive diagnosis and initiate empiric therapy. In certain situations, further diagnostic testing is needed to confirm the diagnosis as well as to assess for complications or alternate causes for the symptoms. GERD complications include erosive esophagitis, peptic stricture, Barrett's esophagus, esophageal adenocarcinoma and pulmonary disease. Management of GERD may involve lifestyle modification, medical therapy and surgical therapy. Lifestyle modifications including weight loss and/or head of bed elevation have been shown to improve esophageal pH and/or GERD symptoms. Medical therapy involves acid suppression which can be achieved with antacids, histamine-receptor antagonists or proton-pump inhibitors. Whereas most patients can be effectively managed with medical therapy, others may go on to require anti-reflux surgery after undergoing a proper pre-operative evaluation. The purpose of this review is to discuss the current approach to the diagnosis and treatment of gastroesophageal reflux disease.

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SYMPTOMS AND EPIDEMIOLOGY

Gastroesophageal reflux disease (GERD) is defined as symptoms or mucosal damage produced by the abnormal reflux of gastric contents into the esophagus or beyond, into the oral cavity (including larynx) or lung^[1,2]. GERD can be classified as non-erosive reflux disease (NERD) or erosive reflux disease (ERD) based on the presence or absence of esophageal mucosal damage seen on endoscopy. The following document will provide a brief overview of the epidemiology, clinical symptoms and complications of GERD as well as a more comprehensive review of the current approach to diagnosis and management.

GERD is one of the most commonly encountered conditions by both primary care physicians and gastroen-

Table 1 Symptoms of gastroesophageal reflux disease

Typical Symptoms	Acid regurgitation, heartburn
Atypical Symptoms	Epigastric fullness, epigastric pressure, epigastric pain, dyspepsia, nausea, bloating, belching
Extracophageal Symptoms	Chronic cough, bronchospasm, wheezing, hoarseness, sore throat, asthma, laryngitis, dental erosions

terologists. To illustrate, a 2005 systematic review found the prevalence of GERD (defined by at least weekly heartburn and/or acid regurgitation) to be as high as 10%-20% in the Western world compared to a prevalence of less than 5% in Asia. There is a trend for higher prevalence in North America compared to Europe, and a trend for higher prevalence in Northern over Southern Europe^[3]. It should be noted, however, that there are limitations in the diagnosis of GERD based solely on patient symptoms as there are patients with endoscopic evidence of GERD (*e.g.*, esophagitis or Barrett's esophagus) who lack symptoms and patients who have symptoms but no objective evidence of GERD. The high prevalence of GERD in combination with the high cost of acid lowering medications results in the significant socioeconomic burden associated with the disease.

GERD can manifest in a wide range of symptoms which can be subdivided into typical, atypical and extraesophageal symptoms (Table 1). In general, symptoms tend to be more common after meals and are often aggravated by recumbency and relieved by acid lowering medications^[1]. Typical symptoms include heartburn and acid regurgitation which have high specificity but low sensitivity for GERD^[4]. Atypical symptoms such as epigastric pain, dyspepsia, nausea, bloating, and belching may be suggestive of GERD but may overlap with other conditions in the differential diagnosis such as peptic ulcer disease, achalasia, gastritis, dyspepsia and gastroparesis. Lastly, there are various extraesophageal symptoms including chronic cough, asthma, laryngitis and dental erosions^[5]. The current belief is that these symptoms are caused by either microaspiration of refluxate or a vagally mediated reflex triggered by distal esophageal acid exposure. The shared vagal innervation of the cough reflex and esophagus is believed to act as the pathway through which distal esophageal acid exposure may lead to coughing, a process known as the esophagobronchial reflex^[6]. However, extraesophageal symptoms could be secondary to a host of other conditions and should not uniformly be attributed to a diagnosis of GERD, especially when typical symptoms are absent.

GERD symptoms have a profound impact on health-related quality of life (HRQoL). A 2011 systematic review of nine studies, including a total of 14774 patients with GERD, showed that persistent reflux symptoms on PPI therapy are associated with reduced physical and mental HRQoL, while reduced mental HRQoL at baseline seemed to impair symptomatic response to PPIs.

The authors recommended that one consider behavioral and psychological factors when making decisions about disease management in those patients with persistent reflux symptoms and reduced well-being despite PPI treatment^[7]. It is therefore important to recognize, diagnose and properly treat patients with GERD in order to avoid detrimental effects on quality of life as well as numerous complications.

GERD-related complications include erosive esophagitis, peptic stricture, Barrett's esophagus, esophageal adenocarcinoma and pulmonary disease. Esophageal adenocarcinoma is thought to be more common in older white males with elevated body mass index and screening for Barrett's esophagus is recommended in this group^[8,9].

DIAGNOSIS

The diagnosis of GERD is typically made by a combination of clinical symptoms, response to acid suppression, as well as objective testing with upper endoscopy and esophageal pH monitoring. For example, the combination of moderate to severe typical symptoms and endoscopic changes (erosive esophagitis or Barrett's esophagus) are highly specific (97%) for GERD (confirmed with pH testing)^[10]. However, a well-taken history alone can prove very valuable in the diagnosis, especially in the setting of heartburn and acid regurgitation which have a very high specificity (89% and 95%, respectively), albeit low sensitivity (38% and 6%) for GERD^[4]. This can allow one to make a presumptive diagnosis and begin empiric therapy, thereby avoiding a comprehensive and costly evaluation in every patient presenting with uncomplicated symptoms^[11]. Additional testing may be necessary, however, for those who do not respond to acid suppression, those who have alarm symptoms (*e.g.*, dysphagia, odynophagia, iron deficiency anemia, weight loss, *etc.*) and those who have suffered from the disease for an extended period of time due to concern for Barrett's esophagus^[1]. The rationale for pursuing additional testing includes confirmation of GERD as well as evaluation of GERD associated complications or alternate diagnoses (Table 2).

Empiric therapy

As mentioned above, those with a history suggestive of uncomplicated GERD manifesting in typical symptoms of heartburn and/or regurgitation can be offered empiric treatment (see treatment section). Typical symptoms that are responsive to acid suppression offer additional evidence for pathologic esophageal acid exposure and it is reasonable to assume a diagnosis of GERD in patients who respond to appropriate therapy^[1]. On the other hand, typical symptoms that do not improve warrant further evaluation to demonstrate the existence of GERD and evaluate for an alternate diagnosis. Likewise, patients with atypical symptoms or non-cardiac chest pain as their primary complaint should also be considered for further diagnostic evaluation prior to empiric therapy. It should be remembered that a minority of patients on even high

Table 2 Diagnostic Testing for gastroesophageal reflux disease

Diagnostic test	Indication
PPI trial	Classic GERD symptoms with no alarm symptoms.
Esophageal pH monitoring	Refractory symptoms where GERD diagnosis is in question, pre-operative evaluation for non-erosive disease
Upper endoscopy	Alarm symptoms (<i>e.g.</i> , dysphagia), PPI unresponsive patients, high risk for Barrett's esophagus
Barium esophagram	Evaluation of dysphagia, otherwise not recommended for GERD evaluation
Esophageal manometry	Prior to anti-reflux surgery to rule out esophageal dysmotility (<i>e.g.</i> , achalasia, scleroderma), otherwise not recommended for GERD evaluation

GERD: Gastroesophageal reflux disease; PPI: Proton pump inhibitor.

dose proton pump inhibition will continue to have objective evidence of pathologic esophageal acid exposure on ambulatory pH monitoring^[12], likely a result of medication non-compliance or PPI resistance.

Ambulatory pH monitoring

Ambulatory reflux monitoring is the only modality allowing direct measurement of esophageal acid exposure, reflux episode frequency and association between symptoms and reflux episodes. It is typically used to evaluate patients with persistent symptoms despite medical therapy, particularly those without endoscopic evidence of GERD, in order to confirm the diagnosis. It can also be employed to monitor the control of reflux in those on therapy with persistent symptoms^[11] and is also recommended in endoscopy negative patients prior to undergoing anti-reflux surgery in order to confirm the diagnosis.

Reflux monitoring is typically performed using either a wireless capsule or a transnasal catheter (pH alone or combined pH-impedance) with the patient either on or off acid suppression. Though there is no uniform consensus regarding the most optimal method, each has its advantages and disadvantages. For either study, diet and activity should remain unchanged in order to capture an accurate depiction of day to day esophageal acid exposure.

Wireless capsule decreases patient discomfort, allows for longer recording time, and may improve accuracy by allowing the patient to resume normal activities without the presence of a transnasal catheter. The test involves endoscopic or transnasal placement of a radiotelemetry pH sensing capsule to the mucosa of the distal esophagus. The capsule (conventionally placed 6 cm above the squamocolumnar junction) measures pH and transmits the data via a radiofrequency signal to a small receiver clipped onto the patient's belt^[13]. Unlike with traditional catheter-based systems, this approach allows the patient to resume normal activity without the conspicuous presence of a transnasal catheter and also allows for additional recording time (typically 48 h compared to 24 h recording with catheter-based monitoring). Another advantage of wireless capsule is the fixed position of the capsule on the esophageal wall in comparison to catheter-based systems where migration due to swallowing or talking has been shown to occur^[14,15]. Potential disadvantages include additional expense due to endoscopic placement (as na-

sal passage can be difficult due to size of capsule), early detachment in a minority of patients, patient discomfort which could require removal via repeat endoscopy, as well as overdiagnosis of GERD due to ingestion of acidic foods^[16]. There is also some data suggesting an increased number of reflux episodes during the first 6 hour period following propofol administration^[17].

Transnasal catheter pH testing is limited by patient tolerance and 24 h monitoring but has the unique advantage of adding impedance which allows distinguishing between acid and non-acid (weakly acidic or weakly alkaline) gastroesophageal reflux. Impedance monitoring detects changes in the resistance to electrical current across adjacent electrodes, allowing it to differentiate the antegrade and retrograde bolus transit of both liquids and gas. Due to the ability to detect both acid as well as nonacid reflux, impedance-pH monitoring has greater sensitivity than pH monitoring alone in the detection of gastroesophageal reflux^[18]. It is the test of choice for on-PPI testing, as these patients have lower rates of acidic reflux with continued episodes of weakly acidic reflux which can then be detected with this modality. In contrast, both wireless capsule and catheter-based systems can be used for evaluation of GERD in patients off acid suppression^[19].

Regardless of the pH monitoring system used, a symptom-reflux correlation is made using either the symptom index (SI) or symptom association probability (SAP), the latter being the preferred statistical calculation^[20]. This allows for measurement of the strength of the association between reflux events and symptoms. A positive association combined with abnormal esophageal acid exposure provides evidence that symptoms are being caused by GERD.

Upper endoscopy

Upper endoscopy is the primary modality used in the evaluation of the esophageal mucosa in patients with GERD and also allows for biopsies of concerning lesions (*e.g.*, Barrett's metaplasia, strictures or masses). It is important though to understand that there are limitations with the use of upper endoscopy in the diagnosis of GERD. For instance, while an endoscopy showing esophagitis or Barrett's esophagus essentially confirms the diagnosis of GERD (high specificity), a normal endoscopy does not refute the diagnosis. In fact, most patients with typical symptoms of GERD will have no endoscopic evidence

of GERD on esophagogastroduodenoscopy. Therefore, an upper endoscopy is not required for the diagnosis and is mostly performed for evaluation of GERD associated complications and alternative diagnoses as well as for placement of wireless capsule pH probes. Patients with multiple risk factors for esophageal adenocarcinoma (age 50 years or older, male sex, white race, chronic GERD, hiatal hernia, elevated BMI, and intra-abdominal distribution of body fat) should receive screening endoscopy for Barrett's esophagus^[8].

Barium esophagram

Barium esophagram was once recommended as a screening test for GERD, but is no longer part of the diagnostic evaluation. A 1996 study of 125 patients compared barium esophagram to esophageal pH monitoring to assess the accuracy of barium screening as a predictor of abnormal esophageal acid exposure. A significantly greater degree of abnormal esophageal acid exposure occurred in patients who had a hiatal hernia or spontaneous reflux on barium radiography. However, the sensitivity and specificity of barium radiography for abnormal degrees of acid reflux were insufficient and therefore this test is no longer recommended in the diagnosis of GERD^[21]. On the other hand, it is frequently used in the evaluation of complications related to GERD (*e.g.*, peptic stricture) as well as in the evaluation of dysphagia in the post anti-reflux surgery patient, in conjunction with endoscopic evaluation.

Esophageal manometry

Esophageal manometry is most useful for the evaluation of dysmotility and has only limited utility in the evaluation of GERD. Although disruption of the anti-reflux barrier (gastroesophageal junction) and dysfunction of esophageal peristalsis are common in GERD patients, these findings are not diagnostic and therefore there is no manometric pattern which is pathognomonic for reflux^[22]. The role of manometry in the evaluation of GERD remains limited to preoperative testing for exclusion of significant motility disorders such as achalasia or scleroderma (clear contraindications to anti-reflux surgery) as well as for assisting in proper positioning of transnasal pH probes. Otherwise, this test is not recommended for the diagnosis of GERD.

TREATMENT

GERD is a chronic disease that typically requires long term management in the form of lifestyle modification, medical therapy and, for a subset of patients, surgical therapy.

Lifestyle changes

Lifestyle and diet modification traditionally have included weight loss, head of bed elevation, avoidance of nighttime meals, and elimination of trigger foods such as chocolate, caffeine and alcohol. A 2006 systematic review

of 16 randomized trials evaluated the impact of lifestyle measures on GERD and concluded that only weight loss and elevation of the head of the bed improved esophageal pH and/or GERD symptoms^[23]. A 2006 systematic review and meta-analysis suggested a positive association between increasing BMI and the presence of GERD within the United States and possibly within other countries as well^[24]. Interestingly, BMI was found to be associated with symptoms of GERD in both normal weight and overweight women and even moderate weight gain among those of normal weight was found to cause or exacerbate symptoms^[25]. Therefore, weight loss is recommended for GERD patients who are overweight or who have had recent weight gain.

For nighttime reflux symptoms, patients should elevate the head of the bed and avoid recumbency 3 h postprandially. A recent study aimed to compare the recurrence rates of ERD and NERD, and determine the risk factors related to the recurrence. Recurrence was diagnosed when patients complained of GERD symptoms requiring additional medication after initial recovery with 4-8 wk of PPI treatment. The authors found that a shorter dinner-to-bedtime interval was the most significant factor influencing the recurrence of GERD and patients who usually slept within 3 h after eating had higher recurrence rates^[26]. Despite strict compliance, lifestyle changes alone are frequently inadequate at controlling symptoms and medical therapy often becomes necessary.

Medical therapy

The mainstay of treatment of GERD is acid suppression which can be achieved with several classes of medications including antacids, histamine-receptor antagonists (H₂RAs) or proton-pump inhibitors (PPIs). Studies have shown more complete healing of erosive esophagitis and heartburn relief with PPIs *vs* H₂RA and this effect occurs nearly twice as fast (healing rate and heartburn relief of 11.7%/wk and 11.5%/wk *vs* 5.9%/wk and 6.4%/wk in the PPI and H₂RA groups, respectively)^[27]. Additionally, studies show that ERD is more difficult to treat with H₂RA compared to PPIs^[28] and patients with ERD tend to have a higher symptom response to PPIs compared to their NERD counterparts^[29]. Therefore, it is recommended to treat erosive reflux disease with maintenance PPI therapy at the lowest effective dose as most will relapse after discontinuation of therapy^[30]. In general, PPIs are felt to be equally effective and patients should be instructed to take these medications 30-60 min prior to meals; the exception to this is dexlansoprazole which can be taken irrespective of food intake.

In contrast, patients with NERD may potentially be managed successfully with on-demand PPI or, alternatively, with less costly therapy such as H₂RAs. A 2001 study set out to determine the feasibility of step-down therapy in patients with symptoms of GERD rendered asymptomatic with PPIs. After 1 year follow up, 58% of patients in the step-down group were asymptomatic on either non-PPI therapy or no therapy at all. Of those

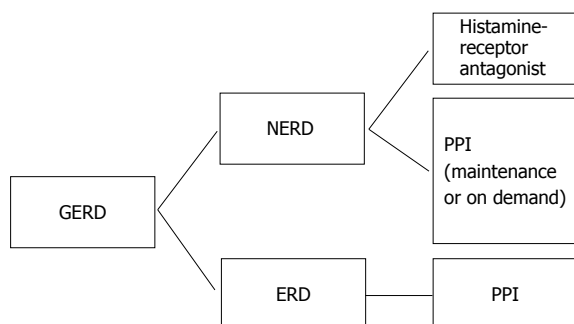


Figure 1 In general, patients with gastroesophageal reflux disease who are found to have evidence of erosive esophagitis on endoscopy should be placed on maintenance proton pump inhibitor due to the high risk of relapse off proton pump inhibitor. However, patients with NERD may achieve symptom control on H₂RAs or, alternatively, with on-demand PPI. If symptoms persist, maintenance PPI should be considered. GERD: Gastroesophageal reflux disease; PPI: Proton pump inhibitor; ERD: Erosive reflux disease; NERD: Non-erosive reflux disease.

who remained off PPIs, 59% required H₂RAs^[31]. Given the high cost associated with indefinite PPI use, attempts should be made to treat patients with the least expensive yet effective medication, particularly in patient with NERD who may be able to be maintained on H₂RAs with control of symptoms. If symptoms recur, then maintenance PPI therapy should be reconsidered (Figure 1).

Patients with PPI-refractory GERD can be challenging to treat and are frequently referred to a gastroenterologist. First, compliance with medical therapy and proper dosing should be addressed. A study involving 10159 patients with Barrett's esophagus and 48965 GERD patients without Barrett's esophagus found that PPI prescriptions were filled by only 66.6% and 60.4% of patients with BE and GERD, respectively^[32]. Given such high rates of noncompliance, an accurate history is important to obtain in order to avoid escalating therapy unnecessarily. If symptoms are truly refractory to proper medical therapy, the dosing can be increased or an alternate PPI can be used. Both methods may lead to further symptom improvement and both appear to be equally effective^[33]. If a patient has predominantly nighttime symptoms, more effective nocturnal acid suppression may be achieved with bid or nighttime dosing of PPIs^[34].

Another approach in the PPI-refractory patient involves the addition of nighttime H₂RAs to bid PPI therapy for persistent nighttime symptoms. Though a contested issue, the benefit from this approach would likely be temporary as studies have shown that after 1 mo of uninterrupted H₂RA therapy, gastric acidity returns to pre-H₂RA levels^[35]. Another well studied medication is the GABA_B agonist baclofen which has been shown to reduce postprandial reflux events and acid exposure in normal individuals and in patients with GERD by inhibiting transient lower esophageal sphincter relaxations, thought to be the primary cause of reflux events^[36]. Unfortunately, side effects often preclude continued use of this medication and include drowsiness (up to 63%),

dizziness (5%-15%), weakness (5%-15%), and fatigue (2%-4%)^[37]. In a recent randomized, cross-over trial it was shown that administering baclofen at bedtime decreases sleep related reflux events and markedly improves objective and subjective sleep parameters compared with placebo. Thus, baclofen appears to have potential benefit for GERD patients with persistent symptoms on PPI therapy, especially those who have persistent nighttime heartburn and sleep complaints^[37]. Finally, with respect to prokinetic therapy, a recent study randomized patients into an omeprazole plus mosapride (5HT₄ agonist) group and omeprazole plus placebo group and found that the addition of mosapride to omeprazole was no more effective at controlling reflux symptoms than omeprazole alone in patients with NERD^[38]. Based on this and several other studies, there is no clear role for the use of prokinetic therapy in the treatment of GERD.

If symptoms persist after attempts at maximizing medical therapy, an evaluation for non-GERD etiologies should be undertaken. An upper endoscopy should be performed next and may reveal an abnormality such as persistent erosive esophagitis, eosinophilic esophagitis, or Barrett's esophagus in roughly 10% of patients in whom empiric PPI therapy fails^[39]. The finding of esophagitis would support the diagnosis of GERD and point towards noncompliance or failure of medical therapy. Most times, the esophagus will appear endoscopically normal and these patients should be further evaluated with pH monitoring to confirm or refute the diagnosis of GERD. Confirming pathologic acid reflux with a positive symptom correlation would indicate PPI failure and need for escalation of medical therapy or consideration of surgical options. The absence of GERD in a patient with typical heartburn symptoms would suggest a diagnosis of functional heartburn^[2].

Surgical therapy

Surgical therapy is another treatment option for long-term therapy in patients with GERD and has become more appealing since the introduction of laparoscopic anti-reflux surgery. Indications for anti-reflux surgery, which typically include laparoscopic fundoplication or bariatric surgery, include unwillingness to remain on lifelong medical therapy, intolerance of medical therapy, medically refractory symptoms with evidence of GERD on endoscopy or pH monitoring, or GERD in the setting of a large hiatal hernia (Table 3).

Proper patient selection is critical to obtain the best possible surgical outcomes and it is imperative that there be objective documentation of GERD. Furthermore, it is well known that the highest surgical response is seen in those with typical symptoms who respond to a PPI or have abnormal pH testing with good symptom correlation. On the other hand, response rates to surgical intervention are lower in those with atypical or extraesophageal symptoms. To illustrate, one study showed that at 69 mo after laparoscopic fundoplication, the majority of patients maintained improvement or resolution of heart-

Table 3 Indications for anti-reflux surgery

Unwillingness to remain on lifelong medical therapy
Intolerance of medical therapy
Medically refractory symptoms with objective evidence of GERD
GERD in the setting of a large hiatal hernia
Medically refractory GERD in the setting of morbid obesity

GERD: Gastroesophageal reflux disease.

burn (90%), regurgitation (92%), and dysphagia (75%) when compared to before surgery. However, the results were less satisfactory in patients with extraesophageal symptoms such as hoarseness (69%) and cough (69%)^[40]. In addition to upper endoscopy and esophageal pH testing, a preoperative workup should include a barium esophagram and esophageal manometry to ensure that there is normal esophageal motility. The combined results of this testing can establish the presence of disease and assist with planning the operative approach^[41].

The short and medium term outcomes of laparoscopic anti-reflux surgery are quite good in terms of improving the typical symptoms of GERD^[42]. However, in the long term it appears these results may diminish. During a follow-up period of 10 to 13 years, one study comparing long term outcomes in medical and surgical therapies for GERD found that 62% of surgical patients took anti-reflux medications on a regular basis, compared to 92% of medical patients. Anti-reflux surgery can be very effective but should not be advised with the expectation that patients will no longer take anti-secretory medications^[43].

Complications from anti-reflux surgery include dysphagia of sufficient severity to require esophageal dilation in about 6% of patients treated with fundoplication surgery^[44] as well as a significant increase in flatulence and inability to belch (gas bloat syndrome). This potential for complications underscores the importance of carefully selecting patients for anti-reflux surgery in order to optimize outcomes.

Due to concern for complications associated with traditional fundoplication, sphincter augmentation using the LINX Reflux Management System was developed. The surgery involves the laparoscopic placement of a bracelet of titanium beads with magnetic cores around the LES which serves to augment the physiologic barrier to reflux without altering gastric anatomy. Studies show that at four years following LINX implantation, 87.5% of patients were satisfied with their present condition, and 80% of patients were free from daily dependence on PPIs^[45].

In view of the invasiveness of surgery, several endoscopic therapies for GERD have been attempted but due to inability to control GERD have been removed from the market. One of the latest endoscopic techniques for treatment of GERD is transoral incisionless fundoplication. A recent study showed that only a subgroup of patients experienced improved quality of life and

reduced need for PPIs at 3 years follow-up, and an unacceptably high percentage of patients required additional medication or revisional laparoscopic fundoplication^[46]. Additional studies in endoscopic therapy for GERD are ongoing.

Finally, when it comes to the obese patient with GERD, a different approach should be considered. Gastric bypass is the recommended treatment for GERD in the morbidly obese patient (BMI > 35 kg/m²) due to concerns over higher failure rates following Nissen fundoplication in this population. Not only does bariatric surgery better address the mechanisms that lead to GERD in obese patients with the potential for a more durable response, but it also reduces obesity-related comorbidities and possibly reduces the long-term mortality risk associated with morbid obesity in an acceptably safe, minimally-invasive, and cost-effective manner^[47]. Although all common bariatric procedures improve GERD, Roux-en-Y gastric bypass is superior to adjustable gastric banding and sleeve gastrectomy^[48].

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Use of methotrexate in inflammatory bowel disease in 2014: A User's Guide

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Crohn's disease; Ulcerative colitis; Immunomodulators; Methotrexate user's guide

Core tip: Methotrexate can be a useful adjunct to the treatment of inflammatory bowel disease, but many practitioners are unfamiliar with its use. Here, we have provided a succinct summary of the data behind the use of methotrexate and a short "user's guide" and algorithm to allow for the busy clinician to become quickly familiar with the drug and information to help prescribe it safely.

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Abstract

Methotrexate has been used as an immunomodulator in many autoimmune diseases, including inflammatory bowel disease. However, many physicians are unfamiliar or uncomfortable with its use in the management of inflammatory bowel disease. We summarize the data for use of methotrexate in common clinical scenarios: (1) steroid dependant Crohn's disease (CD); (2) maintenance of remission in steroid free CD; (3) azathioprine failures in CD; (4) in combination therapy with Anti-TNF agents in CD; (5) decreasing antibody formation to Anti-TNF therapy in CD; (6) management of fistulizing disease in CD; and (7) as well as induction and maintenance of remission in ulcerative colitis. An easy to use algorithm is provided for the busy clinician to access and safely prescribe methotrexate for their inflammatory bowel disease patients.

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Key words: Methotrexate; Inflammatory bowel disease;

INTRODUCTION

Methotrexate (MTX) has a long history for effectively treating rheumatological conditions such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and sarcoidosis^[1-3]. Over the past 25 years there have been numerous studies that evaluated its efficacy in Inflammatory Bowel Disease with varied results. It has to date remained in treatment algorithms as a salvage therapy for patients who have failed, or become intolerant of, azathioprine. The goal of our paper is to summarize the data behind methotrexate for common clinical situations and to provide a quick access guide on prescribing the drug.

MTX PHARMACOKINETICS

The landmark studies demonstrating efficacy of MTX in Crohn's disease (CD) have utilized *sq* or *im* at 25 mg/wk. Smaller non-randomized studies in both CD and UC patients have offered conflicting data and, to an extent

Table 1 Summary of methotrexate trials in Crohn's disease

Study	Dose MTX	Route of admin	n	Study design	Patients	Duration follow up (wk)	MTX response	MTX remission	Placebo or (Comparator) Response	AE MTX	AE Placebo
Kozarek	25 mg/wk	<i>sq</i>	14	Non-Randomized-open Label	CD	12	79%				
Feagan	25 mg/wk	<i>im</i>	141	Double-blind Placebo controlled multi center	Steroid dependent CD	16		39.4% ¹	19.1%	1%	2%
Oren	12.5 mg/wk	<i>po</i>	84	Randomized Double-Blind Placebo Controlled	Active CD	36		38%	46%		
Arora	22.5 mg/wk	<i>po</i>	33	Randomized Double Blind Placebo Controlled	Steroid Dependent CD	52	54%		20%	23%	0
Feagan	15 mg/wk	<i>im</i>	76	Double Blind Placebo Controlled Multi-Center	CD Maintenance	40		65% ¹	39%	1%	2%
Mate-Jimenez	15 mg/wk	<i>po</i>	38	Randomized Single Center	Steroid Dependent CD	76		80% ¹ Induction 66.6% ¹ Maintenance	14% Induction 0 Maintenance	11.5%	0
Lemann	25 mg/wk	<i>im</i>	49	Retrospective	Active CD			84%		49%	
Fraser	20 mg/wk (10-25)	<i>po/im</i>	48	Retrospective	Active CD-Maintenance			62%		27%	
Ardizzone	25 mg/wk	<i>iv</i>	54	Investigator Blind, randomized	Active CD	24		56%	63% AZA	11%	
Mahadevan	25 mg/wk	<i>im</i>	16	Retrospective case series	Fistulizing CD		56%			6%	
Wahed	25 mg/wk Induction 15 mg/wk Maintenance	<i>im/po</i> -Induction <i>po</i> -Maintenance	99	Retrospective	AZA Intolerance/AZA non-responders		62%			8.3%	
Feagan	Wk0-10 mg/wk Wk3-20 mg/wk Wk5-25 mg/wk	<i>sq</i>	126	Double Blind Placebo Controlled Multi-center	Active CD	50		IFX + MTX 56%	IFX + PCBO 57%		

¹*P* < 0.05 vs MTX response. MTX: Methotrexate; CD: Crohn's disease; AE: Adverse events; AZA: Azathioprine.

demonstrate, the relative ineffectiveness with low dose *po* regimens for induction or maintenance of remission (Table 1)^[4,5]. Jundt demonstrated similar bioavailability between *po* vs *sq* vs *im* MTX in RA patients^[6]. The bioavailability of *po* as compared to *im* was 0.85.

Kurnik *et al*^[7] studied the bioavailability of MTX in adult patients with stable Crohn's disease. The patients were administered their weekly doses either orally or *sq* and the MTX levels were measured over the next 24 h. No information on extent of small bowel inflammation was provided. They found that oral bioavailability averages 73% (95%CI: 62%-86%) of that of subcutaneous administration^[7]. Hoekstra demonstrated that the bioavailability of *po* MTX can be boosted by split dosing. RA patients were studied after single dosing of MTX by either *sq* or *po* method. Then the same patient underwent a second measurement after split dosing of MTX (50% of the dose taken 8 h later). The bioavailability of the split

dose was 28% higher compared to the single dose (*P* = 0.007) and was statistically significant. The mean bioavailability after single-dose and split-dose MTX was 0.76 and 0.90, respectively, compared to subcutaneous administration^[8].

Wilson *et al*^[9] updated the Kurnik study using a more sensitive assay. They compared the pharmacokinetic profile of *po* and subcutaneous MTX (25 mg) in 11 CD patients. The bioavailability of *po* MTX compared with *sq* was found to be 0.86 (90%CI: 0.79-0.92). Of note, the 90%CI to meet definition of bioequivalency proposed by the FDA was not met, (lower end of the 90%CI would have had to be 0.80 rather than 0.79), and so this study could not claim true bioequivalency of the oral and *sq* routes of administration.

Although these are small studies and many patient factors were not provided (*i.e.*, extent and severity of bowel disease), the *po* route of administration does ap-

pear to be less bioavailable than *sq* dosing.

WHAT IS THE DATA FOR MTX IN INDUCTION OF REMISSION IN STEROID DEPENDENT CROHN'S DISEASE?

Although Kozarek *et al*^[10] (NEJM 1980) had demonstrated the efficacy of 6-mercaptopurine in the induction of remission of Crohn's disease, the authors noted the response to be delayed and incomplete. The first report of successful induction with methotrexate was reported by Kozarek *et al*^[10] in 1989. This non-randomized, open-label pilot study included 14 patients with Crohn's disease with an unidentified fraction described as failing immunomodulators. Eleven patients (79%) demonstrated a clinical response to 25 mg/wk *im* methotrexate as measured by objective decreases in CDAI, and 5 patients (36%) demonstrated endoscopic mucosal healing. Although this study lacked a control arm, it suggested MTX may have value in inducing remission in patients with Crohn's disease.

Feagan completed a prospective double-blind, placebo-controlled Canadian multicenter study of weekly *im* injections of methotrexate in patients who had chronically active Crohn's disease despite a minimum of 3 mo of prednisone therapy with the primary outcome being the induction of clinical remission^[11]. A total of 141 patients assigned in a 2:1 ratio of MTX to placebo were included in the trial and 37 (39.4%) achieved clinical remission in the methotrexate group compared with 9 (19.1%) in the placebo group ($P = 0.025$). The response among patients requiring high dose prednisone (> 20 mg/d) was equally good as those requiring low doses at study initiation. Prednisone dose was appreciably lower by week 4 in the MTX group and demonstrated the largest difference from week 12 through 16. A greater number of patients withdrew from the treatment arm due to adverse events (17% *vs* 2%). The withdrawals from the MTX arm were due to asymptomatic elevation of serum aminotransferase concentrations (7), nausea (6), skin rash (1), atypical pneumonia (1), and optic neuritis (1).

Oren *et al*^[5] conducted a prospective randomized, double blind, placebo-controlled Israeli multi-center trial to evaluate the effectiveness of oral methotrexate in patients who had required steroids or immunomodulators for at least 4 mo out of the year prior to enrollment. Although it would be difficult to characterize these patients as steroid dependant, they had active ongoing disease as measured by Harvey Bradshaw Index. The study randomized 84 patients to 12.5 mg *po* MTX/week *vs* 6-MP 50 mg/daily *vs* placebo. The lower dose of oral MTX (compared to 25 mg/wk *im* in the Feagan study) was based on reported efficacy in the rheumatoid arthritis literature. Remission rates were 39% and 41% in the MTX and 6-MP groups respectively. However, the rate of remission in the placebo group was 46%, thereby inferring no benefit for either the MTX or 6 MP treatment arm.

Criticisms of this study included presumed underdosing of MTX and 6 MP. Also, no standard steroid tapering regimen was described in this study, although reduction in steroid dose was described as an outcome measure. Although improvement was seen based on intra-patient evaluation (each patient used as their own control), this was not a pre-specified analysis. Hence, these results should be viewed with caution.

A cohort of 38 patients with steroid dependant CD was evaluated by Mate-Jimenez, but the requirement to separate these patients into 3 arms (1.5 mg/kg per day 6MP, 15 mg/wk *po* MTX, or 5-ASA) resulted in a small number of patients in each arm^[12]. However, the large differences in outcomes for induction of remission in both treatment arms (93.7% 6MP, 80% MTX) compared to placebo (14%) was statistically significant. Interestingly, these findings show a degree of benefit that has not been reproduced for either the 6MP or MTX treatment arms. Arora *et al*^[13] evaluated 28 steroid-dependant Crohn's disease patients who received 15 mg/wk *po* MTX *vs* placebo. Dose escalation to 22.5 mg/wk was allowed at the discretion of the clinician. The primary endpoint was clinical exacerbation of Crohn's disease. Although fewer patients in the MTX group (6/13, 46%) experienced exacerbation of CD *vs* placebo (12/15, 80%), the findings did not reach statistical significance. Despite the 43% relative risk reduction in flare frequency between the treatment and placebo, this study was underpowered to find this difference to be significant.

Ardizzone evaluated the efficacy of *iv* MTX in comparison to AZA^[14]. This randomized investigator-blind study enrolled 54 steroid-dependent active (CDAI > 200) CD patients on > 10 mg/d of steroid therapy. Patients were randomized to 25 mg *iv*/wk of MTX *vs* *po* AZA 2 mg/kg per day for 3 mo, after which MTX dosing was changed to 25 mg/wk *po* for an additional 3 mo follow up. The primary outcome considered was the proportion of patients entering steroid-free remission after 3 and 6 mo of therapy. No statistically significant difference was found between the two treatment regimens with respect to remission rate after 3 mo (methotrexate 44%, azathioprine 33%, $P = 0.28$, 95%CI: 0.369-0.147), and 6 mo (methotrexate 56%, azathioprine 63%, $P = 0.39$, 95%CI: 0.187-0.335), respectively. MTX and AZA demonstrated similar rates of adverse events leading to medication withdrawal. While there appeared to be no additional benefit to providing MTX *via* the IV route, MTX at 25 mg/wk appeared to have similar efficacy as weight based azathioprine in inducing and maintaining remission in active Crohn's disease.

A 2011 meta-analysis of MTX in active Crohn's did not include either the Mate-Jimenez or Ardizzone studies (no placebo arm) or Arora studies (categorized the study patients as quiescent)^[14]. Their conclusion that MTX was not better than placebo in active Crohn's was based only on the inclusion of Feagan's positive trial (25 mg/wk *im* MTX) and the negative orally administered MTX (12.5 mg/wk *po*) Oren trial. The Cochrane collaboration

reached similar conclusions a year later, but understood the limitations of the data on oral MTX and suggested further study^[15].

WHAT IS THE DATA FOR MTX IN MAINTENANCE OF STEROID-FREE REMISSION IN CROHN'S DISEASE?

Feagan demonstrated the use of MTX in Crohn's disease for maintenance of remission in a large double-blind, placebo controlled multi-center study with 76 patients in 2000^[16]. Some of these patients were enrolled from Feagan's trial for induction of remission using 25 mg *im*/wk MTX in 1995 and others from an open label trial of 25 mg/wk *im* MTX. The patients were randomized to 15 mg *im* MTX/weekly *vs* placebo and followed for 40 wk. Impressively, no other therapy for Crohn's disease was permitted. At the completion of the trial 65% (26/40) of the MTX group maintained remission compared to 39% (14/36) of the placebo group ($P = 0.04$). A majority (55%) of the relapsers could be re-induced with 25 mg/wk *im* MTX. Adverse events were minimal as only 1 patient discontinued MTX therapy for nausea and vomiting.

The efficacy of oral MTX (10-20 mg *po*) for maintenance of remission in Crohn's and ulcerative colitis was evaluated by a retrospective review by Fraser. Although 1 year remission rates approached 90%, the data for Crohn's and UC were combined and the clinical definition of remission was vague^[17].

Given the dearth of high quality studies of MTX in maintaining remission in Crohn's, the only maintenance study used in the Kahn meta-analysis was Feagan's (15 mg *im*/wk MTX) suggesting benefit with a number needed to treat (NNT) of 4^[14]. Interestingly, the Cochrane meta-analysis of MTX for maintenance of remission, included both the Mata-Jimenez study and Oren studies as part their analysis^[18]. Their main conclusions track the benefit shown by the Feagan's 15 mg/wk *im* MTX and suggest that lower oral doses do not benefit maintenance of remission.

CAN MTX BE USED IN PATIENTS WHO FAIL AZA AND HOW DURABLE IS THE RESPONSE TO MTX?

Despite the widespread use of thiopurines, approximately one third do not respond and another 10% cannot tolerate the drugs^[19]. In the United States, MTX is often reserved for AZA intolerance or failure and fewer physicians are comfortable prescribing it^[20]. AZA Intolerance can include bone marrow suppression, upper GI symptoms, pancreatic dysfunction, abnormal LFT's and nonspecific symptoms including joint aches, hair loss, rash and flu like illness.

A study by Lemann in 2000 evaluated the durability of MTX for maintenance of remission in a population of patients who had (mostly) failed or were intolerant to

AZA and had *already been treated* with MTX for period of at least 6 mo were followed for an additional 18 mo^[21]. Out of 49 patients, 42 had previously failed AZA (85%). Out of the 41 achieving remission, 36 had previously failed AZA (87%). Most of the patients were administered 25 mg/wk *im* MTX, but some physicians changed the dose to oral administration and some were even able to taper it. Despite some patients with oral MTX dosing and despite a heavy proportion of AZA failures in the study population, 71% of the study population remained in remission for 1 year and up to 52% remained in remission after 3 years. Among patients who initially do well on MTX after AZA failure, they are likely to remain well on that therapy over the next several years.

Wahed *et al*^[22] evaluated clinical response of 99 CD patients retrospectively who were placed on MTX due to AZA intolerance or nonresponse. The study suffers from a non-homogenous doses and method of administration of MTX for induction and maintenance. The range of induction dose of MTX was 2.5-25 mg/wk and administration varied as either *im* or *po*. Improvement was based on multiple variables as available from the charts, but was not standardized. With these caveats, clinical response occurred in 18 of 29 patients (62%) refractory to AZA/MP and 42 of 70 patients (60%) intolerant to AZA/MP. This suggests that MTX is effective in CD patients previously treated with AZA who experienced failure or non-response.

At present, there are no high quality trials (prospective, identical induction doses and method of administration, presence of control groups) on which to confidently choose to use MTX specifically in a population of AZA/6MP failures, but it would not be unreasonable to attempt MTX.

DOES COMBINATION MTX AND ANTI-TNF THERAPY TO TREAT CROHN'S DISEASE RESULT IN BETTER OUTCOMES?

The landmark SONIC study demonstrated that patients with moderate-to-severe Crohn's disease who were treated with combination infliximab plus azathioprine were more likely to have a corticosteroid-free clinical remission than those receiving azathioprine or infliximab monotherapy^[23]. Concomitant immunosuppressive therapy also reduces the magnitude of the immunogenic response of infliximab^[24]. It follows that methotrexate, as part of combination therapy with anti-TNF agents, may provide similar benefits.

Feagan *et al*^[25] studied this hypothesis in the COMMIT trial. They performed a 50-wk double-blind, placebo-controlled trial of MTX + IFX *vs* IFX monotherapy in Crohn's patients who had started prednisone therapy within the preceding 6 wk. Patients were not permitted to use any other therapy with the exception of antibiotics for 14 d in the case of active perianal disease. Patients

were initiated on IFX 5 mg/wk and 10 mg *sq* MTX/week (escalating to 25 mg/wk by week 5) or IFX 5 mg/wk and placebo injections. Prednisone was force tapered in all patients by week 14. The primary outcome evaluated steroid free-remission by week 14 or maintenance of remission by week 50. Steroid-free remission at week 14 was 76% (48/63) in combination therapy compared to 78% (49/63) with IFX mono therapy ($P = 0.83$). At week 50, 56% (35/63) *vs* 57% (36/63) maintained remission in the combination arm *vs* monotherapy arm. Mean methotrexate doses at week 50 in the treatment arm was 22.3 mg/wk. This study found that combination therapy with IFX and MTX had no more benefit than IFX alone.

Based on the strongest current body of evidence (SONIC, COMMIT), it seems reasonable to prefer combination therapy using AZA/6MP rather than MTX in those Crohn's patients able to tolerate it.

IS MTX EFFECTIVE IN PREVENTING AUTO-ANTIBODY FORMATION WHEN USED IN COMBINATION WITH BIOLOGIC THERAPY?

A prospective study by Vermeire evaluated the development of antibodies to infliximab (ATI) when combined with AZA, MTX, or placebo^[26]. The concomitant use of immunosuppressive therapy (MTX or AZA) was associated with a lower incidence of antibodies to IFX (53/115, 46%) compared with patients not receiving concomitant immunosuppressive therapy (43/59, 73%; $P < 0.0001$). Furthermore, the incidence of antibody formation was not different between the MTX and AZA groups, 44% compared to 48% respectively. Patients not taking IS therapy had lower IFX levels (median 2.42 mcg/mL) 4 wk after any follow-up infusion than patients taking concomitant IS therapy (median 6.45 mcg/mL) ($P = 0.065$), but there was no difference between MTX or AZA. Sokol *et al*^[27] confirm that patients using co-treatment with immunosuppressives experienced less IBD activity and less need to switch Anti-TNF therapy due to secondary loss of response. In fact, their data suggest efficacy of AZA over MTX, though their patient population included both CD and UC patients, and it is not clear whether any of the UC patients were treated with MTX and included in the analysis.

Although the COMMIT study did not show an improvement in 50 wk outcomes using combination therapy (IFX + MTX *vs* IFX alone), the MTX combination group did achieve statistically significant lower antibody levels (4% compared with 20%, $P = 0.01$) and demonstrated higher median serum trough levels of IFX (6.35 µg/mL *vs* 3.75 µg/mL, $P = 0.08$), similar to what is seen with azathioprine combination therapy^[25]. Whether this would result in fewer instances of infusion reactions or secondary non-response to IFX beyond 50 wk remains to be seen.

CAN MTX BE USED TO MANAGE SECONDARY NONRESPONSE TO BIOLOGIC MONOTHERAPY?

Absah retrospectively evaluated 14 pediatric patients with moderate to severe (CD) eventually failing anti-TNF- α therapy (13 ADA and 1 IFX) who then received concomitant methotrexate (median dose 17.5 mg *sq*/wk)^[28]. Most (12/14) patients had also previously failed AZA therapy (though it is not made clear whether this was as part of combination with biologic). Clinical remission was achieved in 7/14 (50%) of patients on average of 6 wk after MTX initiation with no additional improvement in the other 7 patients during 10 mo of follow up. Unfortunately, no levels of biologic or antibody to biologic were measured in this study, so the mechanism of improvement remains unknown. Further research focusing on the adult population along with mechanism of action would serve to direct therapy in this refractory population often seen in tertiary centers.

DOES MTX TREAT FISTULIZING CROHN'S DISEASE?

To date, only small retrospective series are available to evaluate the efficacy of MTX monotherapy in fistulizing Crohn's disease. A research conducted a retrospective chart review of all Crohn's disease receiving methotrexate 15-25 mg *im* MTX/weekly. This group of patients that had failed or were intolerant to 6MP and were made up of perianal fistulae (9), abdominal wall (3), rectovaginal (1), bladder (1), perianal + rectovaginal (2). Overall, 4/16 (25%) experienced complete fistula closure and 5/16 (31%) had partial fistula closure. Fourteen of sixteen patients received full dose 25 mg *im*/wk of MTX for 3 mo and were switched to *po* for maintenance. The time to response could not be determined in half of the patients, but ranged from 4-13 wk in the other half. Another study found that 8/18 (44%) patients with Crohn's-related fistulas achieved partial or complete response using MTX for 6 mo, but information about success and failure based on oral or *im* administration was not provided^[29]. A pilot study of 12 patients using combination infliximab and MTX found 7 patients had total or partial response to fistula, but there was no MTX only arm and the data seem similar to the benefit achieved with IFX monotherapy^[30,31].

Approximately 10% of peri-anal and abdominal fistulas in Crohn's heal spontaneously^[31]. Given a closure rate well above the spontaneous closure rate, we consider MTX a potentially useful adjunct in management of Crohn's fistulas.

METHOTREXATE AND ULCERATIVE COLITIS

Does MTX work for induction of remission in UC?

Evidence pertaining to the utility of methotrexate in

Table 2 Evidence for induction of remission of ulcerative colitis with methotrexate

Study	Dose (mean)	Route	No. of patients	Study design	Follow-up (wk)	MTX response	MTX remission	Placebo response
Kozarek	25 mg	<i>im</i>	7	Open label	12	5/7 (71.40%)		N/A
Baron	15 mg	Oral	8	Open label	18	3/8 (37.5%)	0	N/A
Oren	12.5 mg	Oral	67	Placebo control	36	14/30 (46.7%)		18/37 (48.6%)
Egan	15 mg	<i>sc</i>	18	Open label	16	7/18 (39%)	3/18 (17%)	N/A
	25 mg	<i>sc</i>	12			4/12 (33%)	2/12 (17%)	N/A
Mate-Jimenez	15 mg	Oral	34	6-MP control	30		7/12 (58.30%)	11/14 (78.6%)
Paoluzi	12.5 mg	<i>im</i>	10 thiopurine resistant/intolerant	Open label	26	10/10 (100%)	6/10 (60%)	N/A
Cummings	19.9 mg mean	Oral	11 AZA failure 31 AZA intolerant	Retrospective	30	3/11 (27%) 18/31 (58%)	14/31	N/A
Nathan	20-25 mg	<i>sc</i> / oral	23	Retrospective	N/A		11/23 (48%)	N/A
Wahed	10-25 mg	Oral, <i>sc</i>	9 thiopurine ineffective 23 thiopurine intolerant	Retrospective	26	7/9 (78%) 15/23 (65%)	N/A	N/A
Manosa	25 mg	Oral <i>sc</i>	7 33	Retrospective	26		24/40 (60%) remission	N/A
Saibeni	20 mg	Oral/ <i>sc/im</i>	23	Retrospective	N/A	11/23 (47.8%)		N/A
Khan	14 mg	Oral	68	Retrospective	60	25/68 (37%)		N/A
	25 mg	<i>sc/im</i>	23			7/23 (30%)		

MTX: Methotrexate; CD: Crohn's disease; AE: Adverse events; SC: Subcutaneous; PO: Oral; AZA: Azathioprine.

induction of remission for ulcerative colitis is conflicting (Table 2). Disparate results reflect disagreement over appropriate dosing and route of administration. To date, only one prospective, randomized placebo-controlled trial examining the efficacy of methotrexate in the treatment of ulcerative colitis exists; Oren *et al*^[5] in 1996 compared 12.5 mg oral methotrexate to placebo in the induction of remission of 67 patients with moderate/severe UC^[5,14]. All patients had active disease with a Mayo score of >7, and were taking steroids for at least 4 mo in the preceding year. The results were disappointing, with clinical remission rates of 46.7% (14/30) in the methotrexate arm in comparison to 48.6% (18/37) for the placebo arm, a non-significant difference. Of those who entered clinical remission, 64.3% of patients in the methotrexate arm had a relapse requiring steroid induction compared to 44.4% of placebo patients, again, an insignificant difference.

Overall, a low remission rate relative to placebo, long time to remission, and a high relapse rate in Oren's study all suggest a lack of efficacy for methotrexate in either the induction or maintenance of remission in ulcerative colitis. Of course, important criticism may be directed at the relatively low dose of MTX used and the oral route of administration.

Otherwise, a number of small open-label and larger retrospective analyses have been conflicting, not least due to differing definitions of response, length of follow up (12 wk-2 years), dose of MTX (7.5-25 mg/wk), and route administered (*po vs im*). None of these studies were considered of sufficient quality to be included in the meta-analysis by Khan *et al*^[14].

The most comprehensive of these was published last year by Khan *et al*^[32], presenting retrospective data regard-

ing experience with methotrexate in the Veterans Affairs (VA) system. A total of 91 patients with ulcerative colitis who were steroid dependent or refractory were commenced on oral (mean 14 mg) or parenteral (mean 25 mg) methotrexate. In the oral MTX cohort, 37% (25/68) were able to successfully wean from steroid therapy, compared to 30% (7/23) of the parenteral cohort.

Overall, looking specifically at induction of remission in ulcerative colitis, response to methotrexate ranged from 27%-100%, and remission rates ranged from 0%-63%. Considering the retrospective nature of most studies, it is impossible to determine the true impact of dose or route of administration. In prospective, open label or randomized controlled trials, response rates similarly ranged from 33%-100%, with remission rates ranging 17%-60%. There are no clear signals regarding the impact of dose, route of administration, or indication for step-up in therapy on remission or response rates in UC.

Does MTX work for maintenance of remission in UC?

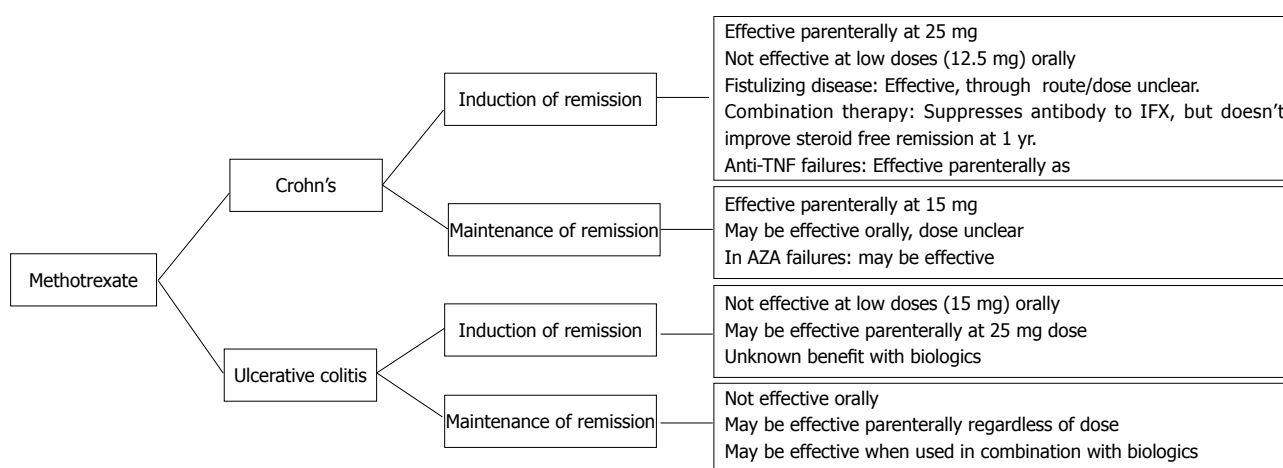
Regarding the maintenance of remission, the results are equally confusing - maintenance of remission rates range from 14%-75% (Table 3). Unfortunately, two open-labeled studies suggesting successful maintenance rates > 60%^[10,33] using parenteral methotrexate did not include a placebo arm as comparison^[10,33]. Oren *et al*^[5] and Mate-Jimenez *et al*^[12] included control arms, but provided disappointing results for the efficacy of oral methotrexate. Whether the route is a factor for better response rates remains to be seen.

There has been no data to date investigating the utility of combining methotrexate with biologic therapy in UC. Increasing interest in using methotrexate as a "synergistic enhancer" - to augment and prolong biologic efficacy -

Table 3 Evidence for maintenance of remission of ulcerative colitis with methotrexate

Study	Dose (mean)	Route	No. of pts	Study design	Follow-up period (mo)	MTX response maintained?	Control response	Significantly effective?
Kozarek	> 7.5 mg	sc	5	Open label	24	3/5 (60%)	N/A	N/A
Oren	12.5 mg	oral	32	Placebo-controlled	9	5/14 (36%)	10/18 (56%)	No
Mate-Jimenez	15 mg	oral	12	6-MP control	18	1/7 (14%)	7/11 (64%)	No
Paoluzi	12.5 mg	im	10	Open label	24	6/8 (75%)	N/A	N/A
Manosa	25 mg	Oral/sc	7/33	Retrospective	24	35%		N/A

MTX: Methotrexate.

**Figure 1** Algorithm for evidence-based use of methotrexate in inflammatory bowel disease. AZA: Azathioprine.

may help define its role in this disease.

PRACTICAL ADVICE ON HOW TO PRESCRIBE MTX IN THE US

Injectable MTX is available in 50 mg/2 mL vials. We prescribe one vial (2 loading dose equivalents) as well as a supply of “tuberculin” 1 mL syringes with 27 gauge, 1/2” needles. The patient draws 25 mg weekly from the vial and injects subcutaneously in either lower quadrant of the abdomen or inner thighs as their preference. After 12 wk, if they have a response, they can be transitioned to oral methotrexate maintenance. A patient friendly resource on injecting MTX is available *via* the Canadian rheumatology association (http://rheuminfo.com/wp-content/uploads/2011/04/METHOTREXATE_INJECTION_SHEET.pdf).

Oral methotrexate is available in 10 and 15 mg strengths as Trexall™. If using oral methotrexate in the induction of remission of IBD, we would recommend starting with 25 mg weekly, reverting to the subcutaneous route in non-responders and those who develop nausea attributed to the oral route.

All patients should be prescribed folic acid 1mg daily as it significantly reduces hepatic toxicity, an infrequent occurrence, and gastrointestinal toxicity associated with MTX^[34,35]. At present, our target population for MTX

are CD patients who are unable to tolerate azathioprine or 6Mercaptopurine due to adverse events, homozygous TMPT mutations, or inefficacy. In the event that methotrexate is required in a woman of child bearing age, we counsel regarding the need for effective contraception (*i.e.*, IUD) and recommend a discussion with their obstetric physician. We advocate obtaining routine blood labs (complete blood count, basic chemistry panel, hepatic function panel) 1 wk after initiation as well as every 8-12 wk subsequently.

CONCLUSION

Given the current evidence an algorithm for MTX can be elucidated (Figure 1). Providers should no longer shy away from using MTX due to concerns of hepatotoxicity and intolerance. Methotrexate demonstrates a similar rate of drug withdrawal as AZA, and may be considered favorable in young males in whom practitioners are reluctant to use AZA (due to concerns of hepato-splenic T-cell lymphoma risk). Determining the optimal dose and route of administration in the various indications for use in IBD is the current priority. MTX is largely used as a second line therapy after AZA failure. It may be useful in combination with Anti-TNF therapy to reduce the risk of immunogenicity and subsequent secondary loss of response to anti-TNF therapy. We eagerly await the results

of two studies that will shed further light; the METEOR trial and MERIT-UC, both randomized, controlled trials of parenteral MTX 25 mg weekly in the induction and maintenance of remission in steroid dependent or refractory ulcerative colitis.

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Visceral hypersensitivity and electromechanical dysfunction as therapeutic targets in pediatric functional dyspepsia

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Abstract

Functional gastrointestinal disorders (FGID) are common clinical syndromes diagnosed in the absence of biochemical, structural, or metabolic abnormalities. They account for significant morbidity and health care expenditures and are identifiable across variable age, geography, and culture. Etiology of abdominal pain associated FGIDs, including functional dyspepsia (FD), remains incompletely understood, but growing evidence implicates the importance of visceral hypersensitivity and electromechanical dysfunction. This manuscript explores data supporting the role of visceral hypersensitivity and electromechanical dysfunction in FD, with focus on pediatric data when available, and provides a summary of potential therapeutic targets.

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Key words: Motility; Visceral hypersensitivity; Functional dyspepsia

Core tip: Functional dyspepsia (FD) is a common disorder of upper gastrointestinal symptoms in adults and children. Etiology and mechanisms of FD are complex, and improved understanding could help direct therapy.

Visceral sensitivity and intestinal electromechanical function both are demonstrated to be altered in some FD patients and are potential targets for treatment. Limited studies in pediatric FD are available, but available evidence supports adult data that targeting visceral hypersensitivity and electromechanical dysfunction is warranted, particularly in the context of the biopsychosocial model. Future studies in pediatrics are needed to determine optimal therapy and appropriate patient application.

Rosen JM, Cocjin JT, Schurman JV, Colombo JM, Friesen CA. Visceral hypersensitivity and electromechanical dysfunction as therapeutic targets in pediatric functional dyspepsia. *World J Gastrointest Pharmacol Ther* 2014; 5(3): 122-138 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v5/i3.122.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v5.i3.122>

INTRODUCTION

Functional gastrointestinal disorders (FGIDs) account for more than 80% of chronic abdominal pain complaints in children. Although additional studies are needed, pediatric FGID prevalence and impact are described broadly in North America^[1,2] and Europe^[3,4], and with increasing recognition in other parts of the world^[5-7]. The impact of pediatric FGIDs on patients and health-care systems cannot be overstated. In one epidemiologic study, 38% of school-aged children in the United States reported abdominal pain weekly and 24% reported abdominal pain persisting for more than 8 wk^[8]. Further, FGIDs frequently are associated with somatic symptoms^[9], decreased quality of life^[10,11], psychological comorbidities^[12], and school absenteeism^[8]. Consequently, the burden on public health care^[13] and associated financial costs are enormous^[14,15].

In the late 1950s, Apley and Naish described an entity of recurrent abdominal pain (RAP)^[16]. RAP was defined

by 3 or more bouts of pain severe enough to interfere with activities and occurring over at least a 3 mo period. Children with a wide variety of clinical presentations and etiologies were included under the single entity of RAP. This entity was rendered inadequate for clinical practice due to broad inclusivity. Over the past decade there was an effort to reclassify RAP into discrete groups that are known as FGIDs. FGIDs are defined by symptom-based clinical criteria set forth by an expert panel generally referred to as the Rome Committee. The committee met for the third time in 2006 (Rome III) to update the criteria^[17]. Rome III defines abdominal pain associated FGIDs in children as pain occurring at least weekly for longer than 2 mo and without identifiable biochemical, structural, or metabolic abnormalities to explain symptoms. However, abdominal pain associated FGIDs are diagnosed even in the absence of laboratory, radiologic, and endoscopic testing, or in the presence of mild chronic inflammation of the intestinal mucosa^[18,19]. Functional dyspepsia (FD) and irritable bowel syndrome (IBS) are among the most common pediatric FGIDs^[20]. FD is diagnosed in children by: (1) upper abdominal pain or discomfort several times a week or more often; (2) upper abdominal pain or discomfort longer than 2 mo duration; (3) pain “sometimes” or less relieved by defecation; and (4) pain “once in a while” or less associated with a change in stool form or frequency. FD is differentiated from IBS in that IBS pain can be upper or lower abdomen, is more often relieved with defecation, and is often associated with change in stool form or frequency. Although distinctions are made within the criteria, it is debatable whether the two disorders are truly distinct in etiology or mechanism and ultimately may be symptom-defined diagnoses sharing a common underlying pathophysiology^[21,22].

In adults, FD is further delineated by two subtypes: postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS). PDS is defined by the presence of upper abdominal fullness or early satiety after normal size meals, whereas EPS is defined by predominance of epigastric pain or burning. PDS and EPS are not included within the pediatric FD symptom definition due to lack of supportive evidence in children. However, subsequent to Rome III, evidence emerged that adult subtypes also may be relevant in the pediatric population. For example, children with PDS-type symptoms have been found to have increased anxiety^[23,24], a phenotype demonstrated in adults with PDS^[25].

FD, as true of other FGIDs, is considered to be etiologically multi-factorial. The biopsychosocial model proposes contributions from and interactions between biologic, psychologic, and social systems. Factors within any of these systems may initiate, exacerbate or alter the course of the pain syndrome. In addition, adverse events early or later in life may lead to brain-gut axis changes, including long-term alterations in visceral electromechanical function, sensitivity, immunity, and brain-gut stress response. Examples of early adverse events span the biopsychosocial spectrum to include infection^[26], inflam-

mation, surgery^[27,28], abuse^[29], and wartime exposure^[30].

We previously reviewed the role of inflammation (specifically eosinophils and mast cells) in pediatric FD^[31]. In this companion review, we explore the role of visceral hypersensitivity and gastrointestinal electromechanical dysfunction in generation and maintenance of FD symptoms or subtypes, as well as their potential as therapeutic targets. Although they will generally be treated as separate entities in this discussion, visceral sensation, motor function and inflammation interrelate and should be considered as such when pursuing patient diagnosis and treatment.

VISCERAL HYPERSENSITIVITY

Visceral sensory output from organs (*e.g.*, intestine, bladder) to the central nervous system occurs continuously. Signals result from stimuli including hollow organ distension, inflammation, traction on the mesentery, and ischemia. Normal physiologic function of the visceral organs, including gastrointestinal distension and contraction, is typically nonpainful. However, the subjective interpretation may change due to increased frequency or amplitude of the visceral stimulus, or increased sensitivity to a typically painful (hyperalgesia) or nonpainful (allodynia) stimulus. Visceral hypersensitivity may result from alterations in the peripheral or central nervous system and has complex but increasingly understood etiology^[32]. Human and animal studies have identified numerous contributing factors to this alteration, with visceral hypersensitivity now considered one of the central mechanisms of FGIDs.

Visceral hypersensitivity in FD may result in early satiety, abdominal pain, and nausea. Results from pediatric and adult investigations strongly suggest that sensory thresholds in FD patients are different than in subjects with other intestinal disorders and healthy controls. Visceral hypersensitivity was studied in 11 FD, 8 IBS and 11 FD-IBS overlap adults utilizing gastric and rectal barostats^[33]. FD patients had predominant gastric (91% of subjects) over rectal (18%) hypersensitivity, IBS patients had only rectal (75%) hypersensitivity, and overlap patients had hypersensitivity to both (82% gastric, 91% rectal). Findings from this study suggest that hypersensitivity in FD may be localized to the stomach. However, other studies have failed to demonstrate these location-specific findings^[34,35]. Differences in findings across studies may be related, at least in part, to heterogeneity in patient selection and/or in hypersensitivity definition.

Assessment of visceral hypersensitivity

Visceral sensitivity of the intestine is measured using a variety of methods in clinical studies. Patients undergo specific interventions, then either subjective pain reports or objective clinical data (*e.g.*, biometrics, functional brain imaging) are collected and analyzed. Tests utilized include water load, balloon distension, and inflammatory/nociceptive challenge. In many studies, tests of visceral sensitivity are conducted in a multimodal design, both to

determine correlation and to validate outcomes. Water load testing requires subjects to drink a maximal amount of water in a brief discrete time period (typically 5 min). Outcomes include subjective symptoms and quantity of water ingested. Balloon distension of hollow organs, including gastric barostat, measures distension thresholds and corresponding signs and symptoms. Of note, balloon distension also is used in animal models of visceral pain, with electromyographic recording included as an additional objective outcome. Inflammatory/nociceptive challenges directly stimulate intestinal mucosal sensory nerves by application of a chemical (*e.g.*, acid or lipid) and measuring subjective pain thresholds. Both water load and balloon distension tests are affected by gastric accommodation and emptying, further demonstrating that separating sensation from function is a practical but artificial distinction.

Water load test: The water load test is advocated as a means of identifying patients with visceral hypersensitivity. Although the water load test may not be useful for identification of pediatric FD due to suboptimal sensitivity, children diagnosed with FD often have abnormal test results^[36]. In a controlled study by Schurman *et al.*^[36], 68 pediatric patients with FGIDs and 26 healthy children completed the Behavioral Assessment Scale for Children-Self-Report Form (BASC-SR) and underwent a rapid water load test (maximal tolerable volume within 3 min). Children with FD, with or without corresponding IBS, had lower water consumption than healthy controls. This was not true of children with IBS only. Using the 10th percentile for water volume consumption in the control group as a lower limit of normal, the water load test had 28% sensitivity and 100% specificity in identifying patients with the diagnosis of FD as determined by the clinician. Consistent with the biopsychosocial model, self-reported anxiety was negatively correlated with volume of water intake; however, it accounted for only 6% of the variance.

A variation on the water load test measuring satiety was evaluated in 28 pediatric patients diagnosed with FD using Rome III criteria^[37]. Participants drank a liquid meal at a constant rate and repeatedly scored satiety until reaching maximal possible score or 5 min time. Total intake volume was decreased in dyspeptic patients compared to healthy controls. Another study of 15 adolescents with FD who consumed a liquid meal at a constant rate to maximal tolerable volume found no statistical difference in total ingested volume or time to satiation compared to controls^[38]. However, total volume was over 10% less and time to satiation over 20% sooner in FD subjects. Additionally, postprandial nausea and bloating were greater in dyspeptics, with 7/15 subjects reporting postprandial pain scores > 99th percentile of scores for healthy adolescents. Of note, in a study of 101 children with functional abdominal pain that utilized multiple validated questionnaires in addition to a water load test, children believing they could modify their own pain (high

problem-focused pain efficacy) had decreased visceral sensitivity compared to those who perceived little control over pain^[39]. Although the direct application to children with FD is unclear given different inclusion criteria, findings support consideration of visceral sensitivity to gastric distension as a possible pathophysiologic mechanism and, further, the potential beneficial role of CNS-mediated inhibition.

Measures of visceral sensitivity are studied more extensively in adult patients with FGIDs including FD. While water load testing in adults with FD has yielded similar results^[40-44] to those reported above for pediatric studies, studies in adults contain expanded data investigating other upper GI conditions, demographic and psychosocial factors, and liquid composition. In one study of adults, patients with FD ($n = 59$), GERD ($n = 101$), and ulcer ($n = 55$) all demonstrated decreased maximal ingested volume of water over 5 min compared to 30 healthy controls^[45]. Although this again supports visceral sensitivity mechanisms, it also raises concern regarding the specificity of the water load test as an assessment for FD. Strid *et al.*^[43] evaluated 35 FD adults and 56 controls. Depressed mood and poor overall health correlated with lower tolerated volumes in FD patients only, again reinforcing the brain-gut connection/biopsychosocial model and the useful but artificial construct of measuring visceral sensitivity in isolation. In contrast, Jones *et al.*^[44] found no correlation between psychological measures and specific water load test outcomes. Composition of the liquid also appears to affect the postprandial symptom profile in FD. Lee *et al.*^[46] compared 30 adults with FD to 12 healthy controls and found that symptoms of bloating and abdominal pain within 30 min following ingestion were greater in FD patients after a nutrient drink as compared to water, while there was no symptom difference between the two liquids in healthy controls^[46]. Interpretation of liquid loading needs to take into consideration the psychologic state of the subject and the nutrient content of the ingested liquid.

Gastric barostat: Barostat testing is the traditional “gold standard” for evaluating mechanical hypersensitivity in adults. In FD, the evaluation utilizes balloon distension of the fundus and subjective scoring of discomfort. Hoffman *et al.*^[47] found that FD children had abdominal discomfort at lower gastric distension pressures compared to healthy young adults. This is consistent with a separate study utilizing barostat testing in which visceral hypersensitivity was identified at a higher frequency in children with RAP as compared to healthy controls^[48]. The RAP group likely included children with FD as well as other abdominal pain disorders.

Gastric barostat studies in adult FD generally replicate, and also extend, pediatric findings. Evaluation of 8 dyspeptic adults found lower sensation threshold to gastric distension compared to controls, although maximal tolerated distension pressure and volume were similar^[49]. These 8 patients had not previously consulted health care

professionals regarding symptoms, suggesting that visceral hypersensitivity to balloon distension is independent of referral bias and certain psychosocial characteristics (such as high anxiety regarding symptoms). FD patient heterogeneity was demonstrated in two other studies, however, suggesting that sensitivity to balloon distension is not universal. Specifically, relative pressure (intraballoon pressure/intraabdominal pressure) to produce discomfort was abnormal in only 37% of 160 consecutive patients with FD when compared to 80 healthy controls and gastric hypersensitivity was found in only 44% of “pain-predominant” and 25% of “discomfort-predominant” FD adults^[50]. Hypersensitivity to balloon distention is enhanced in the postprandial state in FD patients (but not controls) and correlates with preprandial sensitivity, impaired accommodation, and the severity of meal-related symptoms^[51]. Taken together, studies suggest that mechanical hypersensitivity may be associated with an increased prevalence of postprandial pain.

Duodenal infusion: Although chemosensitivity has not been evaluated in children with FD, adults with FD have demonstrated increased symptoms to both duodenal^[52] and gastric^[53] acid infusion. Duodenal acid infusion has most often been associated with nausea but also bloating and pain^[52,54-56]. Duodenal acid infusion decreases antral motility and alters response to balloon distention^[46,55]. In a study of adults with FD, Feinle *et al.*^[57] showed that duodenal lipid exposure affects gastric sensitivity to balloon distension supporting the effect of lipids and cholecystikinin on visceral sensitivity. Lipid infusion, but not glucose infusion, enhances perception to gastric distention and lipid infusion is associated with nausea^[58]. In addition to mechanical sensitivity, chemosensitivity represents another potential therapeutic target.

Mechanisms of hypersensitivity

Visceral hypersensitivity is a complex process which may occur both within the CNS and at the level of the peripheral nervous system. Mechanisms of increased visceral sensitivity to balloon distension have been studied extensively in animal models^[59,60] and in several cohorts of adults with FD, but have not been reproduced in dyspeptic children. Neuroimaging studies conducted in adults with FD support the presence of abnormal CNS processing of pain signals as compared to controls and in FD patients with hypersensitivity as compared to FD patients with normal sensation^[61,62]. Vandenberghe *et al.*^[63] postulated that intense stimulation of low threshold multimodal afferent pathways, as opposed to sensitization of nociceptive pathways, occurs in hypersensitive FD adults. Their conclusion is based on studying 48 FD adults (hypersensitive, $n = 20$) in whom non-pain symptoms were induced at similar distending pressures that resulted in pain. At a peripheral level, hypersensitivity may be induced by a number of factors, including alterations in mediator release (*e.g.*, serotonin) or receptors (*e.g.*, 5-HT or TRPV1), inflammation, or the stress response.

Serotonin (5-HT) is abundant throughout the intestine and is an important neurotransmitter within the brain and the GI tract where it plays a key role in the regulation of motility and sensation. The effects of serotonin are modified by 5-HT receptors and its reuptake controlled by SERT. In adults with FD, plasma levels of 5-HT are decreased in the basal and postprandial states^[64]. This has not been studied directly in children with FD; however, gastric 5-HT content and SERT mRNA do not differ between children with FD and controls^[65]. Due to its important role in sensation, serotonin (broadly or specific serotonin receptors) represents a potentially important treatment target.

Transient receptor potential (TRP) channels survey the gastrointestinal contents for chemicals ingested, produced within the gastrointestinal tract (including those produced by the microbiome), and/or generated by inflammatory responses^[66]. TRP vanilloid type 1 (TRPV1) is a polymodal nociceptor on GI afferent neurons and is the specific sensor for capsaicin. Based on oral capsaicin capsule titration, the majority of adults with FD demonstrate visceral chemosensitivity involving TRPV1 pathways^[67-69]. Repeated ingestion of capsaicin in healthy volunteers initially increases symptoms, but after 4 wk decreases symptoms through desensitization of both chemo- and mechanoreceptors^[70]. The effects on sensitivity appear to be dependent on length of exposure. In healthy volunteers with 7 d exposure, chemoreceptors remain sensitized while threshold of mechanoreceptors to distention decreases^[71]. TRPV1 potentially plays a key role in chemosensation and possibly mechanosensitivity; as such, TRPV1 may represent another therapeutic target.

Inflammation and stress have been implicated in the pathophysiology of visceral hypersensitivity in FD. Consistent with the biopsychosocial model, electromechanical dysfunction may also be influenced by anxiety and the stress response. Anxiety is the most highly implicated psychological contributor to the development and maintenance of FGIDs including FD. Approximately 50% of children and adolescents with FD demonstrate elevated anxiety scores^[72]. Anxiety can trigger the stress response which is mediated primarily through the release of corticotrophin releasing hormone (CRH) from the hypothalamus. The stress response results in physiologic effects relevant to FGIDs including inflammation (particularly mast cell activation), sympathetic nervous system activation, altered gastric accommodation, gastric dysmotility, and visceral hypersensitivity. CRH also alters central processing of nociceptive messages. The effects of CRH on hypersensitivity and electromechanical dysfunction may be direct and mediated *via* CRH1 and CRH2 receptors. Downstream effects of CRH-induced mast cell activation and mediator release can stimulate afferent nerves signaling pain, sensitize afferent nerves resulting in visceral hypersensitivity, and alter electromechanical function. In adults with FD, hypersensitivity is associated with mast cell degranulation after balloon distention of the proximal stomach^[73].

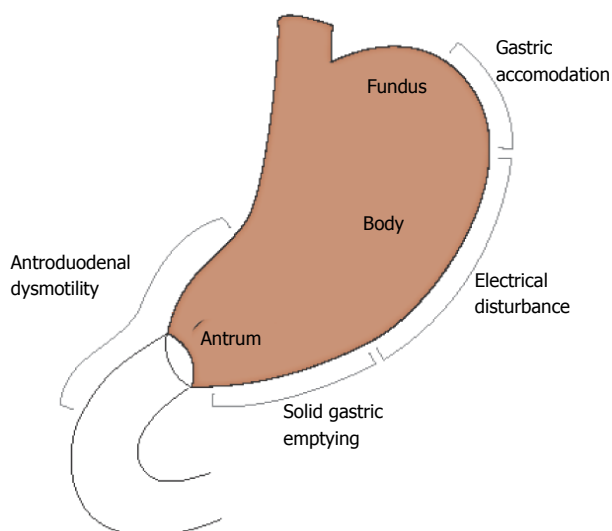


Figure 1 Overview of electromechanical disturbances in functional dyspepsia.

ELECTROMECHANICAL DYSFUNCTION

Visceral hypersensitivity undoubtedly has a role in dyspeptic symptoms, but it is identified in only a fraction of patients diagnosed clinically with FD. In contrast, disordered accommodation, delayed gastric emptying, gastric electrical rhythm disturbances, and altered antroduodenal motility are all physiologically relevant and common in FD (Figure 1). As reviewed by Azpiroz *et al*^[74], gastric motor function is interdependent on visceral sensation and is a complex function affected by both tonic and induced stimuli. Understanding physiologic abnormalities in specific disorders such as FD can guide effective therapy.

Assessment of electromechanical dysfunction

Motor function of the stomach and duodenum is a coordinated activity meant to prepare food for digestion and initiate passage through the small intestine. The stomach serves as a reservoir for ingested food and functions to grind food and then provide passage to the intestine at a rate appropriate for effective nutrient absorption. In the interdigestive period, gastroduodenal motility is modulated by the migrating motor complex (MMC) which is a multiphase action propagated from the gastric antrum into the small intestine controlled by the enteric nervous system, central nervous system, and intestinal regulatory hormones. Gastroduodenal motility depends on prandial state, food composition, presence and type of inflammation, distal intestinal motor function, and both motor and autonomic neural input. Symptoms related to altered gastroduodenal motor function may include abdominal pain, nausea, vomiting, and early satiety and can occur due to rapid^[75] or delayed gastric emptying, or altered proximal stomach accommodation with normal gastric emptying. Gastroduodenal mechanical function can be measured with a variety of tools including scintigraphic or breath gastric emptying study (GES), gastric barostat, antroduodenal manometry (ADM), and electrogastrography

(EGG) as well as newer studies including single-photon emission computed tomography (SPECT), and the wireless motility capsule (WMC). Each test measures related but different aspects of physiology including compliance, accommodation, contractility, coordination, and propagation as highlighted below.

Gastric emptying

Pediatric studies have identified abnormal gastric emptying in FD. In a study of 15 FD adolescents using the ¹³C-*s. platensis* breath test, gastric emptying of solids was significantly delayed^[38]. Solid-phase delays were similarly identified in 26% of dyspeptic children when evaluated with the ¹³C-octanoic breath test^[37]. Emptying function has also been evaluated in pediatric dyspeptics with scintigraphy using 99mTc-sulfur colloid and a standard meal^[76]. Although a majority of the 57 patients had normal gastric emptying at 2- and 4-h post meal, abnormalities of rapid (20%) and slow (20%) gastric emptying were observed. Symptoms did not correlate with emptying rates in these children. Another study utilizing scintigraphy demonstrated delayed solid emptying in 47% of patients, but again there was no relationship between emptying and symptom severity^[77]. In contrast, Devanarayana *et al*^[78] recently used antral ultrasound to correlate gastric emptying after a liquid meal with symptoms in pediatric dyspeptics. Forty-one FD patients had delay in both gastric emptying rate (% change in antral cross sectional area from 1 to 15 min post ingestion) and antral motility index (product of contractile amplitude and frequency) compared to healthy controls. Severity of symptoms correlated negatively with gastric emptying rate ($r = -0.35$), but not with other measures of motility. Gastric emptying appears to have no relationship to satiety in children^[37]. Delays in gastric emptying may also be affected by concurrence of constipation in pediatric dyspepsia^[79]. FD patients with constipation had longer gastric emptying times than FD patients without constipation, and treatment with lactulose over 3 mo resolved the difference.

Abnormal gastric emptying by scintigraphic evaluation has been demonstrated in a significant proportion of adults with FD^[80-82] although findings may be affected by the modality of measurement as well as meal volume and contents^[83]. In adults, there have been no reproducible relationships between impaired emptying and specific symptoms. Some studies have revealed no or only weak associations with symptoms^[84-86]. Other studies have reported variable and highly inconsistent associations with nausea, vomiting, postprandial fullness, and bloating with both positive and negative relationships with regard to pain^[87-91]. Postprandial fullness and nausea, and severe early satiety have been reported with delayed liquid emptying^[88,90].

Gastric accommodation

Gastric accommodation, the ability of the proximal stomach to relax and serve as a reservoir for food, is implicated as a motor abnormality responsible for symptoms in some dyspeptic patients^[92]. Impaired accommodation

has been associated with early satiety in some but not all studies^[84,91,93]. Assessment of accommodation can be conducted with gastric barostat, ultrasound, MRI, and SPECT. Gastric emptying and water-load capacity are certainly affected by accommodation, but neither is a specific measure of fundic relaxation. Impaired accommodation was demonstrated in pediatric RAP patients assessed by 2-dimensional ultrasound^[94]. Participants, most of whom had dyspeptic symptoms, had decreased proximal stomach sagittal area and increased rate of proximal stomach emptying after a liquid meal when compared to healthy controls. A similar assessment of RAP patients utilized 3-dimensional ultrasound to assess antral relaxation and gastric distribution of ingested liquids^[95]. Participants demonstrated decreased postprandial proximal filling (accommodation) and altered liquid distribution favoring the distal stomach despite no difference in gastric emptying rate. Ultrasound evaluation of children with FD also showed increased antral distension after a mixed solid-liquid meal, but without specific evaluation of the proximal stomach^[96]. Adolescents with FD also have a lower postprandial gastric volume change than healthy adults when assessed by SPECT^[38]. No MRI studies of gastric function in pediatric FD patients have been published.

In adults with FD, decreased gastric accommodation and abnormal gastric volumes are widely demonstrated using barostat^[90,97,98], ultrasound^[99,100], SPECT^[80,101,102], and MRI^[83]. Accommodation defects have been reported in 40% of adults with FD as assessed by barostat and in 47% as assessed by SPECT^[93,101]. It is less clear whether symptoms are associated with abnormal accommodation or gastric volumes^[91] and whether newer imaging modalities such as MRI will consistently support these findings^[103].

Electrogastrography

Electrogastrography (EGG) is a noninvasive method to evaluate gastric myoelectrical activity. It can assess rhythmic gastric slow waves associated with frequency and propagation of contractions, as well as superimposed activity (spike/second) indicative of antral contractility. Cutaneous abdominal electrodes are utilized to obtain raw data, then computer analysis is performed to determine targeted values for comparison. Normative data are considered similar in children, adolescents, and adults^[104], but not in neonates or toddlers^[105].

Children with FD have abnormal EGG compared to healthy children, indicating underlying myoelectrical dysfunction. Chen *et al.*^[106] assessed 15 pediatric patients with FD compared to 17 healthy controls using surface electrodes. Children with FD had a lower percentage of slow waves and more time with no rhythmic activity in fasting and fed states. In the postprandial state, frequency of gastric slow waves also increased less in subjects than controls although measures of contractility (power) were similar. In an independent study of 30 children with FD, EGGs were abnormal in 50% and correlated with symptom severity^[77].

Electrogastrogram abnormalities in adults with FD are similar to those described in children^[42,107]. Patients with abnormal EGG also had higher postprandial pain scores, and patients with a history of vomiting had more frequent fasting bradygastria and fewer normal slow waves. This symptom correlation suggests clinical relevance of EGG abnormalities and is consistent with other data correlating EGG and symptoms in pediatric FD^[106,108]. However, the role of EGG abnormalities as a therapeutic target remains to be established.

Antroduodenal manometry

Antroduodenal manometry also demonstrates abnormal motility in children with FD^[109]. A study of 34 children and 35 adults with FD found a majority with abnormal motility with a neuropathic pattern observed most commonly^[110]. Several studies of antroduodenal motility also demonstrate abnormalities in adults with FD^[111,112], but symptoms, intestinal dysmotility, and gastric emptying delays are not clearly correlated^[112]. The relationship between motility studies is made even less clear in that a study of 31 adults with FD showed abnormal EGG was not associated with concurrent abnormalities in antroduodenal manometry^[113], and available pediatric data supports this concept^[114]. The clinical significance of altered antroduodenal motility, particularly as a therapeutic target, is not established.

Wireless motility capsule

The WMC shows promise as a relatively noninvasive, clinically relevant measure of gastrointestinal motility^[115]. It is used to study prokinetic medication efficacy^[116] and to describe an adult irritable bowel syndrome cohort^[117]. Data is not yet available in adult or pediatric dyspeptics, but an initial study suggests the WMC is a sensitive detector of motor abnormalities in pediatric patients with upper gastrointestinal symptoms^[118].

Mechanisms of electromechanical dysfunction

The specific cause of electromechanical dysfunction in FD is unclear, but may be related to immune activation^[119]. Inflammation is implicated as a contributor in dyspepsia-associated dysmotility^[31,120]. However, this effect appears to require specific inflammatory pathways. For example, EGG abnormalities in children and adolescents with FD are independent of chronic gastritis, but associated with antral mast cell and eosinophil density^[121,122]. Likewise, in children with FD, increased antral mast cell density is associated with slower gastric emptying^[121].

As alluded to previously, the stress response also has effects on electromechanical function. Experimentally induced stress has been shown to increase symptoms and inhibit normal postprandial EGG responses in some, but not all studies^[123,124]. Stress is shown to impair accommodation and to decrease gastric emptying^[125,126]. The effect on gastric emptying appears to be mediated primarily *via* CRH receptors.

THERAPIES FOR PEDIATRIC FD

Proper identification of functional dyspepsia using symptom based criteria (Rome III) is the first step in treatment. Diagnostic and screening tests to evaluate for diseases with similar symptoms are sometimes important, but not necessary for FD diagnosis. Providing a named diagnosis (*i.e.*, FD) and the expectation of treatment success potentially increases the treatment response rate. Importantly, the placebo effect may be particularly strong in children with FGIDs and should be considered when interpreting efficacy of studied interventions^[127].

Reassurance and education regarding FGIDs is imperative. Validating that subjective symptoms are real and putting them in the context of the biopsychosocial model aids in directing effective treatment and provides hope for patients and families. Visceral hypersensitivity and electromechanical dysfunction represent potential targets, but patients may be more effectively managed if underlying factors (such as inflammation, anxiety, *etc.*) are considered in the treatment plan. Treating FD, like other FGIDs, in the conceptual framework of the biopsychosocial model necessitates inclusion of both medical and psychological interventions. Effective medical therapy targeted to the specific pathophysiologic mechanism is preferred, but symptom-based therapy may also be useful. Although we will discuss medications in the context of their most likely target, it should be noted that visceral sensation, motor function, and inflammation do not exist in a vacuum; many medications exert an effect on more than one domain of sensation and mechanical function.

Targeting visceral hypersensitivity

Treatment of visceral sensitivity related to distension in FD has focused largely on antidepressant therapy, including tricyclic antidepressants (TCAs), selective serotonin uptake inhibitors (SSRIs), and related medications. Antidepressants may have primary effects on comorbid anxiety/depression that secondarily alter symptom perception, coping skills, arousal thresholds, and/or sleep quality. Alternately, they may affect functional gastrointestinal pain through central nervous system analgesia or a direct effect on gastrointestinal tract sensitivity. Serotonergic neurons have a role in gastrointestinal pain as discussed above, but antinociceptive effects of these medications cannot always be dissociated from their influence on motility and, in some cases, may be integral to effective treatment^[128]. For example, TCAs slow gastric emptying and small bowel transit in healthy patients^[129,130], but do not affect SPECT-determined gastric accommodation or outcomes of the nutrient drink test, except for post-satiation nausea^[129]. Similarly, SSRIs shorten small bowel transit time in healthy patients, but do not clearly decrease gastric sensitivity or compliance^[131,132]. Treatment with TCAs, SSRIs, and related medications must be carefully weighed against potential adverse effects, including cardiac dysrhythmias, suicidality, and anticholinergic effects, and monitored to minimize these relatively rare,

but potentially life-threatening issues.

Several studies have investigated whether TCAs, SSRIs, and related medications alter visceral sensitivity and overall symptoms in adult FD. Data in healthy adult volunteers demonstrate no change in tolerated gastric volume in the nutrient drink test after a short treatment course with desipramine (TCA) or escitalopram (SSRI)^[133]. Although total symptom scores induced by the nutrient drink test were influenced, treatment effects were nullified in multivariate analysis considering age, gender, BMI, and baseline scores. Fluoxetine (SSRI) improved symptom scores in depressed adults with FD^[134], but non-depressed subjects had no change in symptom scores and EGG measures were similar across all groups. Sertraline (SSRI) similarly failed to alter global symptoms or quality of life in adults with FD^[135]. Finally, a randomized clinical trial (RCT) of venlafaxine, a medication with combined SSRI and selective norepinephrine reuptake inhibition (SNRI), demonstrated significant patient drop-out due to medication adverse effects and no differences in symptom scores, health-related quality of life, anxiety, or depression^[136]. Taken together, current evidence does not support a strong direct effect of SSRIs or TCAs on visceral sensitivity in adults. The potential role of these medications in treatment of visceral hypersensitivity, as well as gastroduodenal motility, may be further clarified by an international multicenter placebo-controlled RCT currently underway to compare escitalopram to amitriptyline in adults with FD. This trial has completed enrollment and data collection for the primary outcome of global symptom score, and also is assessing solid gastric emptying, liquid nutrient drink test, and SPECT (<http://clinicaltrials.gov>, NCT00248651).

Limited data exists regarding treatment of pediatric FD with TCAs and SSRIs, and studies typically include a mixed cohort of FGIDs. A double-blind placebo-controlled RCT of amitriptyline (TCA) in 33 pediatric patients with IBS treated for 8 wk demonstrated improvement in QOL and some IBS-associated symptoms^[137]. Symptom improvement was limited to very specific symptoms (*i.e.*, right lower quadrant pain) and the reason for such specificity is not clear. Amitriptyline also was studied in 90 pediatric FGID patients in a multicenter double-blinded placebo-controlled RCT^[138]. Few patients were diagnosed with FD (8% placebo, 13% amitriptyline), but primary outcome of symptom relief was not different when analyzed by diagnosis. No difference in symptom relief, depression, or functional disability was noted, although anxiety was decreased in subjects receiving treatment. Notably, at least “fair” improvement in pain relief was seen in greater than 2/3 of subjects receiving placebo. A retrospective study of 98 pediatric FGID patients ($n = 16$ with FD) treated with TCAs found greater than 75% symptom response rate in all FGID subtypes, but limitations include lack of validated outcome measures, blinding, and control subjects^[139]. A 12-wk open label study of citalopram (SSRI) in 25 pediatric patients with RAP identified improvement in global symptoms, so-

matic symptoms, anxiety, and functional impairment^[140]. There are no published placebo-controlled RCTs of SSRIs for treatment of pediatric FGIDs in general or FD in particular. Given the questionable efficacy in adults with FD, SSRIs should not be viewed as first-line therapy, if at all, in pediatric FD.

In addition to mechanosensitivity, visceral chemosensitivity may represent a valid therapeutic target. Lipid sensitivity may be addressed through diet modification, but there have not been any studies demonstrating long-term benefit from low fat diets. Acid sensitivity may be addressed more directly through acid reducing medications. Acid-suppressive therapy with histamine-2 receptor antagonists (H2RA) and proton-pump inhibitors (PPI) improve pain in adults with FD^[141-143]. PPI therapy may be more effective than H2RA^[144], but studies typically have a mixed cohort without control for presence of *H. pylori* infection, gastroesophageal reflux disease (GERD), or both. A randomized, controlled trial in adults found that PPI therapy improved symptoms only in FD patients with concurrent heartburn^[145]. Whether the therapeutic benefit is related to acid hypersensitivity is not clear as these medications may be treating a component of acid mucosal injury or co-morbid GERD, or may also improve dyspeptic symptoms related to delayed gastric emptying^[146]. Still, acid reduction therapy remains the most common treatment prescribed empirically by pediatric gastroenterologists for FD in children^[147]. In children with abdominal pain, famotidine has demonstrated superiority to placebo in global improvement, and additional benefit is noted in children with FD^[148]. In a large pediatric cohort, omeprazole had no benefit over ranitidine or famotidine in the relief of pain, nausea, or vomiting^[149]. Although acid suppression appears promising, the specific mechanism of action in FD remains unclear.

Treatment of visceral chemosensitivity in FD also has targeted specific nociceptors including TRPV1. As described earlier, healthy adults ingesting capsaicin achieve desensitization following initial increase in symptoms, and FD adults may have increased chemosensitivity to TRPV1 agonists. A double-blind, placebo-controlled trial of red pepper powder in 30 FD adults demonstrated efficacy in decreasing overall symptoms, epigastric pain, and epigastric fullness within 3 wk^[150]. Although some initial discomfort occurred in treatment group patients, only two discontinued the study due to severe pain or burning. Capsaicin or other TRPV1 agents have promise in FD patients with demonstrated chemosensitivity.

Targeting electromechanical dysfunction

Therapies for electromechanical dysfunction in FD can be broken down into those targeting gastric motility/emptying and those targeting gastric accommodation. Therapies to increase gastrointestinal motility and emptying have met with mixed results for FD. A meta-analysis of 1844 adult patients with FD and 1599 controls found that prokinetics were effective in decreasing symptoms^[151]. The authors importantly note that most studies

of prokinetics assess short-term efficacy only. Interestingly, a separate analysis of studies including measures of symptom improvement and gastric emptying found no correlation between the two, suggesting that alternate effects of prokinetics are responsible for symptom improvement^[152].

Prokinetics evaluated in adults include agents primarily targeting 5-HT (5-HT₃ antagonists and 5-HT₄ agonists), dopamine, and motilin receptors. Cisapride, a 5-HT₄ receptor agonist, demonstrated symptom reduction in adults with FD in one meta-analysis, but potential bias and inclusion of specific FD-subtypes may affect applicability of findings^[153]. In pediatric patients with dyspepsia it may normalize gastric myoelectric activity^[154], but data on clinical effects is not available. Cisapride and newer 5-HT₄ receptor agonists regulate intestinal motility through effects on enteric cholinergic neurons, enhancing gastric emptying and accommodation, as well as potentially modulating visceral sensitivity^[155,156]. Although cisapride was withdrawn from the United States and European markets due to concern for potentially fatal cardiac arrhythmias, it is not clear that these effects are common in otherwise healthy children^[157] and the medication can still be used in limited capacity with close supervision. Another serotonergic/anti-dopaminergic compound, levosulpiride, has demonstrated noninferiority to cisapride^[158] with safety and efficacy confirmed in an open-label trial of 279 adults with FD^[159]. A selective 5-HT₄ agonist and 5-HT₃ antagonist (mosapride) has shown mixed results in FD symptom improvement^[160-162]. Cinitapride, a relatively new 5-HT₄ receptor agonist/dopamine-2 receptor antagonist, was demonstrated to relieve symptoms and reduce symptom severity as well as domperidone in a double-blind phase III RCT^[163]. There is a lack of pediatric data regarding agents targeting 5-HT receptors.

Metoclopramide is a dopamine antagonist with a long history of use in FD as an effective promotility agent that reduces dyspeptic symptoms^[164,165], but adverse effects may include irreversible extrapyramidal symptoms. There is evidence that metoclopramide liquid formulation may actually be more effective than the tablet^[166]. Domperidone, a dopamine-2 receptor antagonist that does not cross the blood-brain barrier, is shown to improve symptoms in adults with FD^[167] though it may be less effective when compared to cisapride^[168,169]. Domperidone is currently available for pediatric patients only as an investigational new drug for compassionate use. Itopride, which is anti-dopaminergic and inhibits acetylcholinesterase, did not have promising results in a phase III trial in adults^[170], but a meta-analysis that included a heterogeneous patient population with potential comorbid disease (*i.e.*, *H. pylori*) suggests that it may be effective in symptom reduction^[171]. A lack of proven efficacy and significant potential side effects should limit the long-term use of metoclopramide in pediatric FD.

Erythromycin activates antral and small intestinal motilin receptors, and may have differential physiologic effects in children with underlying gastrointestinal dis-

orders, including FD^[172]. Erythromycin in adults with FD improved bloating and gastric emptying of liquids and solids, but did not affect meal related symptom severity^[173]. The motilin agonist ABT 229 provided no symptom improvement in adults with FD^[174]. Another motilin agonist, mitemincin, showed promise in relieving gastroparesis-associated symptoms in adult diabetics^[175]. Efficacy in a subset of those patients with lower body mass index and hemoglobin A1C suggests a role in nondiabetics with upper gastrointestinal symptoms^[176]. Motilin receptor agonists are known to decrease gastric accommodation and compliance^[177,178] and are susceptible to tachyphylaxis, both factors that may contribute to limited efficacy in FD.

Actiomed is a novel agent that has minimal interaction with serotonin and dopamine receptors. It affects gastrointestinal motility in adult FD, including improving accommodation and gastric emptying^[179], through muscarinic receptor inhibition. This, in turn, increases acetylcholine release and inhibits its degradation. Elimination of meal-related symptoms, and improvement in symptom subgroups and quality of life was demonstrated in a phase III clinical trial in Japan^[180]. Phase III trials are currently in preparation in the US and Europe.

Gastric accommodation represents another potential therapeutic target within the broad category of electromechanical dysfunction. Buspirone, a 5HT_{1A} receptor agonist, increased accommodation and decreased symptom severity, postprandial symptoms, and liquid gastric emptying rate, but did not specifically affect gastric sensitivity to distension by barostat in adults with FD^[181]. Tando-spirone, a partial 5HT_{1A} agonist similar to buspirone, also improved symptom scores in FD adults, but had no effect on early satiety implicating central anxiolytic effects rather than altered gastric accommodation^[182]. Sumatriptan is another 5HT₁ receptor agonist that alters gastric size in dyspeptics, but specific mechanical effect and association with symptom improvement remains unclear^[93,183]. A subset of FD patients also showed improvement in nausea and accommodation when treated with ondansetron, a 5HT₃ antagonist, but mechanical and clinical effects were disassociated^[184]. Tegaserod, a partial 5HT₄ receptor agonist, is shown to enhance gastric accommodation and two large randomized trials showed significant symptom relief compared to placebo^[185]. Paroxetine, an SSRI, has been shown to enhance gastric accommodation in healthy volunteers but has not been studied in FD^[186].

Cyproheptadine is efficacious in improving symptoms in children with FD^[187]. As an antagonist of serotonin, histamine H₁, and muscarinic receptors, it is possible that physiologic effects are due to increased gastric accommodation or decreased gastric hypersensitivity to distension. In a retrospective open-label study of 80 children, Rodriguez *et al.*^[187] showed FD-symptoms significantly improved in 33 (41%) and resolved in 11 (14%) with very good medication tolerance even in nonresponders. It was previously found to be effective in a RCT of children with functional abdominal pain^[188].

Complementary therapies such as ginger^[189,190], peppermint oil^[191], and Iberogast^[192], may also have a role in the treatment of FD. Ginger enhances gastric emptying in healthy volunteers and adults with FD but had no impact on FD symptoms^[193,194]. In healthy volunteers, peppermint oil enhances gastric emptying without effects on sensitivity or accommodation^[195,196]. It was effective for irritable bowel syndrome in children, but has not been specifically studied in FD^[191]. Iberogast (STW 5), an herbal preparation, improves symptoms in FD, but there is not clear data determining whether effects are directly mediated by acceleration of gastric emptying or an alternate mechanism^[192,197].

Non-medication treatments

Gastrointestinal motility can also be influenced by mechanical devices including the gastric electrical stimulator. The device utilizes electrodes implanted into the antrum to deliver high frequency, low amplitude stimulation. Adult studies show the device decreases symptom severity and improves quality of life^[198] and findings were recently replicated in pediatric trials^[10,199]. The study of 24 pediatric FD patients included those who did not improve with conventional medical therapy and most underwent temporary endoscopic gastric pacemaker placement to assess for symptom improvement prior to implantation of the permanent device^[10]. Most patients showed significant gastrointestinal symptom improvement, as well as improved quality of life and global health scores.

Given the interaction between the stress response, visceral hypersensitivity, and electromechanical dysfunction, non-medication treatment of stress and anxiety likely have a role in the management of these patients. Psychological and relaxation interventions studied in children with FGIDs include cognitive behavioral therapy, gut-directed hypnotherapy^[200,201], yoga^[202], and biofeedback-assisted relaxation therapy (BART)^[203]. Children receiving a standardized course of targeted medication plus BART demonstrated better outcomes including decrease in pain intensity, decrease in pain episode duration, and global pain improvement as compared to children receiving only the medication component.

This gives rise to the hope that treatments addressing multiple, complementary targets within the biopsychosocial model can improve outcomes for children with FD, although further research needs to be done with multiple-component treatments to determine optimal combinations for individual children.

CONCLUSION

FGIDs, including functional dyspepsia, are incompletely understood despite high prevalence and significant impact on patient quality of life and healthcare costs. FGIDs are best approached utilizing a biopsychosocial model in which all relevant factors (biologic, psychologic and social) are identified and targeted in treatment. As

mechanisms of disease are further investigated, both in laboratory and clinical models, opportunities arise to target therapies. In addition to inflammation (addressed elsewhere), visceral hypersensitivity and gastrointestinal dysmotility are pathophysiological alterations that may respond to directed treatment. Despite limited evidence in children, the role of pharmacologic agents within a broader biopsychosocial treatment context remains promising.

There remains a need for placebo-controlled trials of therapy targeting visceral hypersensitivity and electromechanical dysfunction in children with FD. Likewise, there is a need to better understand the diagnostic and prognostic utility of various tests of upper intestinal sensory and mechanical function including visceral sensitivity, accommodation, and gastric emptying. Application of knowledge from placebo-controlled trials and specific tests of function may improve directed medical therapy for children with FD.

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Approach to *Helicobacter pylori* infection in geriatric population

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Abstract

The prevalence of *Helicobacter pylori* (*H. pylori*) infection and its complications increase with age. The majority of infected individuals remain asymptomatic throughout the life but 10%-20% develops peptic ulcer disease and 1% gastric malignancies. The incidence of ulcers and their complications are more common in the older population resulting in higher hospitalization and mortality rates. The increased use of medications causing gastric mucosal damage and the decreased secretion of protective prostaglandins in elderly are major factors increasing gastric mucosal sensitivity to the destructive effects of *H. pylori*. Due to higher prevalence of gastrointestinal (GI) malignancies, upper GI endoscopy is mostly preferred in elderly for the diagnosis of infection. Therefore, "endoscopy and treat" strategy may be more appropriate instead of "test and treat" strategy for dyspeptic patients in older age. Urea breath test and stool antigen test can be used for control of eradication, except for special cases requiring

follow-up with endoscopy. The indications for treatment and suggested eradication regimens are similar with other age groups; however, the eradication failure may be a more significant problem due to high antibiotic resistance and low compliance rate in elderly. Multidrug usage and drug interactions should always be considered before starting the treatment. This paper reviews briefly the epidemiology, diagnosis, disease manifestations, and treatment options of *H. pylori* in the geriatric population.

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Key words: *Helicobacter pylori*; Epidemiology; Diagnosis; Treatment; Eradication; Elderly; Geriatrics; Geriatric population

Core tip: *Helicobacter pylori* (*H. pylori*) infection is more common in the older population and may cause significant complications with severe morbidity and mortality. There are similarities but also differences in the diagnosis and treatment of infection in elderly population than non-elderly. Health care providers to the geriatric population should take into consideration these nuances in the management of *H. pylori* infection in the older patients.

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INTRODUCTION

The discovery of *Helicobacter pylori* (*H. pylori*) by Marshall and Warren in 1983 resulted in a breakthrough in the understanding and management of gastric diseases. Currently, it is well known that *H. pylori* infection causes chronic gastritis that may progress into peptic ulcer

disease (PUD), gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma (MALToma)^[1-3]. The ability of this bacterium to persist and establish a low-grade inflammatory state might induce an immunologic response that may influence the occurrence and progression of local and systemic diseases^[4]. Indeed, *H. pylori*, now one of the best models for the investigation of infectious diseases, have been widely studied to the extent of finding its associations with extragastric disorders^[5,6]. Despite the extensive knowledge on the virulence factors and immune manipulation mechanisms of *H. pylori*, there has been little success developing a vaccination for this organism^[7]. Instead, eradication therapy is used for prevention and treatment. Recently, the eradication rates through the standard proton pump inhibitor (PPI)-based triple therapy has declined to unsatisfactory levels of 80% or less, possibly due to antibiotic resistance, poor compliance, and rapid metabolisms of PPI^[8-10]. Therefore, several novel treatment regimens are emerging^[11]. The frequency of *H. pylori* infection, its manifestations, and eradication options are variable and depend on many factors including age. In this review, we discuss the different aspects of *H. pylori* infection and its eradication in elderly.

H. PYLORI INFECTION IN GERIATRICS

Epidemiology

H. pylori infection becomes rarer in recent years especially in young and middle-aged populations due to improvements in the quality of healthcare and effective treatment options^[12]. However, the rate of *H. pylori* infection and its complications are still increasing with age worldwide. Epidemiologic studies report higher prevalence of *H. pylori* infection in elderly with a ratio of over 70% in patients with gastrointestinal diseases and approximately 60% in asymptomatic patients^[13,14]. Although the majority of the infected patients remain asymptomatic throughout the life, about 10%-20% of the patients will develop PUD, and 1% will develop gastric cancer and MALToma in addition to the possible extragastric complications^[15,16]. Particularly elderly patients suffer from more serious complications resulting in higher hospitalization and mortality rates^[17,18]. This difference in the geriatric population can be illustrated by several factors. Firstly, in an older patient, the presentation of *H. pylori* infection may be subtle or atypical, which may delay the diagnosis. With advanced age, the increased presence of concomitant diseases and multidrug therapy, especially medications causing gastric mucosal damage and bleeding (e.g., non-steroidal anti-inflammatory drugs (NSAID), bisphosphonates, antiplatelet drugs, warfarin), can lead to increased and severe complications of *H. pylori* infection^[19]. In particular, NSAID and *H. pylori* are independently the two most important causes of peptic ulcer in adult population^[20]. A meta-analysis showed that the peptic ulcer risk in *H. pylori* infected NSAID takers was 61 times more compared to *H. pylori* negative individuals not taking

NSAID^[21]. In parallel to this study, *H. pylori* infection in elderly NSAID users is also associated with a significantly increased ulcer risk, which should be a concern considering the common use of NSAID in elderly population^[20]. Despite the unclear and rather complex synergy between *H. pylori* infection and NSAID, it is well known that both deteriorate mucosal defense mechanisms considerably. Besides, the decreased secretion of protective prostaglandins, as well as gastric acid (possibly due to fundal atrophic gastritis) with increased age can destruct the mucosal barrier^[22,23]. Clinical studies performed in the United States have shown that the percentage of *H. pylori* screening in hospitalized elderly patients having PUD is only 40%-56%, with a 50%-73% treatment rate after a positive test result^[24]. These results indicate that even if the clinical characteristics and epidemiologic distribution of *H. pylori* infection in the elderly have been extensively reported, the medical attention for the *H. pylori* infection in this population remains low.

Diagnosis

H. pylori infection can be diagnosed by noninvasive or invasive methods. The selection of the appropriate test may vary with the clinical setting^[2,25,26]. Noninvasive tests include ¹³C-urea breath test (UBT), stool antigen test (SAT), and serology. The UBT is a readily available test with an accuracy rate of > 97.9% in elderly patients regardless of the cognitive function, comorbidity, and co-treatment status^[27-29]. The SAT is reported to have a sensitivity of 76%-81% and specificity of 80%-93% in hospitalized elderly patients^[30,31], although these numbers may have been presumably improved with the recent advances in the SAT method. Currently, the laboratory SAT format (ELISA) with monoclonal antibodies is recommended rather than the rapid in-office test due to the significant difference in the accuracy^[26]. Both UBT and SAT can be used for infection follow-up after eradication therapy because of their ability to detect active infection^[26]. The serology test is a widely used and inexpensive test, but its diagnostic accuracy is variable^[32] and only validated IgG tests should be used^[26]. Positive serology may indicate a past infection, and thus it cannot be used for infection follow-up after eradication^[33,34]. In elderly patients with immunodeficiency or protein malnutrition, false negative serology results may occur due to lack of antibody response^[29,35]. However, serology is helpful in patients with low bacterial load (e.g., use of antimicrobial and antisecretory agents, bleeding, presence of malignant lesions, etc.) and therefore remains the only test that is not affected by local changes in the stomach. Also, for all invasive and noninvasive tests except for serology, discontinuation of PPI use for two weeks prior to testing is necessary^[26].

Invasive techniques requiring an endoscopy are usually preferred in elderly patients due to the higher prevalence of gastrointestinal malignancies, as well as for their superiority in analyzing the severity of gastritis and detecting premalignant lesions^[36,37]. *H. pylori* can be detected through histological examination or by indirect assess-

ment of the biopsy specimen with urease test, culture, or polymerase chain reaction (PCR) analysis^[38,39]. The urease test provides inexpensive and rapid detection, however it has lower sensitivity in patients aged 60 years and older^[38]. Cultures can assess the susceptibility of the strain to antimicrobial agents, which is important for the management of the infection^[40]. Nonetheless, false negative results might be obtained with cultures due to frequent antibiotic use in elderly. PCR detection of *H. pylori* infection offers sensitive and accurate results rapidly and it is increasingly becoming popular. PCR assays allow simultaneous detection, quantification, genotyping, and virulence factor identification, as well as determination of antibiotic resistant and cancer susceptible strains of *H. pylori*^[39,41]. Despite the common statement of histopathology being the “gold standard” for diagnosis of *H. Pylori* infection, its accuracy depends on sampling locations and presence of atrophic gastritis^[42]. In addition, the frequent antibiotic and PPI use, as well as active and recent bleeding may alter the sensitivity. Therefore, discontinuation of PPIs two weeks prior to endoscopy, and specimen collection from both the body and antrum are recommended^[42]. In particular, it has been recently reported that in patients with extensive gastric atrophy, the corpus greater curvature is the optimum biopsy site for histopathologic evaluation^[43].

Although current guidelines recommend a general “test and treat” strategy for the uninvestigated dyspepsia^[26], in populations with higher gastric cancer risk like elderly patients, “endoscope and treat” strategy is preferred especially considering the lower accuracy of the noninvasive tests in the elderly^[44,45]. In addition, *H. pylori* infection in elderly might be asymptomatic or present with other symptoms than dyspepsia. For example, the inflammation caused by chronic *H. pylori* infection may result in atrophic gastritis and subsequently vitamin B12 deficiency^[46]. Therefore, a complete work-up for *H. pylori* is not only limited to diagnostic tests for detecting the infection, but also includes the complications and comorbidities of the disease.

EFFECTS OF ERADICATION THERAPY ON *H. PYLORI* ASSOCIATED DISEASE MANIFESTATIONS

Peptic ulcer disease and associated bleeding

H. pylori infection and NSAID/aspirin use have independent and additive effects on the higher prevalence of PUD and ulcer bleeding in the elderly^[47,48]. *H. pylori* positive NSAID users have an almost two fold increased risk of peptic ulcer bleeding compared to NSAID users without *H. pylori*^[49]. Taken together with the increased likelihood of bleeding associated with NSAID use in elderly (approximately 7 times more frequent than young adults)^[20], the concomitant presence of NSAID use and *H. pylori* infection in elderly should raise a potential concern for PUD and associated bleeding.

The eradication of *H. pylori* in elderly patients with

PUD heals ulcers in over 95% of patients^[50], improves symptoms in over 85% of patients^[51], and dramatically lowers the recurrence rate from 41.6% to 2.2%^[52]. For prevention of both duodenal ulcer recurrence (RR = 0.19) and gastric ulcer recurrence (RR = 0.31) *H. pylori* eradication is superior to no treatment^[53].

It is well established that the eradication of *H. pylori* prior to use of NSAID/aspirin is beneficial in prevention of PUD and associated bleeding^[54]. However, the influence of *H. pylori* eradication in NSAID/aspirin users is controversial. Based on multiple studies in this regard, the most recent Maastricht IV/ Florence Consensus Report^[26] have slightly different recommendations for long term NSAID and low dose aspirin users. For NSAID users it is recommended to have continued PPI treatment in addition to *H. pylori* eradication. For low dose aspirin users, *H. pylori* test should be performed if there is a history of PUD. After eradication in these patients, the incidence of gastric bleeding remains low even without gastroprotective agents^[26].

Functional dyspepsia and gastritis

Patients with dyspepsia and *H. pylori* infection are reported to have functional dyspepsia (FD) rather than PUD, although the eradication benefit is less evident in FD in comparison to PUD^[26]. However, the long-term relief of dyspepsia has been shown in one of 12 patients with *H. pylori* and functional dyspepsia after *H. pylori* eradication, which is better than any other treatment^[55].

Prolonged *H. pylori* infection is a well-recognized cause of different phenotypes of gastritis based on the topography of the colonization and inflammation in the stomach, including mild pangastritis, corpus, and antrum predominant gastritis, each with different clinical outcomes^[2,26]. The antrum predominant gastritis, the most common form of *H. pylori* mediated gastritis, is usually associated with a normal to high secretion of gastric acid and an increased risk of duodenal ulcer disease^[2,56]. On the other hand, the corpus predominant gastritis is usually associated with hypochlorhydria and results in an increased risk of developing gastric atrophy, intestinal metaplasia, and ultimately gastric carcinoma^[2,48,57]. As the name implies, the patients with mild pangastritis do not have clinically significant disease. It needs to be noted that the different phenotypes are not completely separate entities, and antrum predominant gastritis may progress into the other types^[2,56]. Regarding the effects of advancing age on gastritis, it has been shown that gastric acid secretion decreases with age only in *H. pylori* positive subjects^[22]. This influence is probably due to the increasing prevalence of fundic atrophic gastritis in elderly^[58]. Evidence suggests that eradication of *H. pylori* infection results in significant decrease in the activity of gastritis in elderly^[59].

Gastric malignancies

H. pylori eradication may prevent gastric cancer^[60]; however, its effects depend on the histological stage and gastric

localization. The progression of the premalignant lesions can be prevented with the eradication^[61], whereas if intestinal metaplasia is established the eradication does not completely prevent the gastric cancer, although it might slow the progression^[62,63]. A meta-analysis has shown that the eradication significantly improves corpus atrophy, but not antrum, and not intestinal metaplasia^[64]. Therefore, the early diagnosis with endoscopy and treatment are important in elderly patients. For low grade MALTomas, *H. pylori* eradication is the first line treatment but the patients need to be followed up after the treatment in case the lymphoma fails to respond to the eradication^[65].

Extragastric diseases

H. pylori has been associated not only with diseases of the gastrointestinal tract but also with extragastric diseases most of which are commonly seen in elderly population^[6]. However, the causal or therapeutic links are stronger in some extragastric diseases than the others. The eradication is indicated in patients with unexplained iron deficiency anemia, idiopathic thrombocytopenic purpura, and vitamin B12 deficiency with significantly clear evidence^[66-70].

Multiple studies reported higher prevalence of *H. pylori* infection in patients with type 2 diabetes mellitus (DM), with one study analyzing the individuals older than 60 year old^[6,71]. Some groups even propose an association between *H. pylori* infection and the metabolic syndrome, supported by the synergistic effect of *H. pylori* infection and higher body mass index (BMI) in increasing the level of glycosylated hemoglobin^[72], the significant association of *H. pylori* seropositivity with both DM and insulin use, as well as the independent association of *H. pylori* positivity with microalbuminuria^[73]. On the other hand, there are some other groups contesting these associations with opposite findings^[74,75]. Therefore, for obesity and DM the evidence is unclear and further studies are warranted.

Some epidemiologic studies suggest the association of *H. pylori* infection and neurologic diseases such as stroke, Parkinson's and Alzheimer's diseases^[6,76-78], as well as ischemic heart disease^[79]. Nonetheless, the evidence is equivocal for *H. pylori* eradication and improvement of these diseases^[26]. Lastly, the bioavailability of thyroxine and Capitalize L-dopa improves with *H. pylori* eradication, although there is no verification of direct clinical benefit to the patients^[80,81].

ERADICATION THERAPY

The triple therapy of PPI, clarithromycin, and amoxicillin (or metronidazole) has been the standard for *H. pylori* eradication since 1997 when the first Maastricht conference report was published^[82]. However, multiple studies have reported suboptimal efficacy of this regimen with cure rates of less than the initial aim of 80%^[8-10,83-87]. The decrease in efficacy might be associated with increased resistance to clarithromycin, high bacterial load, strain types, high gastric acidity, and low compliance^[26,88]. Among these factors, the clarithromycin resistance has

been identified as the major contributor to the eradication failure. To improve the efficacy, different combinations of currently available antibiotics have been assessed^[26,89,90]. Triple therapy with PPI, amoxicillin, and metronidazole has been proposed as an alternative to the standard therapy with cure rates of 82%-94%^[91-94]. Sequential therapy including a 5-d period with PPI-amoxicillin, followed by a 5-d period with PPI, clarithromycin, metronidazole (or tinidazole) is another regimen that has been studied in different countries. A recent systematic review of 22 trials revealed that the sequential therapy is more effective than standard triple therapies, confirming that the sequential administration of drugs is a successful therapeutic intervention for *H. pylori* eradication. Whether the use of the modified sequential therapy with longer duration of sequential regimens is actually more advantageous than that of 10-d sequential therapy requires further studies^[95,96]. Non-bismuth quadruple therapy, also called "concomitant therapy", has been offered as a more convenient regimen for the patient, which involves all three antibiotics to be taken simultaneously together with a PPI for a period of 10-14 d. A recent meta-analysis from 19 studies (2070 patients) on concomitant therapy revealed a mean of 88% cure rate, superior to standard triple therapy, with a safe and well-tolerated profile^[97].

The rate of the clarithromycin resistance is variable in different regions, with a threshold of 15%-20% prevalence to classify low or high clarithromycin resistance^[26]. The clarithromycin resistance determines the approach to *H. pylori* eradication. In regions with low resistance, the standard triple therapy including clarithromycin is still recommended as first line regimen^[26]. Different ways of improving the effectiveness of PPI-clarithromycin-containing regimens have been proposed including increasing the dosage and timing. Significant evidence from multiple studies suggests that high-dose PPIs increase in the cure rates up to 10% in comparison with standard doses^[98]. Extension of PPI-clarithromycin-containing triple therapies from 7-d to 10-14 d has been shown to increase the eradication rate by about 5% without significant difference in the rate of side effects^[99,100]. Bismuth-containing quadruple therapy may be either the first line regimen in a low clarithromycin resistance region or the second line therapy if PPI-clarithromycin containing triple therapy fails. An alternative second line treatment in this population is levofloxacin-containing triple therapy. After two treatment failures, third line treatment should be guided by antimicrobial susceptibility testing^[26].

In regions with high clarithromycin resistance, bismuth-containing quadruple treatment has been suggested as the first line regimen^[26]. This regimen achieved a significantly better eradication rate compared to standard triple therapy (82% *vs* 62%) in a population with high clarithromycin resistance^[101]. If the bismuth-containing quadruple therapy is not available, sequential treatment or a non-bismuth quadruple therapy may be administered. Similar to the low clarithromycin resistance regions, if the first line treatment fails in a high resistance region, it may be followed by levofloxacin-containing triple therapy as

the second line, and antibiotic susceptibility guided treatment as the third line therapy^[26].

In the light of above-mentioned guidelines for *H. pylori* eradication, there are several issues to be emphasized in elderly population. Firstly, antibiotic resistance is particularly important in elderly due to increased prevalence of drug consumption and lower compliance potential in this population^[102,103]. The health care providers should be especially cautious about the emerging levofloxacin resistance primarily in patients with chronic infectious bronchopneumopathy as they may have already received fluoroquinolones^[26]. Structured patient counseling and follow-up might improve the patient compliance and efficacy of the therapy^[104] and therefore, assist preventing antibiotic resistance. Secondly, the drug interaction is of significant importance in elderly population in whom polypharmacy is a common occurrence. Although the choice of PPI in *H. pylori* eradication does not affect the treatment success when used in standard doses^[87], different PPIs might have different drug interactions. Omeprazole is the PPI that is most likely to have drug interactions particularly with cardiovascular drugs and clopidogrel, both of which commonly used in elderly. On the other hand, pantoprazole is the least likely PPI to interact with clopidogrel^[105]. Similarly, frequently used antibiotics for eradication such as clarithromycin, amoxicillin, metronidazole, and tetracycline may also have important drug interactions with commonly used medications in elderly^[106]. Although it is not easy to determine the effects of a particular drug's interaction in the large number of variables, cardiovascular drugs such as statins, antiarrhythmic drugs, and warfarin are among the well-established drugs which may interact with these antibiotics^[106]. If the risk of interaction outweighs the benefit, the eradication treatment should be avoided or suspended. In addition, some co-morbidities in the elderly might require additional modification in the treatment plan. For example, while metronidazole can be used without dosage alteration in patients with renal failure, amoxicillin and clarithromycin require dose adjustment in patients with creatinine clearance less than 30 mL/min. These antibiotics may cause transient and mild elevation in the liver enzymes, but severe hepatotoxicity is unusual particularly in short term usage. Dosage adjustments for PPIs are not necessary in elderly patients or those with renal failure or mild hepatic impairment^[106].

Last but not least, as the complications of *H. pylori* infection are increased with age, the proper follow-up testing needs to be conducted after eradication therapy to prevent further progression of the disease. While patients with gastric ulcer or gastric MALToma, or severe gastritis should be evaluated by endoscopy after therapy, the remaining situations may be followed-up with noninvasive methods (UBT or laboratory-based validated monoclonal SAT)^[26,106].

CONCLUSION

H. pylori infection is a prevalent health problem in the

older patients due to multiple factors increasing the potential damage of bacteria to gastric mucosa. The comorbidities and multidrug therapy can lead to increased and severe complications of *H. pylori* infection. The invasive tests using upper GI endoscopy should be preferred for the diagnosis of infection. The therapeutic approach suggested by the Maastricht IV Consensus Report is also suitable for older patients; however, the eradication failure may be a more significant problem due to high antibiotic resistance and low compliance rate. The expectation from eradication therapy in these patients should meet the therapeutic goals and therefore, the health care providers should take into account the specific characteristics of geriatric population.

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Pharmacological therapy of feed intolerance in the critically ill

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Core tip: Feed intolerance during critical illness must be promptly recognized and treated due to the associated morbidity and mortality. The current first line treatment for feed intolerance is prokinetic therapy with erythromycin and metoclopramide (alone or in combination), which are highly effective and free of significant adverse effects. Although diarrhoea occurs commonly after combination prokinetic therapy, it is not associated with *Clostridium difficile* colitis and settled shortly after stopping the treatment. The use of prokinetic therapy over a long period or for prophylactic purpose, therefore, must be avoided and the indication for ongoing use of the drug(s) should be frequently reviewed.

Abstract

Feed intolerance in the setting of critical illness is associated with higher morbidity and mortality, and thus requires promptly and effective treatment. Prokinetic agents are currently considered as the first-line therapy given issues relating to parenteral nutrition and post-pyloric placement. Currently, the agents of choice are erythromycin and metoclopramide, either alone or in combination, which are highly effective with relatively low incidence of cardiac, hemodynamic or neurological adverse effects. Diarrhea, however, can occur in up to 49% of patients who are treated with the dual prokinetic therapy, which is not associated with *Clostridium difficile* infection and settled soon after the cessation of the drugs. Hence, the use of prokinetic therapy over a long period or for prophylactic purpose must be avoided, and the indication for ongoing use of the drug(s) must be reviewed frequently. Second line therapy, such as total parenteral nutrition and post-pyloric feeding, must be considered once adverse effects relating the prokinetic therapy develop.

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INTRODUCTION

Enteral nutrition is preferred method of nutritional support during critical illness given its major benefit in preserving intestinal mucosal barrier function, cheap and has significantly fewer infective complications as compared to total parenteral nutrition^[1-6]. Unfortunately, gastrointestinal motility is frequently impaired in these patients and consequently, naso-gastric (NG) feeding cannot be tolerated in approximately 50% patients^[1-3] due vomiting, feed reflux or regurgitation, pulmonary aspiration^[3-5]. Not only these feeding complications are associated with higher morbidity and mortality^[4-6], they also prevent adequate delivery of nutrition to meet the daily caloric requirement of these patients. Thus, it is important that feed intolerance is promptly identified and treated.

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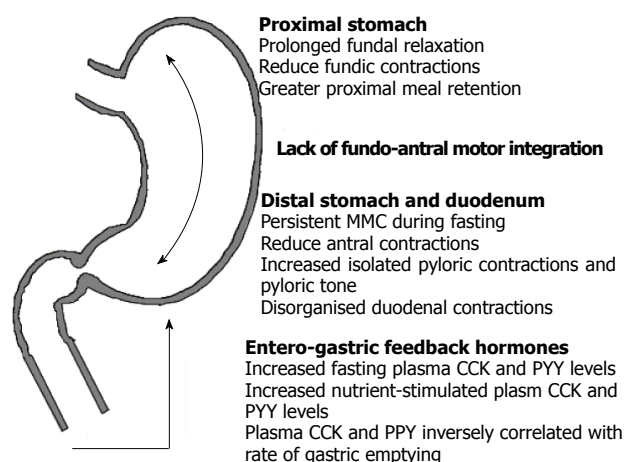


Figure 1 Gastric motor abnormalities reported during critical illness. CCK: Cholecystokinin; PYY: peptide YY.

Prokinetic agents are currently considered as the first-line therapy for feed intolerance given issues relating to parenteral nutrition and post-pyloric feeding^[7-9]. In addition to the technical difficulties related to the placement of post-pyloric feeding tube, the morbidity and mortality benefits of post-pyloric feeding have not been demonstrated in these patients, especially in those who do not have impaired gastric emptying, and thus, routine use of post pyloric feeding is not recommended^[10-12]. Even in feed-intolerant patients who failed to respond to prokinetic therapy^[13], evidence to support post-pyloric feeding in these patients is also lacking. Although total parenteral nutrition offers an alternative approach to deliver nutrition to these patients, it is associated with a higher rate of infective and hyperglycemic complication morbidity, necessitate the need for meticulous care of intravenous lines and blood glucose management. This review aims to provide an overview of the current pharmacological approach to treat feed intolerance and gastrointestinal dysmotility during critical illness.

UPPER GASTROINTESTINAL DYSMOTILITY AND FEED INTOLERANCE DURING CRITICAL ILLNESS

Gastric emptying (GE) is commonly impaired during critical illness with up to 50% of mechanically ventilated patients have delayed GE^[14-19]. Furthermore, antro-pyloro-duodenal as well as intestinal motilities during both fasting and fed stage are also frequently impaired in these patients^[2,20], characterized by (1) an absence gastric phase III motility and a loss of antro-pyloro-duodenal integration during the fasting state^[21]; and (2) a delayed fundal relaxation, a reduced antral motility^[20,22], an increased isolated pyloric activity^[20] and a disrupted motor integration between the proximal and distal stomach^[23] during feeding (Figure 1). Compared to healthy subjects, proximal gastric relaxation is more prolonged in critically ill patients and is associated with a greater suppression of fundic wave activity in response to intestinal nutrient

infusion^[24,25]. There is also a marked reduction in number of antral pressure waves and antral motility index but an increase in the isolated pyloric pressure waves and pyloric tone during a gastric meal^[20,23]. The organisation of the duodenal contractions in these patients is also markedly abnormal with approximately 50% of these contractions being propagated in a retrograde manner^[20,23]. Furthermore, the motor integration between the proximal and distal stomach, which is important for meal distribution and emptying, is also disrupted and leads to increased retention of the meal in the proximal stomach^[23].

The mechanisms underlying gastrointestinal motility dysfunction during critical illness remain uncertain. Overall, the prolonged fundal relaxation, reduced antral activity and increased pyloric activity in response to a nutrient meal is consistent with enhanced motor feedback responses to the entero-gastric reflex. In keeping with this notion, the gastric motor disturbances in critically ill patients are associated with enhanced increases plasma cholecystokinin (CCK) and peptide YY (PYY) concentrations (gut hormones that mediate the entero-gastric motor feedback responses) during both fasting and in response to intestinal nutrients^[26-28]. Together with the known adverse effects of CCK and PPY on gastric emptying, reciprocal relationship between the rate of gastric emptying and plasma concentrations of CCK and PYY suggests a potential role of these hormones in the pathogenesis of impaired gastric emptying during critical illness^[26].

Other potential contributors that have been implicated in the pathogenesis of upper gastrointestinal motor dysfunction during critical illness are: admission diagnosis, severity of the critical illness, pre-existing morbidities, recent abdominal surgery, shock, electrolyte abnormalities, hyperglycaemia, age, gender, and drugs including those that used for sedation (benzodiazepines), analgesia (opioids) and maintaining blood pressure (catecholamines)^[29-31].

Current pharmacological therapy of feed intolerance

Given the impaired gastric emptying is the main cause of feed intolerance, the main aim of the current available drug therapy for feed intolerance is to improve gastric emptying.

Dopamine agonists: Metoclopramide and domperidone are the dopamine agonists that have been used to treat feed intolerance in critical care for a long time. In contrast to metoclopramide, domperidone is a peripherally acting dopamine antagonist, which avoids the central nervous side effects and thus, has little extra-pyramidal adverse effects. Whilst these agents have been reported to improve gastric emptying in these patients, its efficacy in improving feed intolerance remains controversial^[7-9]. The effect of metoclopramide on the gastric residual volume (GRV) was not observed after an orally administered 10 mg dose, and not after the third dose, a modest reduction in GRV was detected^[7-9]. Recently, metoclopramide [10 mg *qid in*] has been

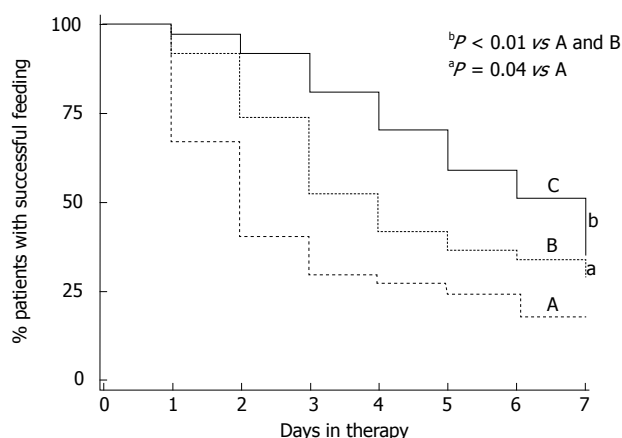


Figure 2 Efficacy of metoclopramide (A), erythromycin (B) and combined erythromycin and metoclopramide (C) in the treatment of feed intolerance of critical illness over 7-d period.

shown to improve feed intolerance during critical illness but its therapeutic efficacy declines progressive over the 7 d (from 85% in the first days of therapy to less than 35% after 7 d of treatment, Figure 2)^[32]. Metoclopramide, however, is not effective^[33] and, in fact, contraindicated^[34] in patients with brain injury, as it can raise intracranial pressure further.

Motilin agonists: Erythromycin is the only available motilin agonist uses in clinical practice. Given at a low dosage, ranging between 3 to 7 mg/kg per day, erythromycin has been shown to increase both gastric emptying and improve feed intolerance in critically ill patients^[7-9]. Whilst enterally administered metoclopramide and cisapride have been shown to have a more rapid onset of action than erythromycin, the overall effect of these agents on GRV in these patients is similar to that of erythromycin^[7-9]. In a recent randomized, double-blind trial, low dose erythromycin (200 mg *bid iv*) was found to be more efficacious than metoclopramide in improving feed intolerance in these patients^[32]. The major issue with both agents, however, is the rapid development of tachyphylaxis, leading to a marked reduction in efficacy after a week of therapy (approximately 30% at day 7)^[32] (Figure 2). For patients who failed to respond to either agent, the use of rescue combination therapy (*i.e.*, erythromycin and metoclopramide) was highly effective and minimized the development of tachyphylaxis^[32] (Figure 3).

The role of combination prokinetic therapy as the first line therapy for feed intolerance has also been evaluated in a double-blind randomized study^[35]. The use of first-line combination therapy to treat feed intolerance was significantly more effective than erythromycin alone, allowing a significantly greater amount of feed to be given to the patients during treatment. First-line combination therapy was also associated with a lesser degree of drug tachyphylaxis and up to 60% of patients remained responsive at day 7 of treatment^[35] (Figure 2). Major adverse effects were not observed in either mono- or dual-therapy groups during both studies^[32,35], supporting the

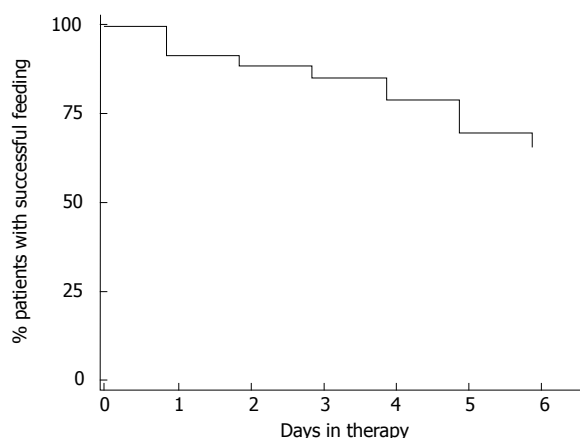


Figure 3 Efficacy of rescue combined metoclopramide and erythromycin in critically ill patients who failed to response to either erythromycin or metoclopramide in the treatment of feed intolerance.

use of low dose erythromycin, particularly in combination with metoclopramide, in the management of feed intolerance during critical illness. Despite these data, routine use of erythromycin for feed intolerance has not been universally recommended amongst the Intensive Care Physicians due the major concern of promoting bacterial resistance development with the widespread use of low dose erythromycin^[36,37].

Serotonin (5-Hydroxytryptamine) receptor agonists:

Serotonin is a monoamine neurotransmitter that acts on a variety of receptor types in the gastrointestinal tract, and has been shown to stimulates peristalsis^[37]. This property leads to the use of this pharmacological agent as a prokinetic drug. Cisapride is the most well known agent in this class, and has dual stimulatory effects on the 5-Hydroxytryptamine (5-HT₄) serotonin receptors and the parasympathetic nervous system, leading to increases in both 5-HT₄ and acetylcholine in the enteric nervous system. The use of cisapride has been shown to associate with improved gastric emptying in critically ill patients as well as reduced the occurrence of feed intolerance^[38-42]. Due to case reports of cisapride induced lethal cardiac toxicity^[43], however, the drug has been withdrawn and is not available for clinical use.

Tegaserod is another serotonin (partial) agonist that have been trialed in critically ill patients. Similar to cisapride, although tegaserod improved gastric motility and accelerated gastric emptying^[44], the drug was withdrawn in 2007 due to cardiovascular adverse effects. Together, evidence indicated that this class of drug is not an ideal prokinetic agent due to the associated cardiovascular side effects, and it use in critically ill patients is not recommended.

Novel prokinetic agents

Opiate receptor antagonists: The use of this class of drug bases on the fact that opiate reduces both gastric and intestinal motility, leading to an increase in feed intolerance during critical illness. Although the regular use of nasogastric naloxone (an opioid antagonist) has been shown

to reduce GRV and incidence of ventilation associated pneumonia (VAP) in critically ill patients who were receiving *iv* fentanyl, it had not impact on the time to wean from mechanical ventilation or the time to discharge from ICU^[45]. Thus, the use of naloxone (8 mg *qid* nasogastric) has not been routinely adopted in clinical practice given its lack of easy administration and increases expense.

Recent data on mu (μ) receptor antagonists are, however, more promising. Unlike naxolone, these peripheral mu-opioid receptor antagonists do not antagonise the analgesic effects of analgesia and induce withdraw effects^[46]. Alvimopan, a peripheral μ -opioid receptor antagonist, has been shown to counteract the inhibitory effect of opiate on small bowel motility^[47] and significant reduce both the time to bowel recovery and time to discharge from hospital in patients with abdominal surgery^[48]. The efficacy of alvimopan in the management of feed intolerance during critical illness, however, has not been formally assessed and warranted further evaluation.

CCK receptor antagonists: Compared to healthy subjects, critically ill patients have increased plasma fasting and nutrient-stimulated CCK concentration, which is further elevated in those who have delayed gastric emptying^[49] or feed intolerance^[50]. Given CCK plays a major role in the negative feedback inhibition of gastric emptying in response to meal, the higher plasma concentrations of CCK in critically ill patients are thought to potentially contribute to the gastric dysmotility and feed intolerance. In health, the use of loxiglumide has been found to associate with enhanced lower oesophageal sphincter function and gastric emptying. Given these findings, the use of CCK receptor antagonist may have a potential role in the management of impaired gastric emptying and feed intolerance during critical illness. Unfortunately, such study has not been conducted.

Ghrelin agonist: Ghrelin, a motilin related peptide, has a number of actions on the gastrointestinal tract, including stimulation of appetite^[51] and gastrokinetic effects^[52]. Physiologically, ghrelin induces phase III gastric contractions and increases the resting tone of the fundus^[53]. Based on these actions, the use of ghrelin analogue has been shown to improved gastric motility and the rate of gastric emptying in diabetic patients with gastroparesis^[54]. Unfortunately, in addition to the prokinetic property, ghrelin also has anabolic effects by induce releases of growth hormones. Given the use of growth hormone has been reported to associate with increased mortality in the critically ill^[55], the potential clinical use of exogenous ghrelin as a prokinetic agent has been cautious, and thus, has not been further evaluation in this patient group.

ADVERSE EFFECTS OF PROKINETIC THERAPY

Cardiovascular side effects

The potentially fatal ventricular arrhythmia relating to

prolongation of the QT interval, *torsades de pointes*, has always been a major concern with the use of currently available prokinetics^[56], as all agents have been shown to block the human ether-a-go-go-related gene currents which is important in mediation of cardiac rhythm^[57,58]. Compared to metoclopramide and erythromycin, cisapride and domperidone are approximately 100 times more potent in the inhibition of human ether-a-go-go-related gene currents and, thus, carry the highest risk of inducing cardiac arrhythmia^[57,58]. The risk of arrhythmia, however, can be potentiated by a number of patient-related factors such as known history of cardiac arrhythmia, structural heart disease, poor left ventricular function and electrolyte disturbances^[59]. In addition, it is important to recognize a number of drug interactions that can increase the risk of cardiac arrhythmia, which is particularly relevant in critical ill setting as poly-pharmacy is common. Concurrent administration of erythromycin and drugs that metabolized by CYP3A4 isoenzyme, such as antifungal and anti-arrhythmic drugs, calcium channel blockers, haloperidol and pimozide can increase the risk of adverse cardiac events and should be avoided^[59]. It is important to recognize, however, that these concerns related to the cardiac adverse effects are extrapolated from reports or studies performed in non-critically ill patients. Thus far, there has been report of cardiac toxicities or arrhythmias with the use of metoclopramide or erythromycin in clinical studies that involved adults or pre-term infants during critical illness^[31,34,60-62].

Another potential cardiovascular side effect of erythromycin is hypotension. In healthy volunteers, a reduction in systolic blood pressure by 10 mmHg has been observed after a single dose of erythromycin^[63]. Such haemodynamic effects of erythromycin may be more relevant in critically ill patients given their cardiovascular function is already compromised. Our study^[62], however, failed to demonstrate any impact of low dose erythromycin on blood pressure and heart rate, reassuring that erythromycin is safe to be used a prokinetic during critical illness.

Neurological side effects

In non-critical illness setting, long-term use of metoclopramide can be associated with somnolence, nervousness, extra-pyramidal dyskinesia, galactorrhea and menstrual disorders in up to 20% of patients^[64,65], especially the elderly females. These side effects are difficult to detect, and thus, not reported during critical illness as the patients are paralyzed for mechanical ventilation. However, the use of metoclopramide is contraindicated in head injury patients as it is not effective^[32] and more importantly, can increase intra-cranial pressure^[33]. Similarly, in patients with a known history of myasthenia gravis, erythromycin can precipitate myasthenia crisis^[66] and should be avoided in these patients.

Gastrointestinal side effects

Up to 25% patients with enteral feeding have watery di-

arrhoea^[67,68] and the majority of cases are not related to infection. The aetiology is likely to be multi-factorial and can relate to increased gastrointestinal transit^[69], reduced intestinal absorption^[70], disturbed carbohydrate fermentation from altered bowel flora^[67], and the hyper-osmolar effects of enteral feeds^[67,68], leading to osmotic diarrhoea in most patients. Consequently, diarrhoea is more frequently observed in patients who receive enteral feeding at a high rate (*e.g.*, greater than 50 mL/h)^[71], which improves when the rate of enteral feeding is reduced^[67,68]. Recently, in a study of 180 critically ill patients who had feed intolerance, 40% patients developed diarrhoea after 10 d after commencement of prokinetic therapy (erythromycin and/or metoclopramide), and was most prevalent in those who received combination therapy [49% *vs* 30% (erythromycin) *vs* 32% (metoclopramide)]^[61]. The diarrhea lasted for a mean duration of 3.6 ± 1.2 d and directly correlated with the amount of feeds delivered^[61]. More importantly, none of the patients with diarrhoea had *Clostridium difficile* infection and the diarrhoea resolved quickly with the cessation of prokinetic therapy^[61].

ISSUES RELATED TO THE DEVELOPMENT OF BACTERIAL RESISTANCE

Given the prokinetic dose of erythromycin is low and in the “sublethal” concentrations of antibiotic effects, the widespread use of this medication as a prokinetic has been cautioned^[35,36] as it can exert selective pressure on bacteria and can lead to the development of bacterial resistance^[72], particularly in the setting of critical illness. The concerns, however, remain hypothetical as there are no data in the current literature to support this hypothesis with the short-term use of low-dose erythromycin^[73]. In order to overcome this issue, a number of motilin derivatives that have no antibiotic property have been developed. Unfortunately, the prokinetic effects of these new agents are less durable, and are most likely due to the rapid development of drug tachyphylaxis^[74-76].

TREATMENT ALGORITHM FOR FEED INTOLERANCE DURING CRITICAL ILLNESS

In addition to pharmacotherapy, there are a number of factors in the general management of the ICU patient which can help to prevent gut dysmotility and avoid its sequelae. These include patient posture, which should be at least 30° head up to reduce aspiration and nosocomial pneumonia in the setting of absent gastro-esophageal pressure^[77,78]. Reduction in the dosage of opiates^[30,45] and catecholamines to minimal tolerated levels and avoidance of the use of dopamine^[79] will also reduce exogenous causes of delayed gastric emptying. If analgesia is required, short-acting agents like fentanyl or remifentanyl are preferred and the uses of morphine should be minimised. Hyperglycemia may contribute to slow gas-

tric emptying and blood glucose should be controlled. Prolonged fasting may affect gastrointestinal motility in healthy subjects^[80], but early initiation of feeding has not been shown to affect subsequent gastric emptying or gastrointestinal hormones^[81] but does improve subsequent nutrient absorption^[82].

If feed intolerance develops despite the above preventive measures, prokinetic therapy should be commenced in patients who have no contraindications. Available data indicate that the combination of low dose erythromycin (200 mg *bid iv*) and metoclopramide (10 mg *qid iv*) is the most effective treatment with the lowest risk of developing drug tachyphylaxis. In Units which are concerned about the risk of bacterial resistance or *Clostridium difficile* infection, metoclopramide can be used the first line therapy and if this agent fails, rescue combination therapy with erythromycin should be adopted. In all cases, prokinetic treatment can be ceased after 7 d of therapy if successful feeding has been achieved or as soon as diarrhoea becomes a problem. In cases where prokinetic therapy fails, particularly with the combination use of erythromycin and metoclopramide, post-pyloric delivery of enteral feed to overcome impair gastric emptying should be considered. If this approach also fails or is not available, parenteral nutrition support with good glycemic care can be adopted.

CONCLUSION

Feed intolerance is common amongst the critically ill patients and, if not treated promptly, can lead to increased morbidity and mortality. Currently, treatment with prokinetics is considered as the first line therapy given the related technical difficulty of post-pyloric placement and the potential infective morbidities of parenteral nutrition. Metoclopramide and erythromycin are the two agents that have been shown to improve feed intolerance in these patients with a relatively good safety profile. Available data suggest that the agents should be used in combination to achieve the highest efficacy with a least incidence of tachyphylaxis. The major but hypothetical concern with the widespread use of low dose erythromycin as a prokinetic in clinical practice, however, is the development of bacterial resistance. Further development and evaluation of novel prokinetic agents, therefore, are warranted to overcome problems relating to drug tachyphylaxis and development of bacterial resistance.

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Renal dysfunction in patients with cirrhosis: Where do we stand?

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Abstract

Patients with cirrhosis and renal failure are high-risk patients who can hardly be grouped to form precise instructions for diagnosis and treatment. When it comes to evaluate renal function in patients with cirrhosis, determination of acute kidney injury (AKI), chronic kidney disease (CKD) or AKI on CKD should be made. First it should be excluded the prerenal causes of AKI. All cirrhotic patients should undergo renal ultrasound for measurement of renal resistive index in every stage of liver dysfunction and urine microscopy for differentiation of all causes of AKI. If there is history of dehydration on the ground of normal renal ultrasound and urine microscopy the diuretics should be withdrawn and plasma volume expansion should be tried with albumin. If the patient does not respond, the correct diagnosis is HRS. In case there is recent use of nephrotoxic agents or contrast media and examination shows shock, granular cast in urinary sediment and proteinuria above 0.5 g daily, acute tubular necrosis is the prominent diagnosis. Renal biopsy should be performed when glomerular filtration rate is between 30-60 mL/min and there are signs of parenchymal renal disease. The acute renal

function is preferable to be assessed with modified AKIN. Patients with AKIN stage 1 and serum creatinine ≥ 1.5 mg/dL should be at close surveillance. Management options include hemodynamic monitoring and management of fluid balance and infections, potentially driving to HRS. Terlipressin is the treatment of choice in case of established HRS, administered until there are signs of improvement, but not more than two weeks. Midodrine is the alternative for therapy continuation or when terlipressin is unavailable. Norepinephrine has shown similar effect with terlipressin in patients being in Intensive Care Unit, but with much lower cost than that of terlipressin. If the patient meets the requirements for transplantation, dialysis and transjugular intrahepatic portosystemic shunt are the bridging therapies to keep the transplant candidate in the best clinical status. The present review clarifies the latest therapeutic modalities and the proposed recommendations and algorithms in order to be applied in clinical practice.

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Key words: Renal dysfunction; Cirrhosis; Assessment; Management; Hepatorenal syndrome

Core tip: Close surveillance, well -classified definitions and scoring systems will be helpful in recognizing the renal dysfunction. Noninvasive biomarkers (NGAL, sCysC) reflect the prospective method in identifying kidney damage and kidney function. The acute renal function is proposed to be assessed with modified acute kidney injury network (AKIN) and the baseline renal function in stable patients with MDRD-6 formula or chronic kidney disease epidemiology collaboration Cys C-Cr equation. MBRS score or RIFLE criteria for AKI evaluation should be tried in critically ill cirrhotic patients, while in candidates for transplantation, glomerular filtration rate should be preferably measured with exogenous markers for accurate assessment of renal function. Amelioration of the underlined liver disease is very impressive in patients with alcoholic liver disease after recovery from

alcoholic hepatitis, and in patients with decompensated cirrhosis due to hepatitis B virus infection after receiving antiviral therapy.

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INTRODUCTION

Physicians involved in the care of patients with cirrhosis recognize that the development of renal dysfunction is associated with significant morbidity and mortality^[1-3]. Methods for early and accurate diagnosis of acute renal failure may assist initiate specific treatment at earlier stage and improve the outcome. Patients with cirrhosis can develop three main forms of acute renal failure and may suffer also from underline chronic kidney disease. Prerenal azotemia is the basis of acute renal injury, which can trigger hepatorenal syndrome type 1 (HRS-type 1) and evolve to acute tubular necrosis - according to the degree of splanchnic vasodilation/renal hypoperfusion and the reduced cardiac output^[4]. HRS type-1 is a prevalently functional disease observed in patients with decompensated cirrhosis, which might remain in a chronic form with less severe renal impairment (HRS-type 2), or progress to acute tubular necrosis^[5-7] and exaggerate systemic inflammatory response resulting in multiorgan failure^[8]. Recently, patients with cirrhosis who have decreased renal plasma flow with normal or low/normal glomerular filtration rate (GFR) before to develop HRS were defined to be in "Pre-HRS" renal disease^[6]. Moreover, the term 'Hepatorenal Disorders' has been proposed to group all forms of kidney disease in patients with cirrhosis so as to describe their prognosis and to assist treatment decisions^[9]. However, in the majority of patients, HRS type-1 still remains a terminal condition of advanced liver disease requiring coordinated affords in the field of diagnosis, pathophysiology and treatment. In this paper, we are going to address the current knowledge on the evaluation and management of acute and chronic kidney failure presented on patients with cirrhosis. All the suggested directions highlight, to the best extent possible, the bibliographic studies, the expert opinions and recommendations.

BASELINE DIRECTIONS FOR ASSESSMENT OF KIDNEY INJURY IN PATIENTS WITH CIRRHOSIS

The appropriate clinical, biochemical and radiological markers with proven sensitivity for the diagnosis of renal disease in patients with cirrhosis have not been established yet. There are only recommendations for the unique form of kidney injury in patients with cirrhosis,

the HRS (Table 1). Renal pathology in patients with cirrhosis includes not only functional abnormalities (developed as a result of changes in hemodynamics, in renal auto-regulation and cardiac dysfunction) but structural abnormalities as well^[5].

Physicians caring for patients with cirrhosis should recognize the acute or chronic character of renal disease; the causes of renal injury; the clinical conditions leading concomitantly to acute kidney injury (AKI) and liver dysfunction, and the prognostic factors associated with the progression of AKI. Hypovolemia (due to diuretics, hemorrhage, diarrhoea), acute tubular necrosis, sepsis, nephrotoxic agents (such as nonsteroidal antiinflammatory drugs, aminoglycosides radiological contrasts) and hepatorenal syndrome-type 1 are the most common causes of AKI in cirrhotic patients^[4]. It is underlined that type-1 HRS is considered a specific form of AKI^[9]. The chronic causes include hepatorenal syndrome-type 2, glomerulonephritis due to hepatitis C virus and hepatitis B virus infection, IgA nephropathy mainly presenting in patients with alcoholic cirrhosis and diabetic nephropathy mainly combined with non alcoholic steatohepatitis^[4]. The situations which may worsen the renal and liver function at the same time might be autoimmune diseases, granulomatous diseases, autosomal dominant polycystic kidney disease, shock, pregnancy induced liver disease and drugs (aspirin, NSAIDs and angiotensin converting enzyme inhibitors^[4,10,11]). Ultimately, factors associated with the progression of AKI were the hepatic encephalopathy, severe liver and circulatory failure, chronic kidney disease (CKD), low serum sodium concentration and high leukocyte count^[12]. This knowledge should be in hand when time for assessment of patients with cirrhosis comes.

In general, differentiation of the main causes of AKI, prerenal "Pre-HRS", HRS and acute tubular necrosis presents great influence on therapeutic decisions and patients' prognosis. An easily applicable algorithm proposed by Angeli *et al*^[5], offer great assistance in clarification of the cause of the AKI in patients with cirrhosis. When there is history of dehydration, excessive use of diuretics and bacterial infection on the ground of normal urinary sediment, proteinuria below 0.5 g daily and normal renal ultrasound, the diuretics should be withdrawn and plasma volume expansion should be tried with albumin. If the patient responds to treatment the diagnosis is prerenal. If the patient does not respond, the correct diagnosis is HRS. In case there is recent use of nephrotoxic agents or contrast media and examination shows shock, granular cast in urinary sediment and proteinuria above 0.5 g daily, acute tubular necrosis is the prominent diagnosis. Furthermore, physicians should take into account that one form may convert into another thus HRS may develop on patient with chronic renal disease or evolve in time^[4,5].

Moreover, the stage of liver disease will provide considerable hints for the evaluation of kidney injury. At the beginning of cirrhosis splanchnic vasodilatation is masked by increased cardiac output thus glomerular filtration rate (GFR) is increased^[13]. Patients with ascites present severe impairment of renal blood flow^[14] and considerable

Table 1 International Ascites Club definition and diagnostic criteria for hepatorenal syndrome^[7,116]

1996 criteria	
Major criteria	
Chronic or acute liver disease with advanced hepatic failure and portal hypertension	
Serum creatinine > 1.5 mg/dL or 24-h creatinine clearance of < 40 mL/min	
Absence of shock, ongoing bacterial infection, and current or recent treatment with nephrotoxic drugs. Absence of gastrointestinal fluid losses (repeated vomiting or intense diarrhea) or renal fluid losses	
No sustained improvement in renal function defined as a decrease in serum creatinine to < 1.5 mg/dL or increase in creatinine clearance to 40 mL/min or more following diuretic withdrawal and expansion of plasma volume with 1.5 L of isotonic saline	
Proteinuria < 500 mg/dL and no ultrasonographic evidence of obstructive uropathy or parenchymal renal disease	
Minor criteria	
Urine volume < 500 mL/d	Urine osmolality > plasma osmolality
Urine sodium < 10 mEq/L	Urine red blood cells < 50 per high power field

fluctuation of serum creatinine (sCr)^[15]. Wide variations may be observed, in regards to volume paracentesis and volume expansion^[15]. Patients with advanced liver disease and high bilirubin show overestimation of GFR if evaluation of renal function is based on sCr, since significant interaction may be observed between serum bilirubin and sCr^[16,17]. Cirrhotic patients admitted to intensive care unit (ICU) have high mortality rates and may present separate predictors and scoring systems for hospital mortality^[18-20]. Emphasis should be given to accurate assessment of renal function in candidates for liver transplantation^[21].

The best method for renal function assessment in patients with cirrhosis is the clearance of exogenous markers such as iothalamate, 51Cr-EDTA and inulin^[15]. However, its application is limited by the cost and complexity while other equivalent methods for estimating the GFR in patients with cirrhosis have not been established^[15]. sCr still remains the key biomarker for the diagnosis of AKI in patients with cirrhosis. Despite all sCr limitations, there have not been detected other widely available and superior serum markers for assessing renal function and predicting outcome in patients with cirrhosis^[15]. sCr is still the most practical serum marker for estimation of renal function in cirrhotic patients, it consists the basis of existing definitions of AKI and it is included in the Model for End-Stage Liver Disease (MELD) score {MELD = 3.8 [Ln serum bilirubin (mg/dL)] + 11.2 (Ln INR) + 9.6 [Ln serum creatinine (mg/dL)] + 6.4}, which is used to allocate patients for liver transplantation^[22]. Nevertheless, sCr should be interpreted with caution, since there is no universal standardized creatinine assay; there are inter-laboratory variations, interactions with bilirubin and great influence by numerous non-renal factors such as body weight, race, age, gender^[23-25]. Moreover, sCr within the normal ranges does not exclude significant renal impairment in patients with cirrhosis^[26] as it overestimates renal function due to decreased creatine production by liver malnutrition and muscle wasting^[27].

RECENT KNOWLEDGE ON EVALUATION OF RENAL DYSFUNCTION IN PATIENTS WITH CIRRHOSIS

So far, the most widely used criterion for the diagnosis

of acute renal failure in patients with cirrhosis is the sCr level ≥ 1.5 mg/dL (133 μ mol/L) (conventional criteria). A propose for the improvement on the current classification of acute renal dysfunction in cirrhosis is the diagnostic criteria developed by the Acute Kidney Injury Network (AKIN)^[28] (Table 2). This is a consensus definition for acute kidney injury (AKI), a new term for acute renal failure, in order to be identified earlier patients with worse prognosis. According to AKIN criteria, AKI is defined as an increase in sCr level ≥ 0.3 mg/dL (≥ 26.4 μ mol/L) or $\geq 150\%$ (1.5 fold from baseline) within 48 h from the first measurement or a urine output of less than 0.5 mL/kg per hour for more than 6 h^[28-30] and is divided in three stages. AKIN criteria in cirrhotic patients have been validated with six prospective clinical trials^[8,12,20,31-33]. The patient population in the five studies included hospitalized cirrhotic patients with or without ascites^[8,12,31-33], while in one study patients with cirrhosis were admitted in ICU^[20]. All studies concluded that AKIN criteria accurately predicted in-hospital mortality, length of hospital stay and organ failure. However, when AKIN criteria compared to conventional criteria, they were not found to be superior^[33]. The authors of this study noted that the addition of either the progression of AKIN stage or the cut off sCr ≥ 1.5 mg/dL to the AKIN improved their prognostic accuracy^[33]. A step forward in this evaluation was made by Fagundes *et al*^[12] who proposed modified cirrhosis-AKI classification and validated it in 375 consecutive patients hospitalized for complications of cirrhosis. Patients with cirrhosis were categorized into three groups: (1) patients with AKI stage 1 and peak of sCr ≤ 1.5 mg/dL; (2) Patients with AKI stage 1 and peak of sCr > 1.5 mg/dL; and (3) patients with AKI stage 2 or 3. By applying this modified classification a better risk stratification for patients with cirrhosis was achieved considering also the cause of AKI.

Serum Cystatin C (CysC) is another marker for evaluation of acute renal dysfunction preferably in female patients with progressive cirrhosis^[34]. It has been shown that in this cirrhotic population (women with cirrhosis Child - Pugh score C)^[34]. CysC presented high diagnostic sensitivity, greater than sCr in detection of acute renal impairment^[34,35]. Indeed it was proved that CysC correlated with the severity of liver fibrosis and with the GFR better than sCr^[35-37], but this has not been confirmed in other studies^[38].

Table 2 Acute kidney injury network and risk, injury, failure, loss, and end stage criteria for the diagnosis of acute kidney injury^[117]

AKIN criteria	Urine output		RIFLE criteria
Serum creatinine	(common to both AKIN and RIFLE)	Class	Serum creatinine or GFR
Stage 1 Increase of more than or equal to 0.3 mg/dL ($\geq$ 26.5 μ mol/L) or increase to more than or equal to 150% to 199% (1.5- to 1.9-fold) from baseline	Less than 0.5 mL/kg per hour for more than 6 h	Risk	Increase in serum creatinine $\times$ 1.5 or GFR decrease $>$ 25%
Stage 2 Increased to more than 200% to 300% ($\geq$ 2- to 2.9-fold) from baseline	Less than 0.5 mL/kg per hour for more than 12 h	Injury	Serum creatinine $\times$ 2 or GFR decreased $>$ 50%
Stage 3 Increased to more than 300% ($\geq$ 3-fold) from baseline, or more than or equal to 4.0 mg/dL ($\geq$ 354 μ mol/L) with an acute increase of at least 0.5 mg/dL (44 μ mol/L) or on RRT	Less than 0.3 mL/kg per hour for 24 h or anuria for 12 h	Failure	Serum creatinine $\times$ 3, or serum creatinine $>$ 4 mg/dL ($>$ 354 μ mol/L) with an acute rise $>$ 0.5 mg/dL ($>$ 44 μ mol/L) or GFR decreased $>$ 75%
		Loss	Persistent acute renal failure = complete loss of kidney function $>$ 4 wk
		End-stage kidney disease	ESRD $>$ 3 mo

For conversion of creatinine expressed in SI units to mg/dL, divide by 88.4. For both AKIN stage and RIFLE criteria, only one criterion (creatinine rise or urine output decline) needs to be fulfilled. Class is based on the worst of either GFR or urine output criteria. GFR decrease is calculated from the increase in serum creatinine above baseline. For AKIN, the increase in creatinine must occur in $<$ 48 h. For RIFLE, AKI should be both abrupt (within 1–7 d) and sustained (more than 24 h). AKI: Acute kidney injury; AKIN: Acute Kidney Injury Network; ESRD: End-stage renal disease; GFR: Glomerular filtration rate; RIFLE: Risk, injury, failure, loss, and end stage; RRT: Renal replacement therapy.

Promising information for acute kidney dysfunction in cirrhotic could be also derived from urine. A novel kidney biomarker associated with early detection of acute tubular injury is neutrophil gelatinase -associated lipocalin (NGAL) measured in blood and in urine. Many studies in several clinical situations^[39–41] have underlined that the NGAL increased two hours after the induction of AKI, before of the sCr elevation. In cirrhotic patients, preliminary studies have reported that NGAL levels were higher in those with HRS^[42] compared to those without renal disease; NGAL was associated with the prediction of short-term mortality^[43,44] and it could be used for differentiation of prerenal azotemia, acute tubular necrosis and HRS^[45]. Urinary NGAL has been found to be 20 ng/mL in healthy population and in prerenal azotemia, 105 ng/mL in HRS, 325 ng/mL in AKI and 50 ng/mL in CKD^[44]. Furthermore, another powerful tool in renal disease detection could be the ratio of urinary sodium to potassium. If that ratio in a random urine sample of patients with decompensated cirrhosis and ascites is less than 1 the diagnosis of renal dysfunction (GFR $<$ 60 mL/min) is possible^[46]. Nevertheless all these findings require confirmation in additional studies.

Ultimately, encouraging method for early acute detection of renal hemodynamic disturbances of patients with cirrhosis showed the measurement of renal resistive index (RI) by renal duplex doppler ultrasound. In general, a RI more the 0.7 is indicative of renal failure, confirming high blood velocity waveform of renal artery and high peripheral arterial resistance^[47]. In patients with cirrhosis RI over 0.7 has been predictor of renal dysfunction and HRS^[48,49] and it has correlated significantly with MELD score, MELD-Na score, sCr and hyponatremia as well^[50].

In addition, it might demonstrate the progress of renal disease since it reached its highest levels in patients with refractory ascites compared with patients with compensated cirrhosis and those with diuretic responsive ascites^[48]. Future research is needed to elucidate RI role in this patient population.

In regards to the evaluation of CKD, the Kidney Disease Outcomes Quality Initiative (KDOQI) Clinical Practice Guidelines have been suggested^[9]. According to KDOQI^[51], CKD is defined as a GFR of less than 60 mL/min for more than three months, calculated using the modified diet in renal disease (MDRD)-6 formula (Appendix 4) supporting its potential usefulness in the decision making for simultaneous liver and kidney transplantation. This hypothesis has been tested in patients with stable cirrhosis in the study of Francoz *et al.*^[52]. They showed that MDRD-6 formula was superior to MDRD-4 and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formulas identifying stable cirrhotic patients with markedly impaired renal function, including those with ascites (Table 3). However, MDRD-6 formula underestimated renal function in patients with GFR more than 30 mL/min subjecting them to possible unnecessary combined kidney and liver transplantation. Recently, CKD-EPI Cys C-Cr equation was shown to be the most accurate GFR-estimating formula compared to sCr or CysC-based formulas in cirrhosis. This formula was proposed to evaluate non AKI in cirrhosis until a brand, radical and specific for this population equation is discovered^[53].

Accurate evaluation of renal function in cirrhotic patients, who are candidates for liver transplantation (LT) is crucial. Kidney disease is the key factor for determination of transplant status and highly affects the choice

Table 3 Formulas for estimating the glomerular filtration rate: modified diet in renal disease-4, modified diet in renal disease-6, chronic kidney disease epidemiology collaboration (mL/min per 1.73 m²)^[118-120]

MDRD-4 formula (1)	$186 \times [\text{creatinine (mg/dL)}]^{-1.154} \times [\text{age (yr)}]^{0.203} \times (0.742 \text{ if patient is female}) \times (1.21 \text{ if patient is black})$
MDRD-6 formula (2)	$170 \times \text{sCr (mg/dL)}^{-0.999} \times \text{age}^{-0.176} \times 1.180 \text{ (if black)} \times 0.762 \text{ (if female)} \times \text{serum urea nitrogen}^{-0.170} \times \text{albumin}^{0.138}$
CKD-EPI equation (3)	$141 \times \min(\text{sCr}/\kappa, 1)^\alpha \times \max(\text{sCr}/\kappa, 1)^{-1.209} \times 0.993 \text{ Age} \times 1.018 \text{ (if female)} \times 1.159 \text{ (if black)}$

MDRD: Modified diet in renal disease; CKD-EPI: Chronic kidney disease epidemiology collaboration.

of simultaneous kidney and renal transplantation, the initial immunosuppression and the survival of these patients^[54-56]. GFR, sCr and serum sodium have been recognized as independent predictors of mortality in patients with decompensated cirrhosis^[57,58]. In this case estimation of GFR should be made accurately by exogenous filtration markers. Particularly for patients with established HRS, a modification of MELD calculation has been proposed, to obtain patients with HRS the right priority in the waiting list, concerning that therapy can reduce their baseline MELD score^[59]. According to this modification, the baseline MELD score before starting therapy should be used in patients with HRS who have been stabilized with therapy; the MELD score considering the pharmacological treatment as dialysis should be applied in patients with continuous recurrence of HRS and the highest MELD-Na over time should be received in patients with repeated recurrence of HRS type-2. Renal biopsy is advisable if GFR is between 30-60 mL/min and there are signs of parenchymal renal disease -hematuria (more than 50 red cells per high power field), proteinuria > 0.5 g/daily and chronic renal abnormalities on the ground of comorbidities such as diabetes mellitus, hypertension and viral infection^[15]. The detection of potential reversible renal disease and vascular lesions- hazardous for calcineurin-inhibitors nephrotoxicity- may be of value for the management before and after transplantation.

Kidney failure at admission or during ICU stay is a crude predictor of mortality in critically ill patients with cirrhosis. Despite supportive treatment measures, mortality was high and the risk for death was multiplied with the increasing severity of the kidney disease^[1,60]. In this patient population, RIFLE classification presents the best predictive ability for ICU and hospital mortality^[18,19]. The RIFLE denomination is an acronym which refers to risk (risk of renal dysfunction); injury (injury or damage to the kidney); failure (renal failure); loss (loss of kidney function); end (end stage renal disease) (Table 2). It was entered by Acute Dialysis Quality Initiative (ADQI) as an attempt to standardize the definition of acute renal failure and to describe the severity of AKI^[61]. It allows the evaluation of the progression of renal injury as AKI is a dynamic process^[62]. However, RIFLE score lack of a uniform approach in a patient population presenting with multiorgan failure, since it is focused only on kidney pathology. In keeping with this, a new score (MBRS) has been introduced combining four parameters: mean arterial pressure, bilirubin, respiratory failure and sepsis displayed an excellent area under the receiver operating characteristic curve (0.898 ± 0.031) for prognosis of mortality. This

tool has been applied in a total of 301 critically ill cirrhotic patients^[63,64] and proved that is an accurate, handy, user-friendly and low-cost scoring system. If it is above 2, cirrhotic patients should be prioritized for LT^[64].

Overall, when it comes to evaluate renal function in patients with cirrhosis determination of AKI, CKD or AKI on CKD should be made. HRS diagnosis is the first which should be excluded by the algorithm of Angeli *et al*^[5]. The acute renal function is proposed to be assessed with modified AKIN and the baseline renal function in stable patients with MDRD-6 formula or CKD-EPI Cys C-Cr equation. MBRS score or RIFLE criteria for AKI evaluation should be tried in critically ill cirrhotic patients, while in candidates for transplantation, GFR should be preferably measured with exogenous markers for accurate assessment of renal function. Serial plasma measurements with delayed sampling to allow equilibrium between plasma and intracellular space, especially ascitic fluid would give a more precise GFR^[65]. All cirrhotic patients should undergo renal ultrasound for measurement of RI, in every stage of liver dysfunction and urine microscopy for differentiation of all causes of AKI. Renal biopsy should be performed when GFR is between 30-60 mL/min and there are signs of parenchymal renal disease (Table 4).

INACCURACIES OF RENAL ASSESSMENT STRATEGIES IN PATIENTS WITH CIRRHOSIS

Regarding the AKIN criteria, the urine volume cannot be applied in patients with cirrhosis since it may be markedly biased. Errors in the timing and the complete of urine collection are very common. Moreover, AKIN overestimate mortality, because they detect earlier patients with worse prognosis^[32]. Since sCr cannot be removed from clinical practice, physicians should use it with caution in patients with advanced cirrhosis. The inadequacies of sCr are more pronounced in this patient group, due to high bilirubin and refractory ascites. The establishment of creatinine levels with enzymatic assays partially overcame this problem, but there are more expensive^[66]. Similarly, none of the creatinine-based mathematical equations are precise acute markers for renal function evaluation in cirrhosis^[67]. The body weight cannot be accurately estimated on the ground of ascites and edema, and there is disproportional high creatinine secretion from the tubules in regards to the level of creatinine filtered by the glomerulus^[23]. Similarly, evidence has not clarified whether sCysC offers clear advantage comparing to sCr in all cirrhotic patients,

Table 4 Recommendations for renal function evaluation in subgroups of patients with cirrhosis

Differentiate prerenal kidney disease, hepatorenal syndrome and acute tubular necrosis	Angeli <i>et al</i> ^[5] algorithm
Acute kidney injury	Modified cirrhosis–acute kidney injury classification sCr increase ≥ 0.3 mg/dL (≥ 26.4 μ mol/L) or more than 150% (1.5 fold from baseline) within 48 h from the first measurement ^[12]
Chronic kidney disease	KDOQI ^[49] guidelines Glomerular filtration rate below 60 mL/min for more than three months, calculated using the modified diet in renal disease-6 formula chronic kidney disease epidemiology collaboration Cys C-Cr equation ^[51]
Critically ill cirrhotic patients	RIFLE score ^[18,19] MBRS score ^[61,62] combining mean arterial pressure, bilirubin, respiratory failure and sepsis
Candidates for liver transplantation	Exogenous filtration markers If there is suspicion for parenchymal disease and Glomerular filtration rate is between 30-60 mL/min consider renal biopsy
Advanced cirrhosis	Cystatin C
Difficulties in differentiation of acute tubular necrosis	NGAL
All patients with cirrhosis in every stage of liver disease	Renal resistive index estimation by renal duplex doppler ultrasound

KDOQI: Kidney disease outcomes quality initiative.

neither improve the predictive power of MELD score^[68]. sCysC may also be influenced by body composition, abnormal thyroid function, systemic inflammation and corticosteroid use, while its assay although easy applicable is of high cost^[41]. In parallel substitution of sCr by sCysC did not improve the prognostic ability of MELD-score and creatinine-based equations^[38,68]. Estimating GFR with the gold standard measures is the method of choice, but in every day routine is expensive, time-consuming, radioactivity transmitter and fatiguing^[21]. Ultimately, renal biopsy is not easily applicable to patients with cirrhosis. Coagulation disorders are common in cirrhotic and the prolonged INR predispose to high risk of hemorrhages. In this situation, transjugular route is preferable than the percutaneous route, since it has been proved equivalent efficient^[69]. Contraindications for biopsy are small size kidneys, large volume ascites and poor cortical differentiation^[15].

MANAGEMENT OF RENAL FUNCTION IN PATIENTS WITH CIRRHOSIS

Research has made an enormous progress by finding treatment directions for HRS, which was previously fatal within a few days or weeks. However, no guidelines have been established for the treatment of patients with cirrhosis and kidney disease. Management options should be based on expert recommendations^[9], proposed algorithms^[33] and knowledge of the nature of renal disease^[6,8]. It is essential to recognize early AKI - mainly diagnosis of HRS, which should be detected within 48 h, following the currently accepted guidelines^[10,59] (Table 1) - to determine the chronic damage of the kidneys and to take the best measures for improving hepatic function. Patients with renal disease due to HRS, have much worse prognosis compared to patients with parenchymal renal disease^[59]. Amelioration of the underlined liver disease is very impressive in patients with alcoholic liver disease after recovery from alcoholic hepatitis, therapy with ba-

clofen^[70] and in patients with decompensated cirrhosis due to hepatitis B virus infection after receiving antiviral therapy^[71-73]. The choice of therapy depend upon the experience of the medical centre, the availability of certain drugs, the unit in which patient is admitted (ICU or not ICU) and whether the patient is a candidate for LV.

First line treatment

First line treatment should aim at the elimination of the potential pathophysiological factors resulting on HRS. Hemodynamic monitoring and management of fluid balance is essential for preventing the relative renal hypoperfusion, maintaining effective circulatory volume and renal perfusion pressure. Traditional measures of intravascular volume evaluation such as right atrial and pulmonary artery pressures are not considered inadequate for this patient group, so continuous central venous pressures and serial indirect or/and direct measurements of cardiac indices are preferable^[9]. The current classification systems are helpful in early recognition of AKI indices and therefore withdrawing the potential causes of renal injury. Patients with AKIN stage 1 and sCr ≥ 1.5 or initial AKIN stage > 1 should be at close monitoring and receive therapeutic measures for maximum two days^[33]. These involve nephrotoxic medications-antibiotics and analgetics-, gastrointestinal bleeding and diuretics, which exacerbate hypovolemia and trigger sympathetic and renin-angiotensin-aldosterone system (RAAS). High level of suspicion is needed regarding the spontaneous bacterial peritonitis since infections very common trigger HRS. Moreover, albumin infusions will correct hypoalbuminemia and partial ascites evacuation will alleviate circulation^[33,74]. In the setting of alcohol-related cirrhosis and ascites, the intestinal decontamination with rifaximin may also improve systemic hemodynamics and renal function^[75]. If the clinical condition of congested patients (the groups previously mentioned) does not improve within two days, differential diagnosis with HRS should be done

Table 5 Recommendations for management of patients with cirrhosis

First line therapy	
Recognize and withdraw all causes of acute kidney disease	
Resolve primary liver disease	
Encounter hypoalbuminemia with albumin infusion and tension ascites with repeated paracentesis plus albumin	
Have a high level of suspicion and treat spontaneous bacterial peritonitis	
Be vigilant and have into close monitoring patients with acute kidney injury network stage 1 and sCr > 1.5 mg/dL (133 μmol/L) or initial acute kidney injury network stage > 1	
If there is no improvement within 2 d, proceed to specific treatment measures	
Second line therapy	
Patients hospitalized at the ward	If the diagnosis of hepatorenal syndrome has been placed: Give albumin and terlipressin in continuous infusion If there is improvement within 4 d continue with oral midodrine When terlipressin is unavailable: Give midodrine plus octreotide plus albumin Norepinephrine plus albumin
Patients admitted to intensive care unit	
Third line therapy	
Patients who qualify for transplant	Consider liver or simultaneous liver kidney transplantation
	Give therapeutic bridges - Dialysis, transjugular intrahepatic portosystemic shunt
Patients who do not qualify for transplant	Continue the combination of terlipressin plus albumin Dialysis, TIPS

and other specific regimens are required^[5] (Table 5).

Second line treatment

Second line therapy encompasses measures undertaken after posing the diagnosis of HRS. The supportive measures are directed mainly into portal hypertension and arterial vasodilatation reversal. Albumin effusion combined with vasoconstrictors is the basic therapy for effective management of hypovolemia^[5,9]. The main effect of albumin is the oncotic pressure increase resulting in volume expansion. However, albumin shows additional effects which make it extremely beneficial for patients with HRS. It shows metabolic, immune and vasoconstrictor effects, through binding of endotoxin, nitric oxide, bilirubin, bile acid and fatty acids^[76,77] and improves cardiac output, through improvement of cardiac contractility, cardiac preload and volume expansion^[78,79]. On the other side, terlipressin is an agonist of renal vasopressin V2 receptors, which reduce splanchnic vasodilatation, increase the MAP and reduce the nitric oxide synthesis during sepsis^[80]. The combination of them leads to renal function normalization in 34%-65% of cases^[81,82], extends the number of patients undergoing LT^[83], additionally improving their outcome^[84] and it increases short-term survival by 34%-43%^[82,85,86]; while it is hypothesized that ameliorates also tubular damage^[5]. They have been applied in a special protocol which has shown efficacy in 59% of cases^[87] and its discontinuation has been followed by HRS recurrence in 15%-22%^[82,85,86,88-91]. The protocol has been proposed to be administered until there are signs of improvement, but not more than two weeks. The decrease of sCr <1.5 mg/dL (133 μmol/L) or the decrease of sCr > 50% but ≥ 1.5 mg/dL (133 μmol/L), the decrease of bilirubin < 10mg/dL and the elevation of MAP ≥ 5 mmHg at day 3 of treatment are the predictors of response^[87,92,93]. If patient respond, some centers continue therapy with midodrine (an oral α1-adrenergic agonist with vasoconstrictive prop-

erties) indefinitely to keep higher MAP and to compensate refractory ascites^[94]. If there is no improvement in renal function after two weeks, the protocol maybe repeated -there have been reports for protocol administration up to eight months^[9,74,95,96] - or other interventional options are applied regarding the patient status and the available treatment options of the centre. Moreover, changes on terlipressin administration modality (given as continuous infusion instead of *iv* pulses) accounted for enhancement of its efficacy^[5,97] (Table 6).

In some cases terlipressin is not applicable. These are when there are contraindications of its use, when there is not available and when the patient is admitted on ICU. In general, the contraindications of terlipressin use are ischemic cardiovascular disease, heart failure, arrhythmias, asthma, respiratory failure and heavy hyponatremia^[4]. Terlipressin use is limited in some countries because of its high cost and the lack of randomized trials proving superiority of terlipressin in comparison to other vasoconstrictors. When patients are admitted to ICU they usually treated with terlipressin^[76,98-100] in patients being in ICU and because the cost of norepinephrine therapy is three times less than the cost of terlipressin^[100]. Norepinephrine is difficult to be administered in the ward since it requires continuous intravenous infusion and hemodynamic monitoring, so instead of terlipressin, other vasoconstrictors maybe used in combination with albumin. These are octreotide, a synthetic analog of somatostatin and midodrine. However, the effect of octreotide, either used alone or with albumin, does not appear to be beneficial for renal function improvement^[99,101] and midodrine alone or in combination with albumin has not been evaluated in patients with HRS type -1. Only when octreotide was used in conjunction with midodrine and albumin has normalized renal function in 49%^[77,102,103], has increased MAP^[77] and survival^[102].

Table 6 Scheme for terlipressin and albumin administration^[5,97]

Terlipressin is given as an intravenous bolus 1 to 2 mg every four to six hours	Albumin is given for two days as an intravenous bolus 1 g/kg per day (100 g maximum) followed by 25 to 50 g/d until terlipressin therapy is discontinued
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Table 7 Published guidelines on selection criteria for simultaneous liver-kidney transplantation

Davis <i>et al</i> ^[121] , 2007
Patients with CKD with CrCl (preferentially iothalamate) of ≤ 30 mL/min for > 3 mo
Patients with AKI and/or HRS on dialysis for ≥ 6 wk
Patients with prolonged AKI with kidney biopsy showing fixed renal damage
SLK was not recommended in patients with AKI not requiring dialysis
Eason <i>et al</i> ^[122] , 2008
Patients with CKD with GFR ≤ 30 mL/min > 3 mo
Patients with AKI/HRS with sCr ≥ 2 mg/dL and on dialysis ≥ 8 wk
Patients with evidence of CKD and kidney biopsy with $> 30\%$ GS or 30% fibrosis
Other criteria that was recommended to be considered: Presence of co-morbidities: Diabetes, Hypertension, age > 65 yr, renal size and duration of sCr > 2 mg/dL
Nadim <i>et al</i> ^[123] , 2012
Persistent AKI ≥ 4 wk with one of the following:
Increase Scr ≥ 3 -fold from baseline or on dialysis
GFR ≤ 35 mL/min (MDRD-6) or ≤ 25 mL/min (iothalamate)
CKD ≥ 3 mo with one of the following:
eGFR ≤ 40 mL/min (MDRD-6) or ≤ 30 mL/min (iothalamate)
Proteinuria ≥ 2 g/d
Kidney biopsy showing $> 30\%$ GS or $> 30\%$ interstitial fibrosis
Note: Higher GFR threshold with MDRD-6 was to account for the approximate 30%-40% overestimation that has been described when compared to iothalamate.

CKD: Chronic kidney disease; CrCl: Creatinine clearance; HRS: Hepatorenal syndrome; AKI: Acute kidney injury; SLK: Simultaneous Liver-Kidney; sCr: Serum creatinine; GFR: Glomerular filtration rate; GS: Glomerulosclerosis; MDRD-6: Modification of diet in renal disease formula calculated using six variables of serum creatinine, serum urea, serum albumin, age, gender.

Third line treatment

When pharmacological measures are insufficient, transplantation is the treatment of choice^[8]. MELD score permits selection of patients needing liver transplant, while patients who are at risk for not -recovery of renal function simultaneous kidney and liver transplant is required^[9]. In the direction of combined liver and kidney transplantation leads the duration of HRS (more than four weeks), AKI on CKD, and baseline diseases (such as hypertension, diabetes and obesity) which predispose to kidney disease progression (Table 7). If the patient meets the requirements to be listed for transplant, dialysis and transjugular intrahepatic portosystemic shunt (TIPS) are the bridging therapies to keep the transplant candidate in the best clinical status. It is essential to resolve HRS since it is associated with many perioperative complications and decreases patient survival.

In general, dialysis procedures have not improved the long -term survival in patients with HRS and they have been associated with high risk of blood pressure decline, hypothermia, bradycardia, tissue hypoxia and clotting^[4]. That is why they are applied under special situations, when there are indications for reversibility of AKI, hyperkalemia, hypervolemia not responding to diuretics, severe metabolic acidosis, acute on chronic liver failure and fulminant liver failure^[9,104,105]. The choice of modality [continuous renal replacement therapy, intermittent hemodialysis, Molecular Adsorbent Recirculating System (MARS)] depends on the abilities and the experience of

the centre, while non standard anticoagulation measures are indicated. Schemes with saline flushing, minimal dose of heparin or minimal dose of citrates are preferable. Peritoneal dialysis may be another option to remove ascites and resolve cirrhosis complications, such as encephalopathy, without exposing patient to anticoagulation and to other dialysis complications^[106,107].

TIPS is an intervention that enhances the return of blood in the right heart and resolve the reduced sympathetic and RAAS activity in HRS type II, suggesting an improvement in systematic hemodynamics^[108-110]. It is indicated in cirrhotic patients with refractory ascites requiring repeated paracentesis^[109-112] because it has conferred positive impact on ascites and renal function amelioration. Nevertheless, it has not improved significantly mortality^[113]. Furthermore, renal function improvement does not come fast, it comes after weeks or months^[114], so very ill patients, without significant liver function reserve (INR > 2 , bilirubin > 5 mg/dL or Child Plug > 11), hepatic encephalopathy and cardiopulmonary disease^[9] should not undergo it. Complications of TIPS procedure are high rates of encephalopathy, liver insufficiency, cardiac failure, infection of the stent and hemolysis^[111,115]. In patients with HRS 1, preliminary studies^[108,111] about TIPS showed improvement of renal function in parallel with survival, but it cannot be applied in clinical practice yet as a main treatment. At present, TIPS can be used in selected patients without severe liver dysfunction as a bridge for LT or in patients with stabilized liver function not enlisted, as a long term -therapy^[74] (Table 5).

CONCLUSION

Patients with cirrhosis and renal failure are high-risk patients who can hardly be grouped to form precise instructions for diagnosis and treatment. AKI is a portentous manifestation of circulatory dysfunction on patients with cirrhosis, which has a detrimental impact on their recovery and survival. Close surveillance, well-classified definitions and scoring systems (AKIN, RIFLE) aim in early recognition of renal disease. Attempts are made to correlate non-invasive biomarkers of kidney damage and kidney function (NGAL, sCysC) to pathological findings. Studies on better using pharmacological and interventional measures are underway promising better and quick recovery. Physicians should be updated on new therapeutic modalities, proposed recommendations and algorithms in order to translate them into clinical practice.

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Inflammatory bowel diseases: Current problems and future tasks

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Abstract

Current knowledge on inflammatory bowel disease (IBD) is mainly endorsed by controlled trials and epidemiologic studies. Yet, we seldom look at the messages from real-world practice. Among a patient population followed since 2008, we looked at an unselected sample of 64 IBD patients [26 Crohn's disease (CD) and 38 ulcerative colitis (UC)] who had been seen as out-patients in the last year. Inducing remission, mesalamines (86% for UC/69% for CD/33%-16% as MMX formulation) prevailed as prescriptions; steroids (55%/19% for UC/CD) ranked second. Prescription of third-party drugs (antibiotics, NSAIDs, biologics) and adherence, were issues in the maintenance. 34% of CD, and 23% of UC patients showed accompanying immunologic diseases: CD-associated familial psoriasis (4:9) ranked first. Main Message. The association between IBD (CD mainly) and psoriasis, now found in our practice, matches current basic science gathering IBD together with psoriasis (and perhaps chronic respiratory disease) under the comprehensive term "barrier organ disease" wherein an epithelial surface with sensor systems rules contacts between outer antigens and a reactive underneath tissue, with the balance between inflammation and quiescence kept at any time by mucosal permeability. IBD is thus viewed as a polyfactorial/polygenic/syndromic

disorder, embedded into a galaxy of immune conditions offering multiple points of attack. This mindset of splitting the IBDs into pathogenic categories may allow overcoming the uniformly targeting of a single cytokine by biological drugs, in favor of demarcating the boundaries between different disease-subtype-specific indications, and paving the way to future personalized strategies.

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Key words: Inflammatory bowel disease; Immunopharmacology; Barrier organs; Future trends in inflammatory bowel disease; Microbiome

Core tip: Long after their description, ulcerative colitis and Crohn's disease (IBD) are still treated but not cured. This somber spell has now begun to be broken by genetic discoveries and by the study of the human microbiome. The former have uncovered hundreds of genetic variants lending support to the clinical hint that IBD is a syndrome encompassing discrete polymorphisms of the immune response pathways, each requiring a personalized approach. The latter has shown the microbiome to be a cell universe which, if disrupted, can provoke IBD together with a myriad of disturbances apparently unrelated with the gut. A frame of mind seeing the IBDS as embedded into a plethora of genetically linked immune disturbances must fuel IBD research from now on.

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STUDY SETTING AND SCOPE OF THE ANALYSIS

Supported by the Italian Health System, in 2008 Grad-

Table 1 Contains patients' demographics and disease characteristics

UC (38, 24 m)		CD(26, 16 m)	
Age, yr	Extension	Age, yr	Extension
18-80	Proctitis, 12	16-73	Ileo-colonic 15
	Sub-total, 11		Colitis, 5
	Left, 8		Universal, 4
	Pancolitis, 6		Ileitis, 2
	IPAA, 1		

UC: Ulcerative colitis; CD: Crohn's Disease; IPAA: Ileo-pouch anal anastomosis.

Table 2 Gives the frequencies of use of the main drugs *n* (%)

Ulcerative colitis	Crohn's disease
Mesalamines 33 (86)	18 (69)
Steroids 21 (55)	5 (19)
Thiopurines 14 (36)	8 (30)
Biologics 1(2.6)	1 (3.8)

enigo Hospital has launched an out-patient service mainly devoted to patients with inflammatory bowel disease (IBD). An interim analysis of the activities of this service has already appeared in 2010^[1]. Eversince its establishment, the service has mostly been conducted by one of us (GCA), enrolling some 200 IBD patients. The scope of the present analysis was to reappraise the data under the light of modern achievements (for example the concept of "barrier organ disease"); to gain more insight into the drawbacks and the limits of traditional therapy with special regard to factors countering maintenance of remission; then, to cast a glimpse into the future of treatment approaches for IBD. We deliberately meant to not loose adherence to our daily clinical experience in this out-patient setting, when either dissecting actual difficulties or visualizing future therapeutic scenarios (personalized treatment for example). At a time when the literature is being "flooded" by a number of large epidemiologic and population studies, we chose to present the limits and the peculiarities of a study that pivots on the narrow environment of an outpatient office conducted by one physician.

STUDY POPULATION

Sixty-four IBD patients, gathered in the most recent interim analysis between 6.6.2012 and 04-24-2013 included 26 Crohn's affections (CD) and 38 ulcerative colitis (UC) cases, corresponding to some 6 IBD patients per month; overall analysis in the previous 31 months had yielded 119 IBD patients. Changes in the core storage system beginning 2010 have imposed a discontinuity in the data collection modalities, a fault that is now mended (Tables 1-3).

Managing chronic remission: open questions

Both medical and budget issues make the maintenance of

Table 3 Illustrates the distribution of the main extra-intestinal affections

	<i>n</i>	Familial	Personal
Ulcerative colitis			
Psoriasis	2	0	2
Inflammatory bowel disease	3	3	0
Asthma	2	1	1
Rheumatoid Arthritis	2	0	2
Crohn's disease			
Psoriasis	4	3	1
Inflammatory bowel disease	3	3	0
Asthma	1	1	0
Rheumatoid Arthritis	1	1	0

remission of IBD a crucial challenge. The relevant literature has particularly expanded on UC^[2]. A variegated list of factors may provoke loss of IBD remission, and we ourselves had the chance to face some of the conditions in our real-world practice. (1) lack of adherence to prescriptions, mostly mesalamine and thiopurine medications. Among the 64 patients in this report, the adherence rate for mesalamines and thiopurines was found to attain 90% and 94%, ranking high with regard to literature data^[2]; (2) unavailability of a non-replaceable drug; we had to face this event for a few patients, who, owing to their intolerance of azathioprine, were prescribed 6-mercaptopurine, at a moment when the latter had become unavailable in our country (see below); (3) toxicity of a pivotal drug (mesalamine, azathioprine). Noteworthy, based on the results of an English survey which was able to reveal only 11 alleged cases of renal damage per million prescriptions, mesalamine is listed among the most tolerated drugs^[3]. Our own present series included a rare case of mesalamine-induced cholestasis^[4,5] which responded to patient's transitioning to balsalazide. As described in various publications^[6,7], we faced a rather common azathioprine toxicity. In a population of 42 UC patients and 37 subjects with CD (females mostly) we recently found an 11% of gastric intolerance to azathioprine. Transition to 6-MP was tolerated in 6 cases which acquired disease control^[8]; (4) undermining of remission because of the introduction of third party drugs: antibiotics and NSAIDs are mostly recognized as capable to reactivate IBD or induce it *de-novo*. Indeed, analysis of our office experience has gathered convincing evidence of a role for antibiotics and/or non-steroid anti-inflammatory drugs (NSAIDs) in active episodes of IBD, requiring the consideration of prescribing physicians^[9]. A specific attention must be devoted to the Crohn's-like colitis^[10] that is not rarely found as an accompaniment to immune-mediated diseases from rheumatoid arthritis to multiple sclerosis: its inciting factors have been recognized in anti-tumor necrosis factor (TNF) formulations and/or rituximab^[11], the impact matching the rising prescription rate of these drugs. In our opinion, these observation are an indicator of the pathophysiologic and genetic commonalities linking the IBDs with their surrounding galaxy of immune disorders of which psoriasis is just the most obvious instance; and (5) the issue of

the ancillary symptoms in IBD. Likewise any other individual, IBD patients may present with bowel abnormalities being due to a plethora of factors from irritable bowel syndrome to celiac disease. Such situations must be borne in mind, in order to avoid prescribing IBD drugs for the wrong indication (so-called over-treatment)^[12].

EXOGENOUS AND ENDOGENOUS FACTORING

Among variables factoring in the management of IBD, smoking is obviously the most studied, with a detrimental action being demonstrated for CD^[13], and a protective one for UC^[14]. Sometimes overlooked in clinical practice, passive smoking must by contrast be given adequate consideration. The causative role of NSAIDs and antibiotics has already been touched on.

Genomic instability is gaining crucial importance among endogenous factors in IBD management, with excessive frequency of hematologic or immune-allergic disorders in the patient or among his/her relatives.

PROGNOSIS

The anticipation that the IBDs that are followed in an out-patient environment might be benign is sometimes contradicted by data. Beginning 2008, for example, in our series we recorded at least three fatalities, including one hematologic malignancy, and two cases of septicemia. One drop-out patient was reported with colonic malignancy from another hospital.

WRAPPING UP SUMMARY

This data were gathered from a random sample of 64 IBD patients (38 UC, 26 CD), who were followed in the last year at an out-patient unit with a 5-year service history. Proctitis was common among the UC patients; mesalamines were the most prescribed drugs, with the MMX formulation attaining 16% in CD and 33% in UC; beclomethasone prescriptions were prominent among steroids, ranking to 12 prescriptions including 9 of local formulations; remission maintenance was a significant challenge, pivoting over two main aspects: the control of third-party drugs, and maintenance of adherence.

At least two patients on biologics presented with superimposed immune disorders: a young female receiving adalimumab for diffuse CD developed psoriasis of the sculp; a young male with juvenile rheumatoid arthritis received three different anti-TNF formulations and developed UC on each of the three^[15]; switched finally to certolizumab presented with psoriasis of the elbows.

The tables hint to an association between psoriasis and rheumatoid arthritis. Such clinical evidence in our opinion launches a few messages of a theoretical and clinical impact, and in the lines to follow we shall try to gain more insight into this matter.

Modern understanding of the anatomy of the gut and

of the pathophysiology of its associated immune system all convey a concept of the IBDs as disorders pivoting on a disrupted balance between the gut mucosal immune tissue and luminal antigens, with gut microbiota as one crucially causative variable in favoring or countering the rise of an inflammatory response; the underlying dogmatic view supporting this reasoning is that while the mucosal immune system has evolved following a tolerization tune, the submucosal lymphoid tissue is highly reactive and can mount a significant inflammatory response should any antigen breach the mucosal barrier.

IBD is now thought to best be described using a concept of a “contextualized syndrome”^[16]. The basis of this concept is double: (1) a uniform curative strategy for the IBDs is yet far from reach; and (2) though often presenting with obvious clinical commonalities, in fact the IBDs do hide distinct serological or genetic subtypes that are best accounted for by a process of splitting rather than one of lumping up^[17].

The frequent observation of a co-morbidity between IBD and psoriasis, such as that observed in our office, served as one of the triggers for this frame of mind. A part of the scientific community has thus begun to conceive IBD as an archetype of “barrier organ diseases” whereby the essential ingredients are a mucosal surface endowed with sensor molecules of the outer environment (see the NOD system for example), and an underneath lymphoid tissue, this mixing being ruled in the background by an abundant metagenomic microbiota load (see below).

At least three systems with similar characteristics have nowadays been defined in human beings: the gut (chiefly the colon); the skin; and respiratory epithelia. It is not by chance that clinical experience has long highlighted that disorders of these three districts might be co-morbid. Our case series recorded hereby emphasize a coincidence between CD and psoriasis, but others have written about chronic obstructive pulmonary disease and IBD^[18]. It is worth noting that the concept of barrier organ has been pioneered in 2005 by the brilliant work of Stefan Schreiber^[19]; the Italian research has recently contributed to this field by a comprehensive dermatologic review^[20] and by a gastroenterologic paper from our own^[21]. As to the state of the art, it seems uneasy to identify a morphological or molecular marker to distinguish those IBDs that associate with psoriasis from those which do not. A few years ago, a North-European group focused their attention on polymorphisms of the IL23 receptor (IL23R) in both IBD and psoriasis, thus perhaps envisaging a genetic link between the two disorders^[22].

Interest in the issue of the systemic positioning of IBD has been fostered by the increasingly frequent observation of ancillary immune diseases arising in patients on biologic treatments: development of IBD in rheumatic subjects receiving etanercept^[23], presentation with IBD of hematologic patients treated with rituximab^[24], and observation of psoriasis in cases of IBD prescribed adalimumab^[25]. The bulk of these observations implies the existence of a galaxy of immune-inflammatory con-

ditions (of which IBD is just one component) spanning from the gut to skin, lungs, and joints. The link between these conditions might be represented by anatomic/physiologic commonalities (barrier organ diseases) or a generic genetic instability perhaps sustained by polymorphisms of STAT transducers^[26].

This scenario recommends that the IBDs no longer be conceived as one nosographic entity. The bulk of the following observations: (1) NOD receptor polymorphism might drive CD phenotypes; (2) there is a link between serologic subtypes and clinical presentations; and (3) some CD presentations do depend on ethnic factors. All of these data contribute to build up a vision of IBD like a non-dichotomic collection of different (though linked) entities that are best described using the definition of “syndrome”^[16].

The implications of this changed frame of mind cannot be ignored. If it is understood that the entity “IBD” contains in fact multiple distinct syndromes along a clinical-serologic-genetic axis, then this must somehow be reflected in differentiated clinical interventions. Such a cutting-edge frame of mind can now hardly fit the widespread recommendation and use of biologic approaches^[27], which target one cytokine in an homogenized-pragmatic attempt to interfere with the common downstream pathways in the mechanisms of IBD.

A GLIMPSE INTO FUTURE TARGETS TO STUDY AND TREAT THE IBDs

Attempts to ensure “sealing” of the gut mucosa with the scope to limit contacts between the immunogenic luminal content and the lymphoid tissue underneath. Partial results of an approach using phosphatidylcholine have already been published^[28].

Triggered by the classic evidence that germ-free animals do not develop IBD, investigators could not neglect the colonic microbiota, which constitutes a heavier meta genome than somatic cells themselves. Various attempts to modify the amount and composition of colic metagenoma have thus proliferated: (1) oral administration of pro-biotic lysates^[29]; (2) fecal transplants^[30]; and (3) diet modifications^[31].

The data from the bulk of these studies is conveying the message that a quantitative or qualitative change of gut microbiota colonization (dysbiosis) might associate with a plethora of (auto)immune and (auto)inflammatory disorders^[32], with a particular emphasis on rheumatoid arthritis (RA)^[33]. Relevant cutting-edge results^[34] are now showing that *Prevotella Copri* (an in-habitant species of the microbiome) might train T-lymphocytes to secrete IL-17, a key mediator in the pathogenesis of RA. To this end, attention is concentrating on the recent claim that NOD receptors on colonic epithelial cells (whether tolerant or reactive against colonic flora at birth) might drive the metagenomic phenotype of the newborn: rather a breakthrough, in view of the ability of colonic species to condition a whole array of affections, from IBD itself to

hepatic steatosis^[35].

Research directed to identify and change factors in the genesis of IBD, such as life style and diet composition^[36].

Along a totally different line, the results have been published of attempts at unraveling genetic IBD surrogates, that though mimicking IBD, might atypically respond according to the signal conveyed by the hidden gene: Behcet mimicking IBD^[37] and familial mediterranean fever are instructive example^[38].

CONCLUSION

Though generated in a limited environment, the analysis of the data from our office has led to general considerations. The IBDs can no longer be considered as autonomous entities, but rather as poly-organic and poly-genic syndromes wherein a critical mass of polymorphic genetic information and environmental factors must interact for full-blown disease to develop^[39]. Visualizing the IBDs like archetypes disorders of the immunological interaction between the “in” and the “out” (together with skin and pulmonary epithelia disorders) to make the umbrella label of “barrier organ disease” seems particularly seminal. This novel positioning of IBD might at first sight increase the degree of complexity, but on the other hand can favor novel therapeutic approaches and pave the way towards the conception of a personalized therapy.

Though apparently stable in the Western World, IBD has two formidable avenues to run. Firstly, Far East populations seem no longer to be immune from the IBDs, and in the next few years may witness an epidemic explosion of these disease^[40]; secondly, populations that immigrate to countries with a higher hygiene standard seem to be particularly prone to develop IBD^[41]. For certain countries, such challenges are not an issue of tomorrow, but are already here today.

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Prevention of hepatocellular carcinoma in patients with chronic hepatitis B

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in achieving profound and durable suppression of HBV DNA levels while improving liver function and histology, robust evidence of other long-term clinical outcomes, such as prevention of HCC, are limited.

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Key words: Chronic hepatitis B; Entecavir; Hepatitis B virus; Hepatocellular carcinoma; Hepatocarcinogenesis; Nucleoside analogues; Risk reduction

Core tip: There is overwhelming evidence for the causal role of hepatitis B virus (HBV) infection in the development of hepatocellular carcinoma (HCC). However, evidence for the role of antiviral therapy in HCC prevention is inconclusive, in part due to the slow course of HCC development, which makes conducting outcome studies very challenging, while the effectiveness of modern antiviral agents in suppressing HBV means that untreated control group comparisons are ethically unacceptable. We review the impact of HBV treatment on the risk of HCC development, with special focus on emerging data for modern anti-HBV drugs such as entecavir and tenofovir.

Abstract

Patients with chronic hepatitis B are at significant risk for hepatocellular carcinoma (HCC). Globally, over half a million people each year are diagnosed with HCC, with marked geographical variations. Despite overwhelming evidence for a causal role of hepatitis B virus (HBV) infection in the development of HCC and a well-established relationship between high baseline hepatitis B viral load and cumulative risk of HCC, the molecular basis for this association has not been fully elucidated. In addition, a beneficial role for antiviral therapy in preventing the development of HCC has been difficult to establish. This review examines the biological and molecular mechanisms of HBV-related hepatocarcinogenesis, recent results on the effect of modern nucleos(t)ides on the rate of HCC development in high risk HBV cohorts and the potential mechanisms by which long-term antiviral therapy with potent inhibitors of HBV replication might reduce the risk of HCC in patients with chronic hepatitis B. Although evidence from randomized controlled trials shows the favourable effects of antiviral agents

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INTRODUCTION

Worldwide, hepatocellular carcinoma (HCC) is diagnosed in over 500000 people each year^[1]. Increasing age, male sex and chronic alcohol consumption are significant risk factors for the development of HCC. Although there is substantial geographical variation, the greatest burden

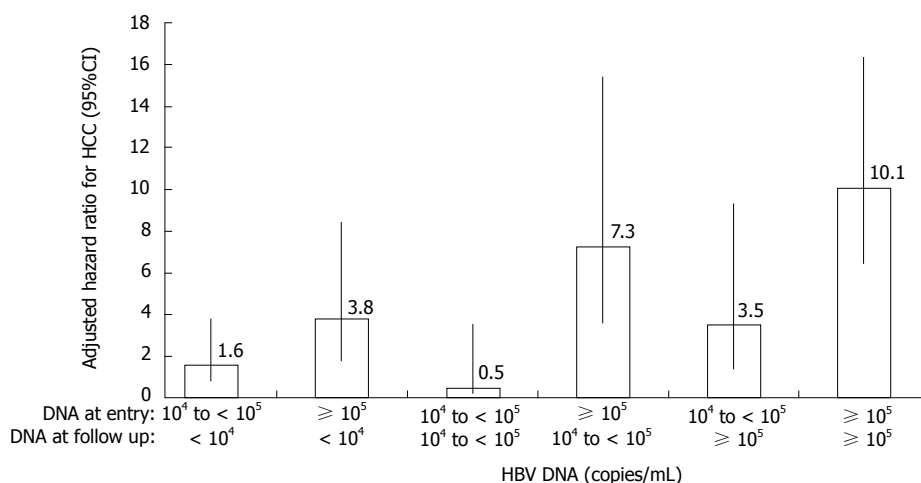


Figure 1 Adjusted hazard ratio for hepatocellular carcinoma by serum hepatitis B virus DNA levels at study entry and last follow-up. Data were adjusted for gender, age, cigarette smoking and alcohol consumption using Cox proportional hazards model. HCC: hepatocellular carcinoma; HBV: Hepatitis B virus. Data sourced from Chen *et al*^[11].

of the disease is in East Asia, Eastern Europe and sub-Saharan Africa, where hepatitis B virus (HBV) infection is highly prevalent^[1-4].

Globally, HBV infection is associated with approximately half of all cases of HCC, and almost all cases of HCC in children^[1]. Chronic HBV infection may progress to cirrhosis and liver decompensation and, the majority (up to 80%) of patients with HBV-related HCC have underlying cirrhosis. The known risk factors for HBV-related HCC can be categorized into host factors, virus factors, and host-virus interactions. Host factors include male gender, Asian race, age older than 40 years, exposure to the mycotoxin aflatoxin, habitual smoking or alcohol consumption, and a family history of HCC^[1-3,5,6]. Virus factors can include coinfection with hepatitis C virus (HCV) or hepatitis delta virus, pre-core (Pre-C) or basal core promoter mutations, high levels of HBV hepatocellular replication, and HBV genotype C. Host-virus interactions include the presence of cirrhosis, prolonged circulating hepatitis B surface antigen (HBsAg) and hepatitis Be antigen (HBeAg), and high levels of DNA-HBV and HBsAg.

Familial aggregation of risk for HCC has been well described in case-control studies in Asia^[7-9]. In one study, the risk associated with having parents and/or siblings with HCC was evaluated in a large cohort of male HBV carriers, in a case-control study of HBV carriers with newly diagnosed HCC and HBV-positive subjects without HCC^[9]. There was an increased risk for both HCC and cirrhosis for mothers and siblings but, of interest, not for fathers of case subjects^[9]. For HCC, the adjusted odds ratios (ORs) according to kinship were 2.64 for mothers (95%CI: 1.60-4.34), 3.73 (2.64-5.27) for brothers, and 4.55 (2.22-9.31) for sisters, while the OR for fathers was only 1.36 (0.86-2.11). Overall, HBV carriers with a family history of HCC had an adjusted OR of 2.41 (95%CI: 1.47-3.95) for HCC if one relation was affected, rising to 5.55 (2.02-15.26) when two or more relations had HCC.

The precise mechanism of this familial aggregation is unclear, but may in part be a result of a higher HBsAg

carrier rate among mothers and siblings of HBV carriers compared with fathers, as a result of vertical transmission. Furthermore, although less well investigated, a family history of HCC also appears to increase HCC risk in Western populations^[10].

There is a well-established relationship between cumulative risk of HBV-related HCC and baseline viral load (baseline serum HBV DNA). Elevated HBV DNA level is strongly predictive of HCC, independent of HBeAg status, serum alanine aminotransferase (ALT) level and cirrhosis, with a cumulative incidence rate of HCC at the end of the 13th year of follow up in a large prospective cohort study of 3653 subjects ranging from 1.30% for subjects with serum HBV DNA level of less than 300 copies/mL at study entry to 14.89% for an HBV DNA level of 10^6 copies/mL or greater at study entry^[11]. A significant biological gradient of HCC risk in patients with higher baseline levels was also observed, independent of viral load achieved after treatment^[11]. Subjects with similar HBV DNA levels at last follow-up but with higher viral loads at study entry had significantly higher risk of HCC than those with lower HBV DNA levels at study entry (Figure 1).

These findings suggest the importance of close clinical monitoring for those with elevated serum HBV DNA, and that effective antiviral treatment may be valuable to lower the risk of HCC in patients with chronic HBV.

This article will review the current knowledge of the mechanisms of HBV-related hepatocarcinogenesis, examine the role of antiviral agents in reducing the risk of HCC, and discuss potential mechanisms for HCC risk reduction during long-term antiviral therapy.

LONG-TERM VIRAL SUPPRESSION AND LIVER-RELATED OUTCOMES IN CHRONIC HEPATITIS B

Long-term suppression of HBV is associated with sub-

stantial histological improvement and reversal of fibrosis or cirrhosis^[12-15]. Strong correlations between viral load and histological grading, and between serum viral suppression and histological improvement have been observed^[15], with indications that a greater than 1 log₁₀ copies/mL change in median serum HBV DNA level will convert into a 2-point change in median histological grade. However, the direct contribution of antiviral treatment to the prevention of HBV-related HCC is less clear-cut. Interestingly, in HBeAg-negative patients, genotype B or C, low HBV-DNA and ALT levels and circulating HBsAg levels > 1000 IU/mL can predict hepatitis flares and progression^[16].

The clinical benefit of first generation nucleoside analogues used in the treatment of HBV, such as lamivudine, is limited by the development of resistance and virological relapse after treatment cessation^[12,17-19]. Entecavir and tenofovir dipivoxil are second generation nucleos(t)ide analogue reverse transcriptase inhibitors with potent activity against HBV and high genetic barrier to resistance^[20-24]. Indeed, the cumulative annual incidence of resistance by year 6 of treatment may reach 76% (lamivudine) 29% (adefovir) and 25% (telbivudine), compared with 0%-1.2% for tenofovir dipivoxil and entecavir, respectively^[25].

A systematic review and meta-analysis found that entecavir, which is an acyclic guanosine nucleoside analogue, and tenofovir dipivoxil, an acyclic adenine nucleotide, are the most effective antiviral agents for the treatment of chronic hepatitis B^[26]. In evaluations of lamivudine, pegylated interferon, adefovir, entecavir, telbivudine, and tenofovir, as monotherapies and combination therapies in treatment-naïve individuals, entecavir and tenofovir dipivoxil consistently ranked in the top five treatments for surrogate outcomes, whereas entecavir was ranked first with regard to improving liver histology, and tenofovir dipivoxil was ranked first for inducing undetectable HBV DNA and normalizing ALT levels^[26].

The long-term efficacy of entecavir was demonstrated in an open-label extension study following two phase 3 clinical studies, in which entecavir for a total duration of at least 3 years significantly improved liver histology, biochemical markers and fibrosis, accompanied by potent viral suppression in nucleoside-naïve, HBeAg-positive and HBeAg-negative patients with advanced fibrosis or cirrhosis^[27]. Similarly, in an open-label extension study after two 48-wk phase 3 studies in patients with advanced fibrosis or cirrhosis, long-term suppression of HBV DNA during treatment with tenofovir dipivoxil for at least 5 years led to regression of fibrosis and cirrhosis^[13]. In these studies, long-term maintenance of viral suppression with entecavir and tenofovir dipivoxil was feasible because of favourable safety profiles and the absence of virological rebound or genotypic resistance^[14,27].

BIOLOGICAL AND MOLECULAR MECHANISMS OF HEPATOCARCINOGENESIS

While there is overwhelming epidemiological evidence

for a causal role of chronic HBV infection in the development of hepatocellular carcinoma, the molecular mechanisms of HBV tumourigenesis remain incompletely understood, although it can be seen as a multi-factorial process involving both direct and indirect components, some of which may act synergistically. A summary of potential mechanisms for the development of HCC in patients with chronic HBV infection is shown in Figure 2. It has been proposed that insertional activation of cellular cancer-related genes by HBV DNA integration, induction of genetic instability by viral integration or by the regulatory protein HBx, and host DNA mutations due to high hepatocyte turnover, cytokine and growth factor release in the setting of chronic liver inflammation, hepatocyte injury, proliferating fibroblasts, and fibrosis/cirrhosis, may be mechanisms associated with HBV-induced carcinogenesis^[28-32].

Among factors implicated in chronic HBV infection and hepatocarcinogenesis, HBx has an important role in activating HBV transcription and replication, and in the development of HCC, because it is involved in the activation of numerous signalling pathways and cellular promoters, activating the expression of genes involved in cell cycle control, oncogenesis, proliferation, inflammation and apoptosis^[28-32]. HBx also modulates the transcriptional activity of CREB (cAMP responsive element-binding protein), which plays an essential role in liver metabolism and proliferation, and is associated with hepatocarcinogenesis^[31].

A key mechanism for hepatocarcinogenesis is the integration of HBV DNA into the host genome and the formation of covalently closed circular DNA (cccDNA). This episomal form of viral DNA, which acts as a template for the transcription of viral genes and is responsible for the persistence of viral replication, is derived *via* a succession of biological steps following the transportation of relaxed HBV DNA into the nuclei of hepatocytes. Both cccDNA and HBV DNA sequences integrated into the host genome have transcriptional activity, resulting in synthesis of HBsAg^[33].

Clearance of intrahepatic cccDNA and/or HBsAg is difficult to achieve but clinically meaningful endpoints for antiviral therapy in chronic hepatitis B, and may be associated with a decreased risk of developing HCC^[33,34]. However, the exact role of antiviral treatment in preventing HBV-related HCC has been difficult to establish. Because of the slow biological evolution of HBV, longitudinal studies may necessitate continuation of antiviral treatment over decades, longer than most researchers or pharmaceutical companies can wait^[2,35]. Furthermore, as modern antiviral agents are effective in suppressing viral replication^[25,26,36-38], untreated control group comparisons are considered unethical and cannot be performed.

Recently, a large Taiwanese study showed that in HBsAg-positive patients, predictors of HCC included age, HBeAg status, HBV genotype, and ALT and HBV DNA levels, but not HBsAg levels; however, in a subgroup of HBeAg-negative patients with viral HBV-DNA < 2000 IU/mL, the risk of HCC significantly correlated with

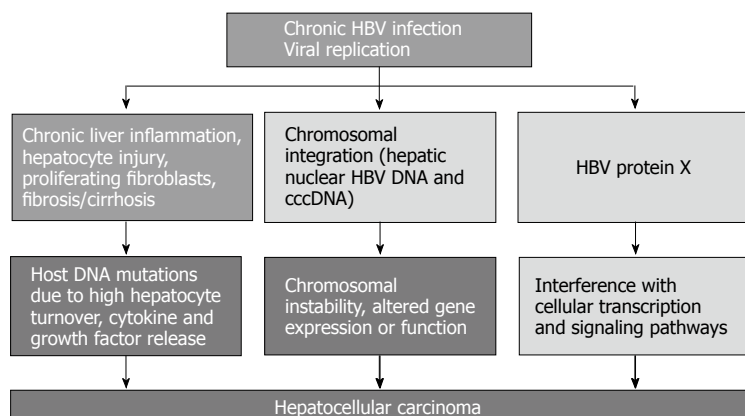


Figure 2 Mechanisms of chronic hepatitis B virus infection-related hepatocarcinogenesis^[27-32]. cccDNA: Covalently closed circular DNA. HBV: Hepatitis B virus.

high HBsAg (≥ 1000 IU/mL), ALT and age, but not HBV-DNA^[39].

ANTIVIRAL TREATMENT AND RISK OF HCC IN PATIENTS WITH CHRONIC HBV INFECTION

A number of systematic reviews and meta-analyses of the role of anti-HBV treatment in the prevention of HCC have been conducted^[26,40-43], without conclusively demonstrating a beneficial impact on the preventing the development of HCC^[2]. This is in part because of the inclusion of studies of older antiviral agents with limited antiviral potency and low genetic barriers, which are, therefore, associated with an increased risk of the development of HBV antiviral resistance mutations. In a recent electronic health records review of 2671 adults with chronic HBV infection enrolled in the Chronic Hepatitis Cohort Study, the adjusted hazard ratio (HR) for HCC risk in those receiving antiviral treatment was (HR = 0.39; 95%CI: 0.27-0.56; $P < 0.001$). In a subgroup analysis of patients with baseline laboratory data for serum fibrosis markers, antiviral treatment was associated with a lower risk of HCC after adjusting for cirrhosis markers of (adjusted HR, 0.24; 95%CI: 0.15-0.39; $P < 0.001$). In another subgroup analysis of patients with HBV DNA viral load data, in patients with HBV DNA > 20000 IU/mL, treated patients had a significantly lower risk of HCC compared with untreated patients^[44].

In a recent meta-analysis of available randomized controlled trials, prospective cohort studies and case-control studies included 3433 treated patients and 4625 controls^[42]. Antiviral treatment was shown to modestly reduce the incidence of HCC in patients with established cirrhosis, but there was no reduction in non-cirrhotic patients. A recent critical review^[40] found that potent and persistent suppression of HBV viral load was more effectively maintained with nucleoside analogues than with other antivirals, leading to reversal of fibrosis and cirrhosis, and indications of a reduction in the incidence of HCC. However, this cannot be taken as high level evidence, as no direct data relating to entecavir and tenofovir dipivoxil were available in this analysis. Of five

studies of oral antiviral agents included in the review (2036 patients treated with nucleoside analogues), all except one were retrospective, and most of were with lamivudine or adefovir, older agents^[40]. However, all studies showed some reduction in HCC. The only randomized trial included in the systematic review was published in 2004, and showed that lamivudine reduced the incidence of cirrhosis and HCC in patients with chronic hepatitis B and advanced cirrhosis^[18]. Ten studies of interferon- α showed inconsistent results, in part because interferon- α was associated with only moderate suppression of HBV DNA. However, recent evidence from two phase 3 clinical trials presented at the 2013 Annual Meeting of the European Association for the Study of the Liver (EASL) suggests that the observed incidence of HCC is lower than expected in patients with chronic hepatitis B treated with tenofovir dipivoxil^[45]. The incidence of HCC was lower than predicted (as assessed by the REACH-B risk model), with a measurable effect in non-cirrhotic patients after 2 years, reaching a 55% reduction at 6 years of treatment ($P = 0.05$)^[45]. Tenofovir dipivoxil had less effect in patients with cirrhosis.

The evidence base for the effect of entecavir on HCC risk is somewhat stronger than that for tenofovir dipivoxil, and will therefore be addressed separately in a subsequent section.

EVIDENCE FOR HCC RISK REDUCTION WITH ENTECAVIR

Although the major goals for therapy in chronic hepatitis B are to delay or prevent progressive liver disease and the development of cirrhosis and HCC^[2], as yet no definitive evidence from randomized controlled trials has shown that antiviral therapy delays or prevents the development of HCC. However, there are a number of recent studies analyzing a potential beneficial impact of entecavir on the development of HCC.

A case-control study that followed a large cohort of Japanese patients with HBV for more than 5 years, compared 472 patients treated with entecavir with a historical cohort without treatment as a control group ($n = 1143$)^[6]. The use of a propensity matching score, applied to match patients from both groups with the same baseline covari-

ates of risk for HCC, minimized study biases. A total of 316 patients in each group (control and entecavir) were matched for comparison. The median follow-up was 3.3 years in the entecavir group and 7.6 years in the historical control group ($P < 0.001$). The cumulative rates of HCC at 5 years were 3.7% in the entecavir group and 13.7% in controls ($P < 0.001$), showing that entecavir significantly reduced the 5-year risk of developing HCC in treatment-naïve patients, compared with control (adjusted HR = 0.37, 95%CI: 0.15-0.91; $P = 0.030$). After multivariate analysis, age, alcohol consumption, pre-existing cirrhosis, HBeAg positive status and platelet count lower than 150000/mL were associated with risk of HCC development. Only entecavir was significantly associated with a reduction of HCC incidence (HR = 0.23, $P = 0.001$). The mutation resistance to drug was 0.8% (4/472) in the entecavir group. The reduction was greater in patients with cirrhosis, and was higher than that observed with a propensity score matched lamivudine cohort^[6]. To assess the impact of entecavir treatment further, the authors applied several established risk models to three studies that utilized HCC risk scales, based on established risk factors for HCC^[6].

Entecavir treatment significantly reduced the risk of HCC development in patients with high risk according to risk scores in the Yang *et al*^[46] ($P = 0.006$) and Yuen studies *et al*^[47] ($P = 0.002$), but not in low score patients. Likewise, in the Wong study^[48], patients with a high risk score had a significant reduction in risk of developing HCC ($P < 0.01$), whereas there was a borderline significance in those with intermediate risk ($P = 0.062$) and no reduction in low risk patients.

Furthermore, entecavir may reduce the risk of HCC recurrence in patients with chronic hepatitis B. In a longitudinal study in patients with newly-diagnosed HCC treated with curative percutaneous radiofrequency ablation (RFA), entecavir administration significantly reduced the incidence of new HCC lesions, compared with patients who did not received treatment after RFA^[49]. The risk of HCC recurrence was significantly lower in entecavir recipients than in nucleoside-naïve patients (OR = 0.077, $P = 0.016$), as well as in those treated with another nucleoside analogue (OR = 0.145, $P = 0.012$). Even when cases of marginal recurrence and recurrence within 6 months of initial treatment were excluded, eliminating the possibility of residual tumour or missed tumour at initial diagnosis, the risk of HCC recurrence was still significantly lower in the entecavir group than in nucleoside-naïve patients (OR = 0.198, $P = 0.004$).

However, in a recent “real life” multicentre Italian study, patients with cirrhosis were still at risk of developing HCC over time, despite profound and durable viral suppression with entecavir. A total of 418 nucleoside-naïve patients with HBV received entecavir for up to 66 mo in the study^[50]. All patients achieved undetectable HBV DNA by year 5, regardless of baseline histology or HBeAg status; 62% achieved HBeAg seroconversion and the HBsAg loss rate was 33%^[50]. Clinical decompensation did not occur during follow-up among the 164 patients

with cirrhosis, indicating that entecavir was effective in preventing the progression of cirrhosis. Nevertheless, despite long-term viral suppression and successful prevention of decompensation of cirrhosis, the cumulative incidence of HCC in cirrhotic patients was still 14% at year 5 (2.8% per year). This suggests that some cellular clones of pre-malignant cells may have already developed before treatment was initiated, and emphasizes the importance of ongoing surveillance for HCC, particularly in patients with cirrhosis. Reviewing the evidence for a multistep model for the process of hepatocarcinogenesis, YN Park^[35] concluded that dysplastic lesions consisting of microscopic dysplastic foci and macroscopic dysplastic nodules may be precursor lesions of HCC. Early detection of precursor lesions may be important in identifying patients at higher risk of developing HCC and, together with diagnosing early HCC, may improve long-term survival for patients with chronic hepatitis B by allowing early initiation of effective antiviral therapy.

As there is stronger evidence that entecavir reduces the risk of developing HCC in patients with associated risk factors such as older age, gender, high HBV viral load, cirrhosis, fibrosis, liver laboratory markers, and core promoter mutations, targeting HCC prophylaxis with entecavir to high-risk patients with chronic hepatitis B may be a rational therapeutic approach. However, this suggestion should be supported by appropriately designed trials.

POTENTIAL MECHANISMS OF HCC RISK REDUCTION IN PATIENTS ON LONG-TERM ANTIVIRAL TREATMENT

In addition to the robust relationship between higher baseline viral load and cumulative risk of HBV-related HCC, several other mechanisms may contribute to reducing HCC risk.

As cirrhosis is in itself a risk factor for HCC development^[1,5], the reversal of cirrhosis associated with long-term HBV viral suppression by effective antiviral therapy may, at least in part, decrease the risk for HCC development. Patients treated with entecavir in two phase 3 studies in nucleoside-naïve patients with HBeAg-positive and HBeAg-negative disease, respectively, and who subsequently were treated in a long-term extension study, underwent liver biopsy after at least 3 years of treatment. Improvement in liver histology was observed in 96% of patients, including all patients with advanced fibrosis or cirrhosis at the phase 3 baseline^[27].

Tenofovir dipivoxil also improved liver histology at week 240 of treatment in an open-label extension study following two 48-week phase 3 trials in which patients received tenofovir dipivoxil plus adefovir^[14]. At the time of a repeat liver biopsy, 73% of HBeAg-positive patients and 85% of HBeAg-negative patients had normal serum levels of ALT, accompanied by profound viral suppression. A total of 87% of patients had histological improvement, including reversal of cirrhosis in 74% of those with cirrhosis at baseline^[14].

These findings of biochemical and histological improvement with entecavir and tenofovir dipivoxil may partly explain a reduction of HCC development in high risk patients.

Inhibition of the intracellular recycling pathway leading to a decrease in levels of intrahepatic cccDNA has been observed during long-term viral suppression, and depletion of cccDNA occurs by hepatocyte turnover as a result of loss by natural liver cell division and/or cell death during injury/regeneration cycles^[33,51]. Currently, determination of cccDNA is not feasible by non-invasive means as a liver biopsy is required, and it has been proposed that serum HBsAg quantification may be used as a surrogate marker for cccDNA levels^[2,33]. However, a recent study showed that, despite profound HBV DNA reduction, HBsAg and cccDNA decline was small on a short-term basis (1 year), and the magnitude of HBsAg reduction did not correlate with cccDNA^[34].

Overall, these results suggest that even when HBV DNA intermediates are suppressed by nucleoside analogues, HBV may still replenish cccDNA by preferentially transporting the viral genome back to the hepatocyte nucleus instead of being enveloped and exocytosed to peripheral blood^[34].

If clearance of cccDNA might contribute to decreased risk of HCC^[23,33], it is likely that long-term therapy is needed to eliminate intrahepatic cccDNA, and it is therefore interesting that 48 wk of treatment with entecavir has very recently been shown to result in significantly greater reductions from baseline hepatic HBV cccDNA levels, as well as total hepatic HBV DNA, than lamivudine^[52]. In this, the ETV-022 trial, cccDNA reduction was related to lower baseline serum HBV DNA and lower baseline necroinflammation. In addition, greater reduction of cccDNA at week 48 was associated with a higher on-treatment reduction in HBV DNA, Knodell necroinflammatory score and serum ALT, as well as higher HBeAg clearance^[52].

CONCLUSION

Although there is overwhelming evidence of the causal role of HBV infection in the development of hepatocarcinogenesis, the evidence for the role of long-term antiviral therapy in the prevention of HCC in patients with chronic hepatitis B is modest. The limited evidence may, in part, be related to the difficulties of conducting longitudinal outcome studies, as HCC develops slowly, necessitating very long-term follow-up studies, and the effectiveness of modern antiviral agents in suppressing viral replication means that untreated control group comparisons are not considered ethically acceptable. However, there is persuasive evidence that entecavir reduces the risk of developing HBV-related HCC, particularly in high-risk patients. Entecavir also lowers the risk of recurrence after radiofrequency ablation of HCC. As HCC development is rare in low-risk patients, longer follow-up durations are needed to fully assess the potential effect of entecavir on preventing the development of HCC in

patients with HBV infection.

In summary, there is emerging evidence suggesting that treatment with entecavir or tenofovir dipivoxil (though less extensive than with entecavir) significantly reduces, but does not completely eliminate, HCC risk in patients with HBV-associated cirrhosis.

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Ischemic heart disease, factor predisposing to Barrett's adenocarcinoma: A case control study

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Abstract

AIM: To define the significance of ischemic heart disease (IHD) (stable angina to infarction) co-existence in Barrett esophagus (BE) patients and patients with esophageal adenocarcinoma (AdE).

METHODS: All BE/AdE patients in Blackpool-Wyre-Fylde area and Trikala prefecture identified from medical records. Patient clinical details were obtained from hospital and General Practitioner records. Additional information was gathered from validated questionnaire.

RESULTS: Forty (33%) AdE and 83 (19%) BE patients had IHD ($P = 0.002$). Eighteen (15%) AdE and 34 (8%) BE patients had suffered a myocardial infarction ($P = 0.03$). Three (3%) AdE and 7 (2%) BE patients had severe heart failure ($P = 0.82$). Thirty-nine (47%) BE with IHD and 8 (20%) AdE patients with IHD consumed aspirin daily ($P = 0.004$). Seventh-seven (93%) BE patients with IHD and 36 (90%) AdE patients with IHD were on statins ($P = 0.86$). Logistic regression analysis: AdE was more frequent in the elderly, with long term

reflux, long BE and concurrent IHD (odds ratio: 2.086, $P = 0.001$) not consuming statins. Eighteen (22%) BE patients with IHD [16 (84%) with myocardial infarction] vs 33 (10%) without IHD died from non-neoplastic causes within 24 mo from BE diagnosis ($P = 0.005$).

CONCLUSION: IHD is more prevalent in AdE than BE patients. Increased prevalence of AdE is related with the presence of myocardial infarction but not severe heart failure, possibly because patients with BE and severe IHD have low life expectancy.

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Key words: Barrett esophagus; Esophageal adenocarcinoma; Ischemic heart disease; Myocardial infarction; Non-steroidal anti-inflammatory drugs

Core tip: Esophageal adenocarcinoma is a major health problem. We performed a population based retrospective comparison, shown that ischemic heart disease is twice as common among patients with esophageal adenocarcinoma than among those with uncomplicated Barrett esophagus. Although myocardial infarction was more frequently acquired in patients with esophageal adenocarcinoma, grade III or IV class heart failure was not, because patients with Barrett esophagus and severe heart failure usually have a low life expectancy and rarely survive longer than 2 years. Patients with Barrett esophagus and ischemic heart disease receive aspirin or nitrates every day more frequently than patients with esophageal adenocarcinoma.

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INTRODUCTION

Gastro-esophageal reflux disease (GERD), a pathology characterized by reflux of gastric juice into the esophagus is rather common^[1]. In case of prolonged and excessive GERD esophageal mucosa is replaced by metaplastic columnar epithelium. This condition is called Barrett's oesophagus (BE)^[2], and represents the main risk factor for esophageal adenocarcinoma (AdE) development^[3].

One of the main macroscopic features of BE is a net of new blood vessels formed within esophageal mucosa. Although Barrett's epithelium is mainly supplied from the submucosal lamina propria vasculature, presence of neovascularization emphasizes why BE is a precancerous lesion and why it can predispose to dysplasia and AdE development^[4]. Barrett epithelium oxygen saturation remains high (approximately 90%) throughout the metaplastic process^[5], because microvasculature density rises stepwise as BE evolves towards AdE^[6]. Esophageal inflammation enriches stromal angiogenesis^[7], while acid reflux causes periodic hypoxia^[8]. Several markers of hypoxia, including oxygen-regulated transcription factor subunit hypoxia inducible factor-1 α and vascular endothelial growth factor, have been related to advanced BE^[9,10]. Neovascularization markers, such as endoglin (CD-105), have been reported to be up-regulated in patients with high-grade dysplasia and AdE^[11].

It is not uncommon, to mix up esophageal with cardiac pain. Therefore GERD may be misclassified as ischemic heart disease (IHD) and vice versa^[12]. Moreover GERD is rather common among patients with IHD, especially those with unstable angina^[13]. Circulating angiogenic markers are increased in patients with IHD especially those with myocardial infarction (MI)^[14] or severe congestive heart failure^[15]. In addition, IHD could alter mucosal microcirculation causing topical ischemia^[16] and through nitric oxide reduction, impairment of the mucosal defense^[17] and mucosal adaptation to noxious stimuli^[18]. Thus, it is expected that IHD might increase the risk of BE patients to develop AdE. Nevertheless, there are no data on the role of concurrent IHD in BE patients.

Our study aimed to calculate the prevalence of IHD (stable angina to infarction) in BE and AdE patients and study its significance in this patient group.

MATERIALS AND METHODS

BE-AdE case finding

The study included all BE or AdE cases, aged over 18 years living permanently either in Blackpool-Wyre-Fylde (BWF) NHS area (318886 inhabitants during 1991 census), between August 1, 1996 and July 31, 2001 or in the prefecture of Trikala (132689 inhabitants during 2001 census), between January 1, 2002 and December 31, 2005. Study design was similar in both study periods^[19]. Endoscopy service was available only in Victoria Hospital in BWF, while it was available both in Trikala General Hospital and private services in Trikala prefecture. Nev-

ertheless we requested private service gastroenterologists to refer both BE and AdE cases in Trikala General Hospital during the study period. To secure complete case identification PT searched patient clinical notes, hospital endoscopy records, histology registers, operating theatre registers and death certificates to ascertain full case identification and gather a full clinical and drug history for every patient. General practitioners (GPs) and adjacent district hospitals were also contacted to provide additional cases who had an endoscopy outside the study hospitals during the study period as well as additional clinical information.

All BE or AdE subjects provided and completed an adapted and validated version of Reflux Symptom Questionnaire^[20], on their first visit after endoscopic and histological case verification. The study questionnaire provided clinical and drug details as described elsewhere^[19]. For deceased patients the closest relative provided information to complete the study questionnaire. Thirty percent of patients failed to return the study questionnaire. We contacted them by phone and collected relevant data during the phone call.

For any discrepancy between questionnaire data and clinical records we favor the latter with the exception of over the counter non-steroidal anti-inflammatory drugs (NSAIDs) consumption, and deliberate ignorance to GP prescriptions.

Patient characteristics

Patient characteristics definition has been described in detail elsewhere^[19]. Thus, we recorded as active smokers all cases reporting any cigarette consumption the 10 year period preceding case recording. Total cigarette consumption recorded separately in pack-years. We recorded as alcohol abusers all cases consuming daily more than 50 g of pure alcohol the 10 year period preceding case recording. We recorded as NSAIDs consumers all cases consuming NSAIDs at least once a week the 10 year period preceding case recording. Daily NSAID consumption for at least 2 years NSAIDs users was recorded as daily one. Aspirin and non-aspirin NSAID consumption was recorded separately. Patients were considered users of nitrates, calcium channel blockers, beta blockers and statins if they consumed them at least 3 d/wk, the 10 year period preceding case recording. To avoid reverse causality, any medical therapy started less than 4 years before the study period was disregarded.

Based on body mass index (BMI) all cases were classified in 4 grades: grade 0: BMI < 20, grade 1: 20 $\leq$ BMI < 25, grade 2: 25 $\leq$ BMI < 30, grade 3: BMI $\geq$ 30.

We calculated the mean of frequency and duration of reflux recordings checked in every patient visit.

Diagnosis of ischemic heart disease (IHD) was based on clinical (angina), electrocardiographic, echocardiographic, scintigraphic and coronary arteriographic data. Hospitalizations for unstable angina or MI were recorded separately. PT and PI discussed objective findings and agreed IHD diagnosis. We used New York Heart Association Functional Classification to classify heart failure^[21].

We calculated socioeconomic status, as described by Ford *et al.*^[22], utilizing patient residential postcode and data from 1991 census (for BWF or 2001 Greek census for Greek patients). According to their socioeconomic status all cases were classified in three classes: lower, middle and high socioeconomic status.

Endoscopy

We defined BE endoscopically as salmon pink mucosa extending at least 2 cm above the proximal end of the gastric folds. We measured BE length during endoscope withdrawal, and calculated tumor size measuring the distance between the two tumor edges and the incisors. Only adenocarcinomas co-existing with BE were included in case analysis and only when the centre of the tumour was over or above the gastroesophageal junction.

We recorded only hiatal hernias greater than 3 cm of length.

Histology

During endoscopy we obtained biopsies in BE patients every 2 cm from all 4 quadrants. Presence of goblet cells and villi defined specialised epithelium^[23]. We grades dysplasia as negative, low grade, high grade^[24]. Cases with high grade dysplasia were not recorded as AdEs.

Two pathologists reviewed the pathology of all resected AdE specimens. Mucinous tumors, adenosquamous cancers, and poorly differentiated tumors not expressing cytokeratins 7 and 13 were excluded from the analysis.

Ethics

Both BWF Ethics Committee and Trikala Hospital Scientific Council standing for Trikala Hospital Ethics Committee approved the study. All cases signed informed consent before entering the study.

Statistical analysis

We used chi-square test with Yates' correction for non-parametric comparisons and student's *t*-test for parametric values. We overcame biases due to known risk factors using logistic regression analysis. Dependent parameters entered in the analysis were: age (per decade), male gender, BE length (per 5 cm), hiatal hernia length (per 5 cm), duration of reflux, daily use of aspirin, use of statins, high socioeconomic status. All of them represented well known risk factors for AdE development. We also evaluated the role of IHD. For each parameter we calculated the odds ratio (OR) and the corresponding 95%CI of OR.

Taking into consideration the results of a pilot study performed in BWF^[25] and found that 20% of BE and 41% of AdE patients had IHD, we calculated that the study should include at least 36 BE patients with IHD to reach a power of 80%.

RESULTS

Patients

We found 193 patients with a lower esophageal adenocar-

cinoma in BWF. After histologic evaluation we excluded 30 (18%) patients with a tumor of the gastric cardia, 30 (18%) with an AdE without any co-existing BE, and 19 (12%) AdEs with scarce traces of BE. In the latter it was impossible to calculate Barrett length. We also found 10 lower esophageal adenocarcinomas in Trikala prefecture. We excluded 2 (20%) patients with a tumor of the gastric cardia and another 2 (20%) with an AdE without co-existing BE. Thus from the two hospitals 120 AdE patients were entered the study.

We identified 869 patients with salmon pink mucosa in the lower esophagus in BWF, compatible with BE. We excluded 238 (27%) patients because histologic definition was unavailable and 249 (39%) because histology reported the presence of fundic, cardiac or junctional mucosa instead of specialized columnar epithelium. We found another 78 patients with endoscopic BE in Trikala prefecture. We excluded 34 (44%) of them because histology identified only fundic, cardiac or junctional mucosa. Thus 426 BE patients were entered the study.

Both BE and AdE patients who entered the study were not different than those excluded (Table 1).

Patients with AdE were older than BE ones; presented a longer BE; which was less frequently co-existed with a hiatal hernia and they were complained for heartburn for a longer period of time (Table 2). Main demographic and BE related characteristics were independent to reflux complaints (Table 3).

Patients with IHD

Forty (33%) AdE and 83 (19%) BE patients had IHD ($P = 0.002$). Of them 18 (15%) AdE and 34 (8%) BE patients had suffered a MI ($P = 0.03$), while 3 (3%) AdE and 7 (2%) BE patients had grade III or IV class heart failure ($P = 0.82$).

Patients with IHD and AdE, when compared to BE patients with IHD were less frequently diabetics had consumed fewer cigarettes and had a longer reflux history. Forty-two (51%) BE patients with IHD and 12 (30%) AdE patients with IHD were on aspirin treatment ($P = 0.03$). Of them 39 (47%) BE and 8 (20%) AdE patients consumed aspirin daily ($P = 0.004$). Twelve (14%) BE patients with IHD and 20 (50%) AdE patients with IHD were on clopidogrel ($P < 0.0001$). All of them persistent dyspepsia, when they have tried aspirin short-term. Twenty-nine (35%) BE patients with IHD had stopped antiplatelet treatment, due to persistent ulcerative lesions (14 duodenal ulcers, 8 gastric ulcers and 10 esophageal ulcers). Eight (20%) AdE patients with IHD had abandoned antiplatelet treatment ($P = 0.14$); 3 due to persistent duodenal ulcer, 1 due to persistent gastric ulcer and 4 due to persistent esophageal ulcer. Seventy-seven (93%) BE patients with IHD were on statins for hyperlipidemia. Thirty-six (90%) AdE patients with IHD were also on statins ($P = 0.86$). Six (7%) BE and 4 (10%) AdE patients had stopped statin treatment due to side effects ($P = 0.86$), mainly elevation of transaminases, resolved after medication cessation. Seventy-nine (95%)

Table 1 Clinical effects of aspirin in high risk population (clinical trials) *n* (%)

Characteristics	Patients in the analysis	Patients excluded from the analysis	<i>P</i>
Patients with esophageal adenocarcinoma			
<i>n</i>	83	120	
Age [mean (SD)], yr	73 (SD = 11.3)	73 (SD = 11.5)	1.00
Male gender	73 (61)	58 (70)	0.19
Current smokers	44 (37)	33 (40)	0.66
Cig. Cons. (in PY) [mean (SD)]	22.5 (SD = 30.2)	22.1 (SD = 27.3)	0.92
Alcohol abusers	32 (27)	21 (25)	0.96
BMI ≥ 25	57 (48)	40 (47)	0.96
Presence of hiatus hernia	73 (61)	52 (63)	0.91
Ischemic heart disease	40 (33)	27 (33)	0.97
Use of aspirin	17 (14)	12 (14)	0.88
Low socioeconomic status	26 (22)	18 (22)	0.87
Dur of reflux [in Y-mean (SD)]	28.5 (SD = 10.1)	27.8 (SD = 12.2)	0.66
Freq of refl (d/wk) [mean (SD)]	5.4 (SD = 2.4)	5.4 (SD = 2.6)	1.00
Patients with Barrett's esophagus			
<i>n</i>	426	521	
Age [mean (SD)], yr	68 (SD = 14)	68 (SD = 13)	1.00
Male gender	264 (62)	316 (61)	0.73
Current smokers	136 (32)	174 (33)	0.68
Cig. Cons. (in PY) [mean (SD)]	19.8 (SD = 28.4)	20.3 (SD = 29.4)	0.79
Alcohol abusers	108 (25)	130 (25)	0.95
BMI ≥ 25	232 (54)	283 (54)	0.98
Barrett's length (in cm)	6.6 (SD = 3.9)	6.6 (SD = 3.7)	1.00
Presence of hiatus hernia	304 (71)	371 (71)	0.98
Ischemic heart disease	83 (19)	99 (19)	0.92
Use of aspirin	87 (20)	109 (21)	0.91
Low socioeconomic status	69 (16)	83 (16)	0.98
Dur of reflux [in Y-mean (SD)]	16.1 (SD = 9.9)	16.4 (SD = 10.2)	0.65
Freq of reflux (d/wk) [mean (SD)]	5.1 (SD = 2.3)	5.1 (SD = 2.5)	1.00

Cig. Cons.: Cigarette consumption in total throughout life; PY: Pack-years; BMI: Body mass index; Dur of reflux: Duration of reflux; Freq of refl (d/wk): Frequency of reflux episodes in days/week.

Table 2 Comparison of the main demographic endoscopic and clinical characteristics between patients with Barrett's esophagus and esophageal adenocarcinoma *n* (%)

Characteristics	Barrett's esophagus (<i>n</i> = 426)	Esophageal adenocarcinoma (<i>n</i> = 120)	<i>P</i>
Age [mean (SD)], yr	68 (SD = 14)	73 (SD = 11)	0.0003
Male gender	264 (61)	73 (61)	0.82
Smokers	136 (32)	44 (37)	0.39
Cig. Cons. (in PY) [mean (SD)]	19.8 (SD = 28.4)	22.5 (SD = 30.2)	0.36
Alcohol abusers	108 (25)	32 (27)	0.86
Barrett's length (in cm)	6.6 (SD = 3.9)	7.5 (SD = 4.2)	0.03
Presence of hiatus hernia	304 (71) ¹	73 (61)	0.04
BMI ≥ 25	232 (54)	57 (48)	0.21
Low socioeconomic status	69 (16)	26 (22)	0.21
Dur of reflux (in Y) [mean (SD)]	16.1 (SD = 9.9)	28.5 (SD = 10.1)	< 0.0001
Freq of reflux (d/wk) [mean (SD)]	5.1 (SD = 2.3)	5.4 (SD = 2.4)	0.21

¹Thirty/fifty (60%) of patients with short segment Barrett present a hiatus hernia, as well as 5/10 (50%) with AdE on short segment Barrett esophagus. Y: Years; Cig. Cons.: Cigarette consumption in total throughout life; PY: Pack-years; BMI: Body mass index; Dur of reflux: Duration of reflux; Freq of reflux (d/wk): Frequency of reflux episodes in days/week.

BE patients with IHD and 37 (93%) AdE patients with IHD were on beta-blockers ($P = 0.85$). Sixty-two (75%) BE patients with IHD and 19 (47%) AdE patients with IHD were on sphincter relaxing medication ($P = 0.005$). Of them 57 (69%) BE and 14 (35%) AdE patients were on nitrates ($P = 0.0004$), while 15 (18%) BE and 15 (38%) AdE patients were on calcium channel blockers ($P = 0.02$). Main risk factors and treatment receiving in BE

and AdE patients with IHD are presenting in Table 4.

Logistic regression analysis

Logistic regression analysis in the whole study population revealed that AdE was more frequent in the elderly; in those with long term reflux complaints; with longer BE and and in those with concurrent IHD (odds ratio: 2.086, 95%CI: 1.339-2.257, $P = 0.001$), AdE was less frequent

Table 3 Comparison of the main demographic endoscopic and clinical characteristics between patients with Barrett esophagus without reflux symptoms and those with gastroesophageal reflux *n* (%)

Characteristics	Asymptomatic patients <i>n</i> = 40	Patients with GERD <i>n</i> = 386	<i>P</i>
Age [mean (SD)], yr	68 (SD = 8)	68 (SD = 15)	0.50
Male gender	28 (70)	236 (61)	0.35
Smokers	17 (43)	119 (31)	0.18
Cig. Cons. (in PY) [mean (SD)]	22.7 (SD = 29.6)	17.4 (SD = 25.6)	0.12
Alcohol abusers	13 (33)	95 (25)	0.37
Barrett's length (in cm)	7.4 (SD = 5.1)	6.6 (SD = 3.9)	0.12
Presence of hiatus hernia	26 (65)	278 (72)	0.45
BMI $\geq$ 25	18 (45)	214 (55)	0.27
Low socioeconomic status	7 (18)	62 (16)	0.99

GERD: Gastroesophageal reflux disease; Cig. Cons.: Cigarette consumption in total throughout life; PY: Pack-years; BMI: Body mass index.

Table 4 Main demographic and disease related characteristics and treatment received in patients with ischemic heart disease and Barrett esophagus or esophageal adenocarcinoma *n* (%)

Characteristic	BE <i>n</i> = 83	AdE <i>n</i> = 40	<i>P</i>
Age [mean (SD)], yr	75 (SD = 10)	78 (SD = 10)	0.12
Male gender	54 (65)	23 (58)	0.54
Active smokers	23 (28)	10 (25)	0.92
Alcohol abusers	18 (22)	8 (20)	0.98
Cig. Cons. (in PY) [mean (SD)]	43 (SD = 25)	33 (SD = 17)	0.02
Low socioeconomic status	21 (25)	12 (30)	0.74
Diabetes	16 (19)	1 (3)	0.02
Barrett's length (in cm)	6.4 (SD = 3.6)	7.1 (SD = 4.4)	0.35
Presence of hiatal hernia	55 (66)	27 (68)	0.95
Hyperlipidemia under treatment	77 (93)	36 (90)	0.86
Hypertension	58 (70)	30 (75)	0.71
Dur ref (in years) [mean (SD)]	19 (SD = 10)	28 (SD = 10)	< 0.0001
Freq ref (d/wk): [mean (SD)]	5.3 (SD = 2.3)	6 (SD = 2)	0.1
BMI $\geq$ 25	45 (54)	19 (48)	0.61
Use of beta-blockers	79 (95)	37 (93)	0.85
Sphincter relaxing medication	62 (75)	19 (47)	0.005
Low dose aspirin	42 (51)	12 (30)	0.03
Low dose aspirin daily	39 (47)	8 (20)	0.004

Cig. Cons.: Cigarette consumption in total throughout life; PY: Pack years; BMI: Body mass index; Dur ref: Duration of reflux; Freq ref (d/wk): Frequency of reflux episodes; BE: Barrett esophagus; AdE: Esophageal adenocarcinoma.

in statin consumers (Table 5).

Follow-up

Nineteenth (23%) BE patients with IHD *vs* 33 (10%) without IHD died from non-neoplastic causes within 24 mo from BE diagnosis (*P* = 0.002). Sixteen (84%) BE patients with IHD who deceased within 2 years from BE diagnosis, had suffered a MI or had grade III or IV class heart failure (*P* = 0.01).

DISCUSSION

We performed a population based retrospective study and found that IHD was almost twice as frequent in AdE patients as those with uncomplicated BE. Although MI was more frequently acquired in AdE patients, grade III

or IV class heart failure was not, because the majority of BE patients with severe heart failure do not survive longer than 2 years. BE patients with IHD consumed aspirin daily and nitrates more frequently than AdE patients and calcium channel blocker less frequently.

Despite its population-based design and thorough case evaluation our study has several drawbacks. It is retrospective, and not large enough to draw strong conclusions.

Patients with AdE have no choice but to come to medical attention. On the other hand BE patients are usually referred for endoscopy only if they present severe persistent GERD. It is very difficult to exclude reference related biases, nevertheless a small minority of BE patients without GERD were referred for endoscopy^[26] and we can speculate the features of BE population escaping medical attention by studying this population. We have shown that patients with BE and reflux symptoms were not different than BE patients without reflux in various demographic and disease related characteristics. Thus, we expect that our study population might be representative of the total BE population in BWF.

It is still uncertain whether the presence of specialized epithelium in the lower esophagus is exclusively related to AdE development^[27]. Some authorities believe intestinal metaplasia absence is only a reflection of sampling error and that it will invariably be present if meticulously searched^[28]. Nevertheless, the risk of non-columnar intestinal metaplasia to progress to AdE is still debatable^[29]. By excluding patients without a histological verification of intestinal metaplasia, we limited our BE population and increasing bias due to BE underreporting. On the other hand, because there was no difference between patients included and those excluded from the analysis in any demographic or disease related characteristic, we avoided bias related to poor defined cases or overestimation of BE length due to esophageal inflammation.

Over-expression of various angiogenetic factors, such as hypoxia-inducible factor or vascular endothelial growth factor permits human myocardium to adapt to coronary ischemia^[30]. Nevertheless, as those angiogenetic factors enter general circulation they can produce BE

Table 5 Logistic regression analysis, in the whole study population, for known risk factors for esophageal adenocarcinoma development, various conditions co-existing with non-steroidal anti-inflammatory drugs use and various subgroups of non-steroidal anti-inflammatory drugs use

Variable	Odds ratio	CI of odds ratio	P
Age (per decade)	1.315	1.220-1.514	<0.001
Male gender	0.946	0.622-1.437	0.75
BE length (per 5 cm)	1.289	1.043-1.547	0.045
Length of HH (per 5 cm)	0.924	0.847-1.007	0.06
Duration of reflux (in decades)	1.848	1.686-2.060	<0.001
IHD	2.086	1.339-3.257	0.001
Daily aspirin use	0.623	0.346-1.111	0.65
Use of statins	0.576	0.356-0.918	0.02
Low socioeconomic status	1.411	0.844-2.351	0.43

IHD: Ischaemic heart disease; BE: Barrett esophagus.

hyperproliferation and augment BE malignant potential^[4]. After all, tissue hypoxia has been related to cancer development^[31] and epidermal growth factor up-regulation due to cardiac ischemia^[32], can favor carcinogenesis within BE^[33]. Finally, oxidative phosphorylation up-regulation^[34] and subsequent reactive oxygen species overproduction, due to peripheral hypoperfusion increases the mutagenic pressure and raises genetic instability^[35]. Thus, we expected and we found that IHD is more frequently acquired in AdE than BE patients, especially those suffered an MI.

Old age is more prevalent in AdE patients and IHD is a disease of old age^[36]. Thus it is possible that higher IHD incidence in AdE patients is solely a result of old age. Nevertheless, IHD was an independent risk factor for AdE in multiple regression analysis and pathogenetic mechanisms support a deleterious effect of IHD in BE patients.

Deleterious effect of IHD on BE progression to malignancy is balanced by reduced life expectancy of those patients, especially those with severe heart failure^[37]. In concordance to Moayyedi *et al.*^[38] we have reported a high mortality in BE patients with concurrent IHD, especially those with a MI or with severe heart failure.

Observational study data from BE patients are disappointing concerning aspirin protective effect. Both our case control study in BE/AdE patients^[19] and Kastelein *et al.*^[39] prospective study identified no protection from low-dose aspirin use in BE patients. Opposing our findings in general BE population, daily aspirin use in BE patients with IHD seems to be beneficial, possibly because of it improves cardiac and peripheral circulation and prevents over-expression of angiogenetic factors.

Epidemiological data agree that statin use could protect BE patients from AdE development^[39-42]. Although use of statins was less frequent in AdE than BE patients, its use was almost universal in patients with IHD, preventing identification of their possible beneficial properties.

We have already reported, in concordance with Ladanchuk *et al.*^[43] that nitrates have no influence in BE patients^[19]. Nevertheless, we found that nitrates had a beneficial role in BE patients with IHD. Beneficial role of nitrates/sphincter relaxing medication in BE patients with

IHD could be incidental, mirroring not a truly protective relationship but the small number of patients studied. Nevertheless it could also be a result of cardiac and peripheral perfusion improvement after nitrate use.

In conclusion IHD is more prevalent in AdE than BE patients. Use of low-dose aspirin and nitrates in this study group is encouraging. More studies are needed to show if IHD is more frequent in BE patients because they are older or verify that IHD is deleterious for BE patients and unveil the pathogenetic mechanisms (increase of angiogenetic and growth factors) beneath it. Those studies should be prospective, multicentric and large enough to overcome possible biases faced in our study.

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COMMENTS

Background

Gastro-esophageal reflux disease is a common condition resulting from reflux of gastric or intestinal contents into the esophagus. Prolonged reflux may lead to replacement of esophageal lining by pathological lining resembling large bowel, a condition known as Barrett's oesophagus. The most serious complication of Barrett's oesophagus is the development of esophageal adenocarcinoma. Barrett's lining is characterised by the presence of pathological vessels and overproduction of various substances promoting the production of pathological vessels. Such substances are overproduced in ischemic heart disease. No studies today have addressed any correlation of Barrett's esophagus to ischemic heart disease. People only know that ischemic heart disease is the main cause of death in Barrett's patients.

Research frontiers

Various substances promoting the production of pathological vessels have a key role in the development of esophageal adenocarcinoma in patients with Barrett's esophagus. Population studies suggest that aspirin and statins, to cornerstones of ischemic heart disease treatment can prevent the development of esophageal adenocarcinoma in patients with Barrett's esophagus.

Innovations and breakthroughs

This study has shown that ischemic heart disease was almost twice as frequent in cancer patients as those with uncomplicated Barrett's esophagus. Myocardial infarction, as severe complication of ischemic heart disease was more frequent as well. Severe heart failure was not, because the majority of Barrett's esophagus patients do not survive longer than 2 years. Barrett patients more frequently used daily aspirin and nitrate use with ischemic heart disease than patients with esophageal adenocarcinoma.

Applications

Patients with Barrett's esophagus and ischemic heart disease deserve more frequent endoscopies in order to identify esophageal adenocarcinoma early. Aspirin and statin treatment is useful in this patient group and can reduce the risk to develop esophageal adenocarcinoma.

Peer review

This is an excellent study as it's the first report to explore the relationship be-

tween ischemic heart disease and Barrett esophageal adenocarcinoma. The case control study was well designed and carried out, and the manuscript is clearly written. The results are believable, and the conclusions are acceptable.

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Statins and their role in acute pancreatitis: Case report and literature review

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Abstract

Statin induced pancreatitis has historically been considered a diagnosis of exclusion, with literature references typically in the form of case reports and observational studies. Recently, larger studies have challenged the correlations made by earlier case reports, and instead demonstrate a mild protective effect in statin users. We present a case report of likely statin induced pancreatitis in a 58-year-old male (which we have attributed to drug-drug interaction with resulting inhibition of hepatic cytochrome P450 enzymes) and have reviewed the apparent dichotomy in the available literature.

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Key words: Statin; Pancreatitis; CYP450; Inflammation; Toxic

Core tip: Statins may reduce the risk of developing an acute episode of pancreatitis through anti-inflammatory perturbation of the systemic inflammatory response pathway. However, it appears that these drugs may also carry a concomitant long-term risk of pancreatitis through a buildup of toxic metabolite/s.

Etienne D, Reda Y. Statins and their role in acute pancreatitis:

INTRODUCTION

Drug-induced pancreatitis has historically been considered a relatively uncommon cause of acute pancreatitis, accounting for 1.4%-2% of all cases^[1,2]. However, recent studies indicate that the diagnosis of drug-induced pancreatitis may be underestimated^[3,4]. Among the many drugs that have been associated with pancreatitis, lipid-lowering agents-in particular, statins-have been increasingly reported as a cause of acute pancreatitis^[5]. More recently, a large population based case control study and meta-analysis have called into question the prevailing consensus regarding the role of statins in the development of acute pancreatitis. This apparent dichotomy in the literature warrants that we re-examine what is known about the role of statins in acute pancreatitis. We present a case of a 58-year-old male incidentally found to have acute pancreatitis in the setting of background statin therapy.

CASE REPORT

A 58-year-old Caucasian male with a past medical history of traumatic brain injury at the age of five with a history of complex partial seizures and renal cell cancer status post right partial nephrectomy presented with syncope. His initial complete blood count (CBC) and electrolyte panel were normal. Head computer tomography (CT) was negative for any intracranial processes. The patient was subsequently managed for vaso-vagal syncope secondary to severe coughing spells. On the day of planned discharge the patient complained of vague pain in his right upper quadrant and epigastrium that had been progressively worsening for the past month. Physi-

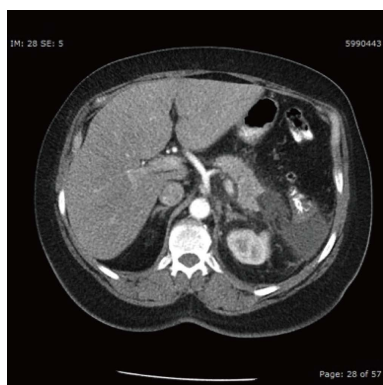


Figure 1 Cross-sectional view of computer tomography abdomen with contrast showing pancreatitis of the body and tail.

cal exam revealed a negative Murphy's sign and labs and imaging were ordered. The ultrasound was negative for gallstone disease, pericholecystic fluid and pericholecystic thickening. Liver function tests (LFTs), white blood cell count, serum creatinine and calcium levels were also within normal limits. Interestingly, lipase and amylase levels were noted to be elevated at 702 units/L (normal values 28-350 units/L) and 417 units/L (normal values 27-117 units/L), respectively. Triglyceride levels were found to be 317 mg/dL, which would unlikely account for an episode of acute pancreatitis (hypertriglyceridemia is typically considered a risk for pancreatitis when levels are > 1000 mg/dL)^[6]. In addition, the patient denied any history of alcohol use. He did not have any travel outside of the United States. CT of the abdomen was performed and found to be consistent with an acute episode of pancreatitis without evidence of structural anomaly (Figure 1). After extensive review of his history and the relevant literature, we found that the patient was on three medications [valproic acid (class 1A), omeprazole (class 1B) and simvastatin (class 1A)] that could potentially cause pancreatitis^[5]. In this patient's case, venlafaxine (a potent inhibitor of neuronal serotonin and norepinephrine reuptake and weak inhibitor of dopamine reuptake) was started six weeks prior and is extensively metabolized by the same hepatic enzyme (CYP3A4) as simvastatin-which he had been taking for more than 10 years. Omeprazole is extensively metabolized by CYP2C19 with only minor contributions from CYP3A4 while valproic acid is not metabolized by CYP3A4. We accordingly held his simvastatin with subsequent decline in lipase levels and resolution of symptoms in the next 24-48 h.

Notably, the standardized Naranjo Adverse Drug Reaction Probability Scale was used to assess the strength of the suspected link between acute pancreatitis and the above-mentioned drugs (venlafaxine, simvastatin, omeprazole and valproic acid) in this patient. In each case, we deduced the probability to be possible for an adverse drug reaction causing acute pancreatitis^[7].

DISCUSSION

Although the mechanism of action of statin induced pan-

creatitis remains ill defined in the literature, an immune-mediated inflammatory response, direct cellular toxicity and metabolic effect have all been postulated as possible culprits^[8]. Three case reports have identified drug-drug interaction as the most likely precipitant. Wong *et al*^[9] documented a case of multiple organ toxicity, including acute pancreatitis, which was due to the interaction between lovastatin and erythromycin. Likewise, Abdul-Gaffar and El-Sombaty reported a case of acute pancreatitis with rhabdomyolysis due to the interaction between lovastatin and gemfibrozil^[10]. Acute pancreatitis was also reported in the context of interaction between simvastatin and fenofibrate^[11]. Interestingly, with regards to combined simvastatin and fenofibrate therapy, Stefanutti *et al*^[12] reported no serious adverse effects in 45 patients using this double-drug regimen over a 12 mo period. The above data and previously reported cases of statin-induced pancreatitis during the last 2 decades are reported in Table 1.

These cases are predicated on the inhibitory effect of these drugs on the oxidative metabolism of statins *via* the hepatic cytochrome P450 enzymes, in particular CYP3A4^[13]. This is the mechanism that we have postulated in the case above. Venlafaxine is metabolized predominantly by CYP3A4 and was likely the reason that Simvastatin, which was being used for years, had precipitated an episode of acute pancreatitis. Interestingly, fibrates have also been found to inhibit the glucuronidation and non-CYP3A-mediated oxidation of statins^[14]. It is important to note that in the case presented above, other more common causes of acute pancreatitis such as alcohol, mechanical ampullary obstruction *via* gallstones, hypercalcemia, hypertriglyceridemia, post-endoscopic retrograde cholangiopancreatography (ERCP) and trauma were initially ruled by history, laboratory tests and gallbladder ultrasound. CT of the abdomen also excluded congenital pancreatic anomaly-which is rather unlikely to have primary occurrence in the 6th decade of life. Initial workup for other less common causes such as autoimmune (IgG4 related) pancreatitis, vasculitis from systemic lupus erythematosus and polyarteritis nodosa was negative.

As an aside, it is noted that the patient above had right partial nephrectomy secondary to a history of renal cell carcinoma. While this has been shown to alter the pharmacokinetics (*e.g.*, decrease in renal metabolism/excretion of drugs) in patients with resultant chronic kidney disease, the above patient did not have evidence of renal impairment and thus this condition was not expected to significantly impact renal drug metabolism^[15,16].

Singh and Loke have postulated that there exists differences in the safety profiles of the various statins that may correlate with the degree to which they inhibit cytochrome P450 CYP3A4 as well as the degree of their lipophilicity^[17]. A subsequent meta-analysis demonstrating a lower incidence of adverse drug reactions with pravastatin (which is the only statin not metabolized by CYP3A4) versus with atorvastatin (which inhibits CYP3A4) gives credence to this idea^[18]. Miltiados *et al*^[19] have also documented a case in which acute pancreatitis may have been caused by the interaction between atorvastatin and

Table 1 Previously reported cases of statin-induced pancreatitis

Ref.	Patient (age, yr/gender)	Associated drug/s	Drug rechallenge	Outcome
Abdul-Ghaffar <i>et al</i> ^[10]	55/Female	Lovastatin and gemfibrozil	No	Complete recovery
Wong <i>et al</i> ^[9]	73/Male	Lovastatin and erythromycin	Yes: no recurrence	Complete recovery
Belaïche <i>et al</i> ^[22]	63/Male	Atorvastatin	No	Complete recovery
Tysk <i>et al</i> ^[13]	36/Male	Fluvastatin	Yes: Recurrence	Complete recovery
McDonald <i>et al</i> ^[11]	70/Male	Simvastatin and Fenofibrate	No	Fatal
Miltiados <i>et al</i> ^[19]	60/Male	Salicylate and Atorvastatin	No	Not available
Anagnostopoulos <i>et al</i> ^[20]	56/Male	Pravastatin	Yes: Recurrence	Complete recovery
Singh <i>et al</i> ^[23]	77/Female	Atorvastatin and Rosuvastatin	Yes; Recurrence with Rosuvastatin	Complete recovery
Antonopoulos <i>et al</i> ^[41]	58/Male	Salicylate and Simvastatin	No	Complete recovery
Tsigrelis <i>et al</i> ^[25]	50/Female	Pravastatin	No	Complete recovery
Chintanaboina <i>et al</i> ^[21]	67/Female	Rosuvastatin	Yes: Recurrence	Complete Recovery
Current report	58/Male	Simvastatin and Venlafaxine	No	Complete Recovery

salicylates, however no possible mechanism of action has been put forward.

Understandably, reintroduction of the likely offending drug following the resolution of symptoms has been largely unfeasible due to the risk of recurrence. As such, there remains a dearth of concrete experimental evidence regarding the precise mechanism of action for the reported cases of statin-induced pancreatitis. Interestingly, the majority of documented instances in which statins have been reintroduced, demonstrate reproducibility of acute pancreatitis and/or symptoms consistent with this diagnosis^[8,13,19-21]. However, these findings have not been universal as Belaïche and colleagues have documented a patient who tolerated pravastatin prior to and following an episode of atorvastatin-induced pancreatitis^[22]. Furthermore, the latency period from initiation of treatment with a statin to onset of pancreatitis also varies between different statins, ranging from one day to several months^[13]. Thus, there is lack of consensus in the literature regarding whether statins exert a class effect or carry distinct and individual risk profiles^[13,22,23]. Observations from Singh *et al*^[17] however suggest that statin induced pancreatitis rarely occurs early and most commonly occurs months to years after statins have been started. As one would expect, this predilection for later onset favors the buildup of toxic metabolite as an etiologic factor. A more recent cross-sectional study also found that statin use was more frequent among patients with idiopathic acute pancreatitis than in patients with other known etiologies of acute pancreatitis (*e.g.*, alcohol and gallstone-induced). The inherent positive correlation does not however prove causality as it is noted that statin users were more likely to suffer from diabetes, obesity and dyslipidemia-which are all risk factors for acute pancreatitis^[24].

A systematic review of observational studies and case reports yielded interesting results as statin-induced pancreatitis was found to have no correlation with the cumulative ingested dose of statins^[17]. Analysis of the data revealed that the development of statin-induced pancreatitis was independent of duration of therapy even though it occurred more commonly months to years after treatment with statins. Although statins are generally used more frequently in older individuals, age of the patient

was not found to be a major susceptibility factor^[17]. It also appears that the majority of cases of statin-induced pancreatitis usually follow a relatively mild course with only a few severe or fatal cases reported^[17,25]. This mirrors the natural history of other documented cases of drug-induced pancreatitis^[1].

However, as noted above, lack of consensus regarding the precise causal link between statin use and the development of acute pancreatitis still exists. With regards to pathophysiology, acute pancreatitis involves local pancreatic inflammation as well as activation of the systemic inflammatory response system (SIRS)^[26]. The latter system is characterized by the activation of multiple cellular processes and humoral cascades which supports the notion that acute pancreatitis results from an imbalance of pro-inflammatory and anti-inflammatory cytokines^[27]. Thus, any targeted- intervention should, in theory, be capable of attenuating several arms of the inflammatory cascade. Statins have a diverse range of potent anti-inflammatory properties which are believed to modify the pathogenesis of acute pancreatitis. To this end, Almog *et al*^[27] have proposed the following possible effects of statins as it relates to the inflammatory cascade: (1) statins could disrupt ligand receptor interaction step thereby hindering the SIRS cascade; (2) statins could blunt the acute-phase response and its immediate consequences; (3) statins could exert a protective effect on the elegant sequence of endothelial activation, dysfunction; and (4) apoptosis statins may also help create a favorable balance between constitutive nitric oxide synthase and inducible nitric oxide synthase so that maintenance of hemodynamic stability is favored^[28-32].

In addition to the above theoretical benefits, Choi *et al*^[33] have demonstrated an increase in Heat Shock Protein (HSP) 60 (HSPs are responsible for maintaining cellular homeostasis and help cells survive stress conditions by repairing damaged proteins) and decrease in the release of inflammatory mediators (*e.g.*, IL-1 β , TNF- α and IL-6) when statins were used in rats with cholecystokinin-octapeptide (CCK)-induced pancreatitis. Subsequent animal studies have also demonstrated benefit of statin therapy in acute *via* reduction of IL-10 levels and myeloperoxidase activity^[28]. Thus, these studies may indicate an anti-inflammatory role-*via* the modulation of various pro

and anti-inflammatory cytokines-for statins in acute pancreatitis, however no long term protective benefit have been yet demonstrated.

In a population based case-control study involving three Danish counties, Thisted *et al*^[34] found no strong causative effect of statins on the risk of developing acute pancreatitis. Instead, they found that former statin users (those patients who used statins greater than ninety days prior to hospital admission for acute pancreatitis) were at increased risk of acute pancreatitis. Furthermore, no increased risk among new users (those patients who filled their first statin prescription 0-90 d prior to hospital admission for acute pancreatitis) was shown, arguing against a direct short-term toxic effect of statins. These authors also cite a possible mild protective effect of statins as their results indicated an inverse relationship between the number of filled statin prescriptions and the risk of acute pancreatitis^[34]. This finding does not lend support to the theory of a long-term accumulation of a toxic metabolite and may be mediated by the cholesterol and-to a lesser extent-triglyceride lowering effects of statins (it is noted that statins are not the first line therapy for hypertriglyceridemia)^[34,35].

More recently, a meta-analysis conducted by Preiss *et al*^[36] demonstrated that statin use was associated with a reduced risk of pancreatitis in patients with normal or mildly elevated triglyceride levels. This study also suggests a possible protective effect of statins, citing both the reduction of bile cholesterol levels and reduced risk of gallstone formation in statin users as corroborating evidence^[37,38]. However, this meta-analysis is likely to be effected by multiple issues such as the failure of the trials to include pancreatitis as a primary end point, the lack of standardization when recording episodes of pancreatitis, the inability to examine specific causes of pancreatitis such as gallstones, and lack of access to individual-participant data. In addition, because exclusion criteria in the trials tended to exclude patients with marked hypertriglyceridemia, the findings may not be generalizable to that specific group of patients^[36].

In light of the evolving evidence regarding statin induced pancreatitis, we believe that statins may reduce the risk of developing an acute episode of pancreatitis through anti-inflammatory perturbation of the systemic inflammatory response pathway. However, it appears that these drugs may also carry a concomitant long-term risk through a buildup of toxic metabolite/s. That being said, the overall mortality benefit of statin use (*e.g.*, especially in patients with recent acute coronary syndrome and established coronary artery disease) clearly outweighs the risk of developing acute pancreatitis based on current evidence^[39,40]. Further prospective double blinded trials with statin challenge and re-challenge are necessary to clarify the precise relationship between statin use and the development of acute pancreatitis.

COMMENTS

Case characteristics

A 58 years old male with presenting with sudden onset abdominal pain.

Clinical diagnosis

Characteristic epigastric abdominal pain and tenderness radiating to the back.

Differential diagnosis

Includes acute cholecystitis, gastroesophageal reflux disease, peptic ulcer disease and abdominal aortic dissection.

Laboratory diagnosis

Lipase and amylase levels were elevated at 702 units/L (normal values 28-350 units/L) and 417 units/L (normal values 27-117 units/L), respectively.

Imaging diagnosis

Computer tomography of the abdomen with contrast demonstrated inflammation of the body and tail of the pancreatitis highly suggestive of acute pancreatitis.

Treatment

The offending agent, which in this case was simvastatin, was discontinued in addition to bowel rest and pain control.

Related reports

Please refer to Table 1 for previously reported cases of statin-induced pancreatitis during the last 2 decades.

Experiences and lessons

Careful examination of drug profile and drug-drug interactions is necessary when other more common causes (*e.g.*, gallstone disease, alcohol, *etc.*) of pancreatitis have been excluded.

Peer review

The authors report a case of pancreatitis during treatment with statin, quickly improved after stopping statin intake, and review literature concerning this topic. The manuscript is of sufficient interest, considering the limited knowledge currently available on the possible correlation between use of statins and pancreatitis.

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Rhabdomyolysis after midazolam administration in a cirrhotic patient treated with atorvastatin

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Key words: Rhabdomyolysis; Chronic liver disease; Statins; Midazolam; Alcoholic liver cirrhosis

Core tip: When dealing with alcoholic liver disease, clinicians need to pay particular attention to the administration of drugs, their dosage, interactions and metabolism to avoid severe adverse reactions. Cirrhotic patients on treatment with statins (particularly atorvastatin) are at high risk of developing fatal rhabdomyolysis and acute renal failure when midazolam is used to allow gastric endoscopy.

Abstract

The administration of statins in patients with liver disease is not an absolute contraindication. Hepatotoxicity is a rare and often dose-related event and in the literature there are only a few described cases of fatal rhabdomyolysis in patients with chronic liver disease after statin administration. During treatment with 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, the factors responsible for myopathy may either be related to the patient, or due to interactions with other medications that are metabolic substrates of the same isozymes and therefore able to increase blood statin concentration. The most important side effects consist of increased transaminase levels, abdominal pain or muscle weakness, increased serum levels of creatine kinase and rhabdomyolysis. In this article we report a case of fatal rhabdomyolysis with acute renal failure after gastric endoscopy, where midazolam was used as a sedation agent in a patient with chronic liver disease treated with a high dose of atorvastatin. Therefore, we suggest paying particular attention to the potential risks of associating atorvastatin and midazolam in patients with chronic liver disease who need to undergo gastric endoscopy.

Gigante A, Di Lazzaro Giraldi G, Gasperini ML, Barbano B, Liberatori M, Sardo L, Di Mario F, Giorgi A, Rossi-Fanelli F, Amoroso A. Rhabdomyolysis after midazolam administration in a cirrhotic patient treated with atorvastatin. *World J Gastrointest Pharmacol Ther* 2014; 5(3): 196-199 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v5/i3.196.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v5.i3.196>

INTRODUCTION

Statins are widely used to treat hypercholesterolemia, therefore reducing cardiovascular risk. Currently, there are no trials on the safety of statins in chronic liver disease. Although hepatotoxicity represents a rare event (< 2%) and is often dose-dependent, adverse effects or even death have been described in patients suffering from liver disease.

The concomitant use of other drugs that are metabolic substrates of the same isoenzymes, as cytochrome P-450 and isoenzyme CYP3A4, can increase statin concentration and consequently elevate the risk of myopathy. The most important side effects consist of increased transaminase levels, abdominal pain or muscle weakness,

Table 1 Biochemical analysis on different days

	ER	Before EGDS	1 st day after EGDS	2 nd day after EGDS	6 th day after EGDS	ICU
ALT (UI/L)	146	52	70	130	207	827
AST (UI/L)	125	117	191	240	927	2075
Total bilirubin (mg/dL)	1.2	2.73	3.39	4.51	6.1	7.44
Direct bilirubin (mg/dL)	0.2	1.86	2.12	3.01	3.8	5.87
INR	1.75	1.7	1.58	1.6	1.68	6.11
Fibrinogen (g/L)	3.52	-	-	-	-	0.58
Platelets (mm ³)	115	54	49	45	70	40
D-dimer (ng/mL)	2557	-	-	-	-	9000
ATIII (%)	-	-	-	-	-	20
Myoglobine (ng/mL)	175	198	22.899	25.981	> 30.000	> 30.000
CK (U/L)	81	95	3.298	5.876	38.289	89
CK-MB (ng/mL)	4.2	4.1	14.01	25.03	73.59	68.72
Troponin T HS (mg/L)	0.022	0.025	0.061	0.043	0.189	1.53
LDH (UI/L)	240	286	358	470	1603	2084
Creatinine (mg/dL)	1.1	1.3	1.3	1.3	3.14	3.8
BUN (mg/dL)	45	60	61	59	161	78
Calcium (mg/dL)	8.8	8.4	-	-	6.7	4.4
Diuresis (cc)	-	2000	1300	1000	150	300
BP (mmHg)	90/50	115/60	105/55	95/60	75/50	60/40

EGDS: Esophagogastroduodenoscopy; ICU: Intensive care unit; ALT: Alanine transaminase; AST: Aspartate aminotransferase; ATIII: Antithrombin III; CK: Creatine kinase; INR: International normalized ratio; Troponin T HS: High sensitivity troponin T; LDH: Lactate dehydrogenase; BUN: Blood urea nitrogen; BP: Blood pressure; ER: Emergency room.

increased levels of creatine kinase and rhabdomyolysis^[1,2].

CASE REPORT

A 67-year-old man was admitted to an internal medicine ward at our hospital for syncope. In the emergency room (ER) the patient was oriented, afebrile and had pale skin. His blood pressure was 90/50 mmHg, and he had arrhythmia (80 bpm), swollen abdomen and peristalsis.

Laboratory findings demonstrated abnormal alanine transaminase (146 IU/L), aspartate aminotransferase (125 IU/L), D-dimer (2557), creatine kinase-MB (4.2 ng/mL), platelets ($115 \times 10^3/\mu\text{L}$), glucose (195 mg/dL), myoglobin (175 ng/mL), international normalized ratio (1.75), and high sensitivity troponin T (0.022 mg/L) values (Table 1).

Chest X-ray and brain computed tomography (CT) scans were performed, which showed no notable findings. The patient was admitted to our department for further investigation and treatment. This patient had a history of hospitalization for myocardial infarction three months earlier (treated by percutaneous transluminal coronary angioplasty with implantation of two drug-eluting stents), arterial hypertension, type II diabetes mellitus (diagnosed about 7 years earlier) and a more recent diagnosis of chronic atrial fibrillation (AF). Patient's medications included carvedilol 50 mg/d, digoxin 0.125 mg, ramipril 10 mg, doxylamine 75/100 mg, furosemide 25 mg, canrenon 100 mg, pantoprazole 40 mg, insulin, and atorvastatin 40 mg. Our examination revealed that the patient presented with low blood pressure, and physiological anamnesis outlined a history of alcohol abuse (about 2 L of wine for the last 30 years). Electrocardiogram (ECG) showed AF at a frequency of 73 bpm, and a Holter mon-

itor confirmed AF. Ambulatory blood pressure monitoring showed recurrent episodes of hypotension. Carotid ultrasonography and electroencephalography showed no abnormalities consistent with syncope. For this reason, we reduced the dosage of antihypertensive medications (ramipril 5 mg, carvedilol 25 mg) and treated the patient with intravenous fluid administration. During hospitalization, because of persistently elevated transaminase levels, the patient underwent hepatobiliary ultrasonography, which showed increased liver size with heterogeneous echogenicity, irregular surface, but no focal lesions. The average velocity in the portal vein was 8.2 cm/s (normal values ≥ 14 cm/s). Spleen size was increased, and mild ascites was present.

Therefore markers for viral hepatitis were sought and found to be negative, and thus the patient was diagnosed with alcoholic liver cirrhosis.

During his second day of hospitalization, the patient reported localized muscle pain in the lower limbs associated with intense weakness. Since statin-induced myopathy was suspected, muscular enzymes were assayed and the results were within reference intervals. Nonetheless, atorvastatin administration was discontinued. The next day the patient underwent esophagogastroduodenoscopy (EGDS) under sedation with midazolam (at a dose of 2 mg), which revealed congestive gastropathy in absence of esophageal varices. The day after the examination the patient complained of a further increase in muscle pain with extension to the upper limbs: muscular enzymes levels increased, as showed in Table 1. In the following days, despite the discontinuation of the statin, muscle pain did not regress, and neither did muscle enzymes levels return within reference values (Table 1).

Suspecting a possible drug interaction, digoxin was

suspended too, which is a known common substrate of atorvastatin cytochrome (CYP 3A4). However laboratory test values and the patient's condition did not improve. Moreover, six days after EGDS, clinical findings of myoglobinuria, oligo-anuria, acute kidney injury and elevated levels of muscle enzymes (Table 1) suggested the diagnosis of rhabdomyolysis, with the indication to begin hemodialysis (HD).

The patient underwent HD treatment for 5 consecutive days with worsening of pain and persistently elevated muscle enzymes. The condition eventually deteriorated into disseminated intravascular coagulation (DIC). ECG showed a new-onset diffuse ST and T wave changes, and prolonged Q-T interval (0.54 s) associated with severe metabolic acidosis.

The patient was transferred to the intensive care unit (ICU). Biochemical values are reported in Table 1. The patient died the next day, 9 d from the diagnosis of rhabdomyolysis.

DISCUSSION

The use of statins in patients with chronic liver disease is not an absolute contraindication: recommendations suggest to start with low doses, making sure that the patient does not consume alcohol and does not suffer from acute hepatitis. In the literature there are only a few described cases of fatal rhabdomyolysis in patients with liver disease treated with statins^[3]. Recent studies suggest that even in liver disease patients, especially those suffering from non-alcoholic steatohepatitis, the indication to use statins stands strong because of the increased cardiovascular risk in these subjects^[4].

During treatment with statins, the factors responsible for myopathy may be related to the patient (age, female sex, alcoholism, hypothyroidism, systemic diseases, family history of myopathy, high consumption of grapefruit juice, large physical activity, major surgery, *etc.*) or to interaction with other medications (fibrates, cyclosporine, antifungals, macrolides, protease inhibitors, nefazodone, amiodarone, verapamil, *etc.*)^[1]. In our case, we assume that the development of rhabdomyolysis was related to several contributing factors such as the high dose of atorvastatin in a patient with undiagnosed chronic liver disease.

The benefits associated with the use of statins in lowering cholesterol levels and preventing cardiovascular disease still remain superior to their potential risk of hepatotoxicity in patients with chronic liver disease. However, in the course of acute viral or alcoholic hepatitis, HMG-CoA reductase inhibitors should be avoided until liver function is restored^[5]. In fact, although major trials have excluded patients with a history of active liver disease, other studies recommended to start with low doses of statins, making sure that the patient does not take alcohol, and to check serum transaminase levels after the first two weeks of therapy, and then each month for three months, eventually reducing the interval to four times a year. If serum transaminase levels are doubled or tripled

compared to reference, therapy should be discontinued until normalization of liver enzymes, and then the use of another statin is reconsidered^[1,2].

In our case, the trigger for the onset of rhabdomyolysis followed by overt DIC and multi organ failure may have been the use of midazolam, metabolized by the same isoenzyme that is responsible for the metabolism of atorvastatin.

Statins are inhibitors of HMG-CoA reductase undergoing first-pass hepatic metabolism. Excluding pravastatin, other molecules of this class are subject to phase 1 hepatic metabolism mediated by CYP 450 isoenzymes. Isoenzyme CYP3A4 is responsible for atorvastatin, lovastatin and simvastatin metabolism, while fluvastatin and rosuvastatin are metabolized mainly by CYP2C9 isozyme.

Although serum levels of atorvastatin and midazolam were not checked, we assume that the concomitant use of drugs that are substrates of the same CYP isoenzymes, as midazolam and atorvastatin, can dangerously increase statin concentration in the blood and consequently the risk of myopathy.

There are some reports of rhabdomyolysis caused by propofol^[6] and its interaction with other statins^[7], but this is the first case report documenting rhabdomyolysis after atorvastatin and midazolam administration.

Furthermore, patients with alcohol use disorders (AUD) are at high risk for rhabdomyolysis secondary to toxic effects of ethanol in the muscle, metabolic disturbances, alcohol withdrawal syndrome and sepsis.

In this case report, the fatal outcome of drug-induced rhabdomyolysis may have been promoted by the presence of pre-DIC condition due to liver cirrhosis: the association of both conditions escalated to multiple organ failure.

In conclusion, particular attention must be paid to the potential risks of associating statins, such as atorvastatin, with other drugs especially in patients with AUD and chronic liver disease. The use of midazolam as a sedation agent should be avoided in patients needing EGDS while treated with statins.

COMMENTS

Case characteristics

The patient complained of muscle pain in the lower and upper limbs associated with intense weakness.

Clinical diagnosis

Clinical findings included elevated transaminases and muscle enzymes, myoglobinuria, oligo-anuria, acute kidney injury, disseminated intravascular coagulation, prolonged Q-T interval (0.54 s) and severe metabolic acidosis.

Differential diagnosis

Rhabdomyolysis in the presence of pre-disseminated intravascular coagulation (pre-DIC) condition due to secondary toxic effects of ethanol in the muscle, metabolic disturbances, alcohol withdrawal syndrome and sepsis.

Laboratory diagnosis

Findings demonstrated elevated transaminases, muscle enzymes, serum creatinine, myoglobinuria, and disseminated intravascular coagulation.

Imaging diagnosis

Hepatobiliary ultrasonography showed increased liver size with heterogeneous echogenicity and irregular surface, and the average velocity in the portal vein

was low. Increased spleen size and mild ascites were also present.

Treatment

Atorvastatin and other drugs metabolized by the same cytochrome isozyme were discontinued; fluid IV administration and hemodialysis were given.

Related reports

The concomitant use of substrates of the same isozymes (CYP3A4), such as midazolam and atorvastatin, can increase statin blood concentration and consequently the risk of myopathy.

Experiences and lessons

The indication for the use of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors in cirrhotic patients must be evaluated by physicians on the basis of clinical necessity. It is correct to start with low-dose drug administration while monitoring transaminases. Finally, it is appropriate to evaluate simultaneous administration of other drugs metabolized by the same cytochrome, therefore reducing the risk of moderate and severe interactions.

Peer review

It was a nicely written case report. It suggests that rhabdomyolysis may have been related to the simultaneous administration of atorvastatin and midazolam in a patient with alcoholic liver disease.

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REVIEW

- 200 Evaluation of gastrointestinal bleeding: Update of current radiologic strategies
Parekh PJ, Buerlein RC, Shams R, Vingan H, Johnson DA
- 209 Protein kinases are potential targets to treat inflammatory bowel disease
Yang L, Yan Y

Contents

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APPENDIX I-V Instructions to authors

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Evaluation of gastrointestinal bleeding: Update of current radiologic strategies

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Core tip: The purpose of this review will be to discuss to provide an evidence-based update on the most current diagnostic and interventional radiologic modalities available and provide clinicians with an algorithmic approach to gastrointestinal bleeding.

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INTRODUCTION

There are over 500000 patients hospitalized annually for gastrointestinal bleeding (GIB) in the United States, which carries an inpatient mortality rate of 3%^[1]. Patients presenting with GIB are considered as having either overt or occult GIB, depending upon the presence of visible or non-visible bleeding^[2]. Overt GIB presents as visible bleeding which constitutes melena, hematochezia or hematemesis. Occult GIB manifests as positive immunohistochemical staining or as iron deficiency anemia^[3]. Irrespectively, the initial evaluation of either overt or occult GIB includes an esophagoduodenoscopy and/or colonoscopy. Obscure GIB (OGIB) refers to the patient population with persistent or recurrent GIB where the initial endoscopic evaluation was negative, estimated to be the case in approximately 5% of patients^[3]. As a result, these patients undergo extensive testing with the objective of localizing and potentially treating the bleeding lesion. Estimates suggest that an average of \$33630 is spent per Medicare patient for further evaluation of OGIB^[4]. While radiologic advances have changed the ap-

Abstract

Gastrointestinal bleeding (GIB) is a common presentation with significant associated morbidity and mortality, the prevalence of which continues to rise with the ever-increasing aging population. Initial evaluation includes an esophagoduodenoscopy and/or colonoscopy, which may fail to reveal a source. Such cases prove to be a dilemma and require collaboration between gastroenterology and radiology in deciding the most appropriate approach. Recently, there have been a number of radiologic advances in the approach to GIB. The purpose of this review is to provide an evidence-based update on the most current radiologic modalities available and an algorithmic approach to GIB.

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Table 1 Common causes of obscure gastrointestinal bleeding based on location

Upper GI lesions	Middle GI lesions	Lower GI lesions
Cameron erosions	Less than 40 years old	Angiodysplasia
Peptic ulcer disease	Tumors	Neoplasm
Fundic varices	Meckel diverticulum	Diverticular disease
Angiodysplasia	Crohn's disease	
Dieulafoy lesion	Dieulafoy lesion	
Gastric antral vascular ectasia	Celiac disease	
	More than 40 years old	
	Angiectasia	
	NSAID enteropathy	
	Celiac disease	
	Uncommon lesions	
	Hemobilia	
	Hemosuccus pancreaticus	
	Aortoenteric fistula	

GI: Gastrointestinal; NSAID: Non-steroidal anti-inflammatory drug.

proach to GIB, the lack of an algorithmic approach and understanding of the available modalities have made it difficult for clinicians to practice cost-effective medicine. Diagnostic and therapeutic modalities differ in the setting of upper GIB (UGIB) *vs* lower GIB (LGIB). This article is intended to present the newest radiologic modalities available as it pertains to UGIB and LGIB as well as provide an algorithmic approach to GIB and the role of endoscopic markings.

ETIOLOGY

Small bowel pathology accounts for up to 75% of patients presenting with OGIB^[5-8], with angiodysplasia being the most common culprit accounting for up to 60% of these cases^[9]. Table 1 subdivides the most common causes of OGIB by anatomic location. Upper lesions are characterized by those that are proximal to the ligament of Treitz, mid lesions are distal to the ligament of Treitz extending to the terminal ileum, and lower lesions are distal to the terminal ileum^[10].

UPPER GI BLEED

Approximately 100000 are patients hospitalized annually for an UGIB, which carries a mortality rate as high as 11%^[11]. The cornerstone for diagnosis and therapeutic intervention in UGIB revolves around endoscopy. There are no radiologic approaches that have demonstrated utility in the diagnosis of an UGIB. There are, however, radiologic interventions that may be used when endoscopic therapy has proven futile, namely transjugular intrahepatic portosystemic shunt, retrograde transvenous obliteration, and transcatheter selective embolization.

Transjugular intrahepatic portosystemic shunt

Transjugular intrahepatic portosystemic shunts (TIPS) utilizes angiographic guidance to create a low-resistance communication between the hepatic vein and the intrahepatic portion of the portal vein^[12]. The expandable metal

stent maintains patency of the tract allowing for continued systemic circulation. The indication for TIPS in the setting of an UGIB include acute esophageal, gastric, or ectopic variceal hemorrhages and in the prevention of recurrent variceal bleeding.

García-Pagán *et al*^[13] evaluated the use of TIPS in 63 patients with cirrhosis (Child-Pugh class B or C) presenting with persistent bleeding. Patients were randomly assigned to treatment with TIPS (early-TIPS; *n* = 32) or continuation of vasoactive therapy with propranolol or nadolol and long-term endoscopic band ligation (pharmacotherapy-EBL; *n* = 31) with future consideration of TIPS as rescue therapy if necessary. Re-bleeding or persistent bleeding occurred in 14 patients in the pharmacotherapy-EBL group as compared to 1 patient in the early-TIPS group during a median follow up at 16 mo (*P* = 0.001). The 1-year likelihood of remaining free from re-bleeding or persistent bleeding was 50% in the pharmacotherapy-EBL group compared 97% in the early-TIPS group (*P* < 0.001). The 1-year survival in the pharmacotherapy-EBL group compared to the early-TIPS group was 61% and 86%, respectively, which led the authors to conclude that early use of TIPS was associated with significant reduction in treatment failure and mortality in patients with cirrhosis hospitalized for acute variceal bleeding. A retrospective study in 2013 validated these results confirming a favorable 1-year mortality in patients who received early-TIPS when compared to those who received vasoactive and endoscopic therapy at 86% and 70% respectively (*P* = 0.056)^[14].

It has recently been postulated that the use of embolotherapy may further prevent recurrent variceal bleeding and stent dysfunction following TIPS creation^[15]. A recent prospective study randomized 106 patients with cirrhosis and recurrent variceal bleeding to TIPS in conjunction with prior embolotherapy *via* the jugular vein (TIPS + E, *n* = 54) or TIPS alone (*n* = 52). The 6-mo rate of shunt patency and overall rate of recurrent variceal bleeding was significantly lower in the TIPS-E cohort as compared to those patients who received TIPS alone. The 3-year cumulative rates of shunt patency, recurrent variceal bleeding and mortality were not statistically significant amongst the two groups, however, leading the authors to conclude that the TIPS-E regimen may reduce the rate of recurrent variceal bleeding during the first 6 mo but the long term benefit requires further investigation.

Retrograde transvenous obliteration

Gastric varices are an important manifestation of portal hypertension and are associated with a very high mortality rate approaching 55%^[16]. Originating from short gastric and gastroepiploic veins, cardiofundal gastric varices present with a unique vascular anatomy as they have a tendency to develop spontaneous splenorenal or gastrorenal shunts^[17]. Thus the use of cyanoacrylate *via* endoscopic sclerotherapy on gastric varices carries a risk of systemic migration through the inferior vena cava. TIPS has been performed for this reason, however is only as-

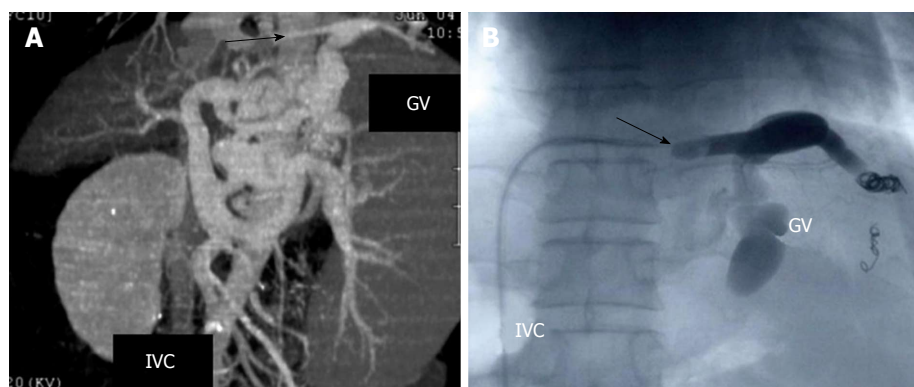


Figure 1 Balloon retrograde transvenous obliteration^[59]. A: Multidimensional computed tomography revealing varices supplied by the left gastric vein (GV) and subsequent drainage into the subphrenic vein (illustrated by the arrow) which is connected to the inferior vena cava (IVC); B: Fluoroscopic image of B-RTO depicting placement of the occlusive balloon catheter into the bleeding vessel (demonstrated by the arrow). B-RTO: Balloon-occluded retrograde transvenous obliteration.

sociated with a success rate of 50% in the regression of gastric varices and is associated with an elevated risk for the development or exacerbation of underlying hepatic encephalopathy^[18]. As a result, a new minimally invasive procedure known as retrograde transvenous obliteration (B-RTO) has been developed. This technique utilizes a sclerosing agent to thrombose gastric varices *via* a balloon catheter typically introduced through the femoral vein and left renal. Figure 1 depicts the use of B-RTO in thrombosing gastric varices.

A recent meta-analysis evaluated the outcomes for B-RTO for gastric varices^[19]. A total of 20 suitable studies were included totaling 734 patients from multiple centers. Cumulative rates for gastric variceal recurrence were reported to be 2.6% (0%-7.2%) with 1-year, 3-year, and 5-year survival rates to be 92.2% (83.1%-100%), 82.6% (71%-100%), and 67.9% (53.7%-85%), respectively. This led the authors to conclude B-RTO should be considered in the management of gastric varices when a gastorenal shunt is present. Several other retrospective studies have demonstrated technical success with B-RTO concluding it to be effective in obliterating bleeding gastric varices with good short-term outcomes^[20-22]. The use of ethanolamine oleate as a sclerosant when performing B-RTO should be with caution as recent studies have implicated it in pulmonary function disorders^[23]. The total amount of ethanolamine oleate has been shown to have a direct correlation to the extent of pulmonary damage, and thus careful respiratory monitoring may be necessary in patients undergoing B-RTO, particularly when anticipating the use of large volumes of ethanolamine oleate.

Transcatheter selective embolization

Rösch *et al*^[24] first introduced the use of transcatheter arterial embolization (TAE) in 1972 as an alternative to surgery. The advent of metallic coils, gelfoam, and surgical glue has greatly improved clinical outcomes. The procedure entails a transfemoral or brachial approach with introduction of a 5-french sheath into the common femoral artery. Subsequent arteriography is performed to delineate the anatomy and identify the culprit lesion *via* contrast

extravasation. In the event that the initial approach does not yield a culprit, then superselective catheterization of the gastroduodenal artery, left gastric artery, or splenic artery is performed depending on clinical suspicion^[25]. Once the lesions are identified then operator has a plethora of embolization techniques at his disposal, depending on the clinical scenario. The choice of the best embolic agent, however, remains an area of debate. Figure 2 depicts the use of TAE in a gastrointestinal (GI) bleed.

Recently, there have been several retrospective analyses comparing the efficacy of TAE to surgery as salvage therapy following failed endoscopic therapy in the setting of UGIB^[26,27]. These studies found TAE to be efficacious in controlling life threatening bleeds from an UGI source in high-risk patients who would otherwise be subjected to emergency surgery, which carries a substantially higher mortality rate (nearly 5 times) when compared directly to TAE.

Intra-arterial vasopressin infusion

Vasopressin elicits contractions of smooth muscles in mesenteric vasculature, thus decreasing perfusion pressure to the bowel and potentially thrombosing the bleeding site^[28]. Vasopressin is directly infused into the suspected artery at a rate of 0.2-0.4 U/min until control of bleeding is observed on angiography. Once control is documented, vasopressin is infused into the mesenteric artery for another 12-48 h depending on severity.

A review of the literature demonstrated an initial success rate of 70%-80% with use of intra-arterial vasopressin infusion^[28]. Unfortunately, the rate of re-hemorrhage with refractory bleeding ranged from 20%-40%. There were various theories that emerged regarding vasopressin failure, namely the rich collateral supply to the upper GI tract, however the exact reason remains unproven. At present time, the emergence of embolotherapy has put vasopressin infusion out of favor.

The bottom line

Endoscopy is the point of focus in identifying the culprit of an UGIB and in delivering initial treatment. There are

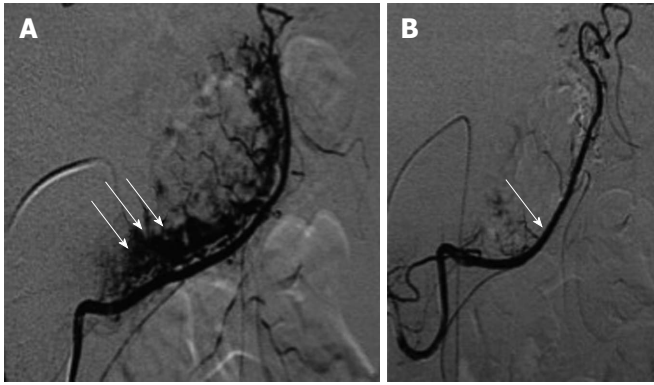


Figure 2 Selective transarterial embolization^[60]. A: Selective catheterization of the right gastro epiploic artery in the setting of angiodysplasia. The arrows depict the pathologic vessels at the greater curvature of the stomach; B: Post embolization angiogram revealing control of the bleeding vessel.

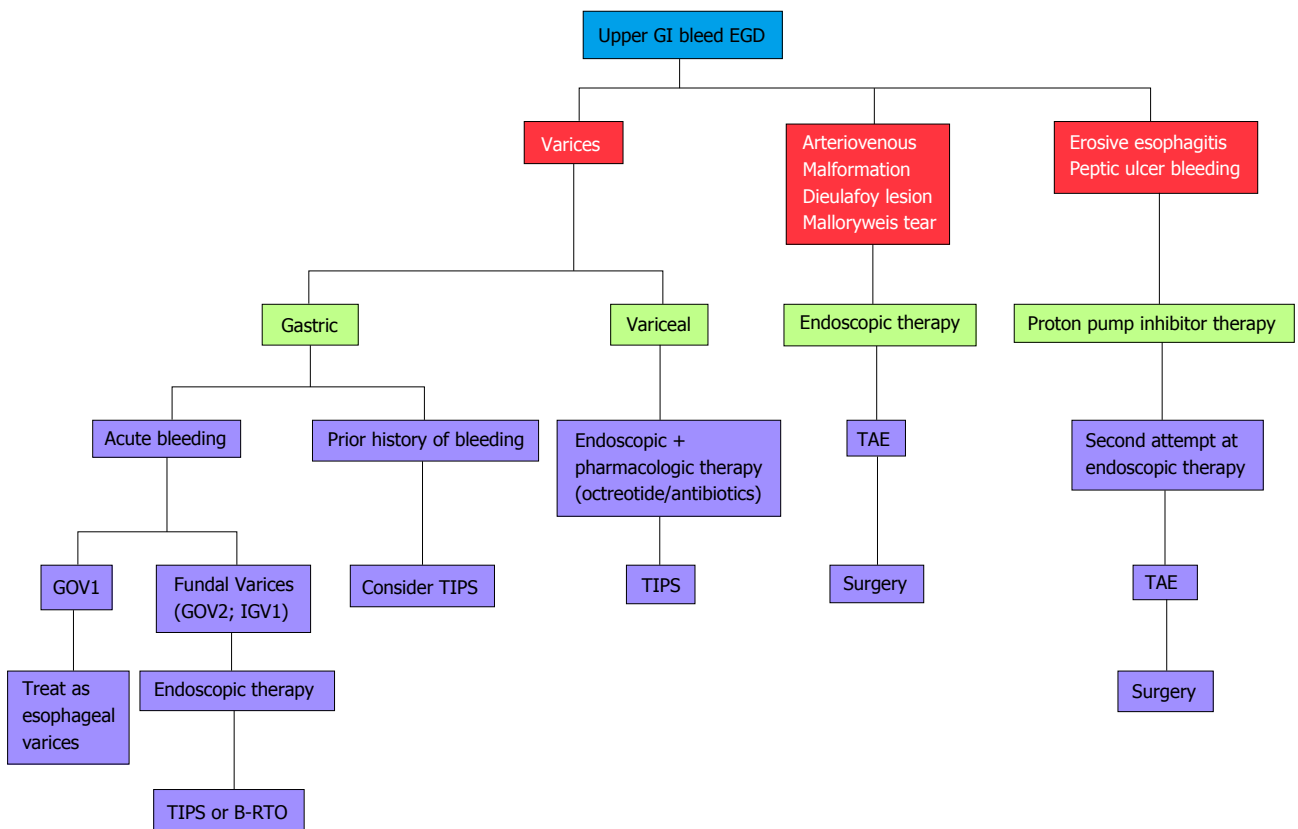


Figure 3 Algorithmic approach to upper gastrointestinal bleeding. EGD: Esophagoduodenoscopy; TAE: Transcatheter arterial embolization; TIPS: Transjugular intrahepatic portosystemic shunt; GOV1: Gastroesophageal Varices 1; B-RTO: Balloon-occluded retrograde transvenous obliteration; GI: Gastrointestinal; IGV: Isolated gastric varices.

no radiologic modalities that have been proven to show diagnostic utility in the setting of UGIB. When endoscopy proves unsuccessful, however, there are several radiologic alternatives that can potentially treat the underlying lesion including TIPS, B-RTO, and TAE. Figure 3 provides an algorithmic approach to a patient with UGIB.

LOWER GI BLEED

The incidence of LGIB is approximately 20 per 100000 persons^[29] with an associated all cause mortality of 3.9%^[30].

In addition, patients who experience LGIB during a hospitalization for another condition seem to have a higher mortality when compared to those who are admitted with LGIB as their chief complaint^[31]. The differential diagnosis of LGIB ought to include UGIB being that 10%-15% of patients with severe hematochezia are found to have an upper GI source^[32]. Hemorrhoidal bleeding is the most common cause of LGIB followed by diverticular bleeding, and next by vascular ectasia^[33]. This section will dissect the various tools available for approaching a patient with a LGIB.

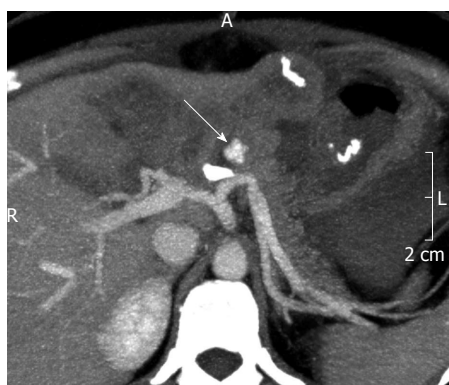


Figure 4 Unenhanced computed tomography showing contrast media extravasation anterior to the splenic artery^[61].

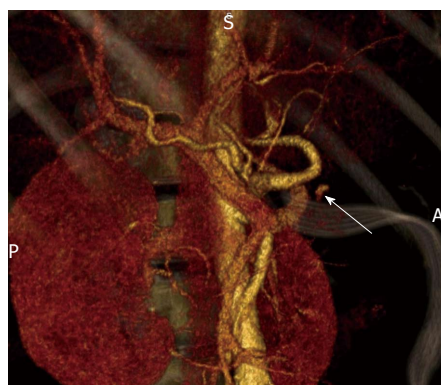


Figure 5 Multiphasic multidetector computed tomography angiography (arterial phase) showing extravasation anterior to the splenic artery^[61].

Techetium-labeled red blood cell bleeding scan

Techetium-labeled red blood cell bleeding scan (RBC scan), or scintigraphy, is typically the first approach utilized in the localization of an active LGIB. Red blood cells tagged with technetium (99 mTc) are re-injected into the patient with sequential monitoring for extravascular activity. Frequently, images are obtained over 30 to 90 min, and then, if necessary, every few hours for up to 24 h.

Brunnler *et al.*^[34] performed a retrospective analysis of 92 patients evaluating the role of techetium-labeled red cell bleeding scan in patients with OGIB. Scintigraphy was able to demonstrate a positive result in all cases, with a false positive rate of only 4%. Notably, a heparin provocation test (a diagnostic approach to localizing the lesion in OGIB which will be later discussed in further detail) increased the diagnostic yield by 46% in patients with a primary negative scan. Scintigraphy ($\pm$ heparin provocation) is a reliable modality in localizing the lesion in approximately half of OGIB cases.

Advantages and disadvantages

There are two major advantages to the use of scintigraphy. The first is that scintigraphy only requires an active bleeding rate of 0.1 to 0.4 mL/min making it the most sensitive modality in detecting an active GIB^[35,36]. The second benefit is the immediate ability to test patients without any need for preparation prior to the procedure.

There are two major disadvantages to the use of scintigraphy. First, it does not offer any therapeutic capabilities, therefore in the setting of a positive scan a second procedure, *i.e.*, endoscopy, catheter directed angiography or surgery must subsequently be performed^[37]. A second major detriment to the use of scintigraphy is that it can only localize an active bleed to a general area and not a specific location. This occurs as blood moves secondary to peristaltic or anti-peristaltic actions. As a result, accuracy rates in the literature have varied ranging from 24%-91%^[38-40]. Hunter *et al.*^[41] demonstrated these difficulties by evaluating the outcome of 203 patients who underwent scintigraphy for LGIB. Scintigraphy was positive in 52 patients (26%), who subsequently underwent further evaluation. A definitive bleeding site was identified in

22 patients with only 8 cases correlating to scintigraphy.

Multiphasic multidetector computed tomography angiography

Until recently, computed tomography only played a minor role in the evaluation of GIB. The introduction of multiphasic multidetector computed tomography angiography (MDCTA) transformed this modality into an important tool in detecting, localizing, and characterizing active GIB. Most institutions utilize MDCTA with a 64-detector-row scanner in three phases: unenhanced, arterial, and portal-venous^[42]. Comparing the arterial and portal-venous phase images to the unenhanced images is critically important in avoiding false-positive results. Active GIB often appears as extravasated contrast material in the bowel lumen or bowel wall during the arterial phase and increases throughout the portal-venous phase (> 90 HU, but typically ranging from 115-300 HU)^[42-45]. Figures 4 and 5 depict the various phases of MDCTA in the setting of an active arterial bleed anterior to the splenic artery.

Wu *et al.*^[46] performed a meta-analysis evaluating the accuracy of CTA in the diagnosis of acute GIB, which included a total of 9 studies with 198 patients in total. The pooled results showed a sensitivity of 89% and specificity of 85% proving MDCTA to be an accurate tool in the setting of an acute GIB. Recently published were the results of a 5-year prospective trial evaluating the utility of MDCTA in active GIB^[47]. There were a total of 113 patients enrolled with clinical signs of active GIB who underwent MDCTA. The investigators found the overall sensitivity, specificity, positive and negative predictive values of MDCTA to be 86%, 100%, 100%, 61% and 89%, respectively, concluding that MDCTA is an accurate first line screening method for detection and localization of GIB.

Advantages and disadvantages

Angiography requires active blood loss of at least 0.5 mL/min^[48] in order to visualize the bleeding vessel. The major advantage of MDCTA over other modalities is that it does not require any preparation and yet localization is accurate.

A major disadvantage to the use of MDCTA is the substantial radiation exposure the patient incurs. Other serious complications include cardiac arrhythmias, bowel ischemia, and a rebleeding rate in as high as 50% of patients^[49]. Lastly, the use of contrast media often times precludes patients with renal failure. As always, the risk benefit ratio must be taken into account of worsening renal insufficiency *vs* on-going GIB. The literature varies in defining a creatinine clearance (CrCl) (most institution use a threshold value for creatinine of 1.7 and CrCl of 45 mL/min) precluding patients from MDCTA, however pre-procedural hydration and administration of n-acetylcysteine has been proven to prevent contrast induced nephropathy and worsening renal function in high-risk patients^[50]. It should be noted that there are no contraindications to the use of intravenous contrast for end-stage renal patients on chronic hemodialysis.

Computed tomography enterography and magnetic resonance enterography

Computed tomography enterography (CTE) and magnetic resonance enterography (MRE) are non-invasive alternatives used to determine the etiology of OGIB, particularly small bowel pathology^[51]. Huprich *et al*^[52] evaluated the findings of CTE in 22 outpatients with OGIB^[53]. This retrospective study compared findings on CTE with capsule and traditional endoscopic, surgical, and angiographic findings. CTE findings were positive for a bleeding source in 45% of patients, with 80% of findings also positive with subsequent capsule endoscopy or clinical diagnosis. In addition, CTE correctly identified 3 lesions undetected by capsule endoscopy. A recent prospective study evaluated the use of CTE in 35 patients with OGIB, both overt ($n = 20$) and occult ($n = 15$)^[54]. They found positive findings in 46.9% of CTE scans and as a result 12 patients underwent subsequent laparotomy. The surgical findings in all 12 cases were in conformity with the findings on CTE leading the authors to conclude CTE to be a useful diagnostic tool in the evaluation of both overt and occult OGIB. Since MRE is relatively new to clinical practice, there is limited data available for its use in GIB and thus may be an area of further research in the future.

Advantages and disadvantages

There are several advantages to the use of CTE and MRE, namely the ability to visualize the thickness of the bowel wall in its entirety and provide a global overview of visceral structures within the abdomen^[54]. This allows for not only diagnosis but also staging of small bowel pathology.

The major disadvantage of CTE, but not MRE, is the radiation exposure to the patient^[48].

Another detriment to the use of both CTE and MRE is that they do not have the necessary capabilities to perform therapeutic maneuvers. In addition, pre-existing high attenuation material within the bowel may decrease the diagnostic yield of CTE. Lastly, patients with on

chronic hemodialysis should be precluded from receiving gadolinium due to risk of nephrogenic systemic fibrosis.

Provocative angiography

Provocative angiography is a salvage method used to localize the source of bleeding in patients with OGIB when all other radiologic and endoscopic approaches have been exhausted^[55]. The technique involves the addition of anticoagulants, vasodilators and fibrinolytics during angiography to induce a hemorrhagic state with intent to localize the bleeding vessel.

The use of provocative angiography remains controversial due to theoretical increased risk of exacerbating the bleed. A recent prospective analysis evaluated the diagnostic yield and complication rate in 36 patients with LGIB who underwent provocative angiography^[56]. Each patient was subjected to heparin followed by selective transcatheter injection of a vasodilator and plasminogen activator into the vessel of high suspicion. They found that 31% of angiograms resulted in visible extravasation and the identification of a source of LGIB in 33% of cases overall. A total of 31% underwent successful definitive treatment of LGIB with only one embolization-related complication requiring surgical resection. This led the authors to conclude provocative angiography to be a safe and effective means for eliciting the source of occult LGIB leading to a definitive therapy in up to 33% of patients.

The bottom line

Colonoscopy remains the mainstay to diagnose the etiology of LGIB in most patients who are hemodynamically stable. In a hemodynamically unstable patient with active bleeding, scintigraphy and MDCTA are two radiologic approaches that can be utilized to identify the source. In a patient with intermittent GIB or OGIB, scintigraphy and MDCTA may prove futile. In this case, capsule endoscopy, single-balloon/double-balloon/spiral enteroscopy, and CTE are modalities used to identify and potentially treat the underlying lesion. Provocative angiography is a last resort tool when all other endoscopic and radiologic alternatives have been exhausted. There is relatively limited data on the use of MRE in OGIB, and thus may be an area requiring further research. Figure 6 provides an algorithmic approach to a patient with LGIB.

THE ROLE OF ENDOSCOPIC MARKINGS

Gastroenterologists may facilitate interventional radiologic procedures through the placement of endoscopic markings typically involving a MRI compatible metallic clip. Eriksson *et al*^[57] recently evaluated the utility of endoscopic marking with a metallic clip in TAE for patients with UGIB. They placed a metallic clip in the fibrous edge of the ulcer adjacent to the bleeding point in 13 patients. In 10 patients, subsequent TAE was indicated due to persistent or recurrent bleeding. The artery was embolized with microcoils in close proximity to the clip. Of

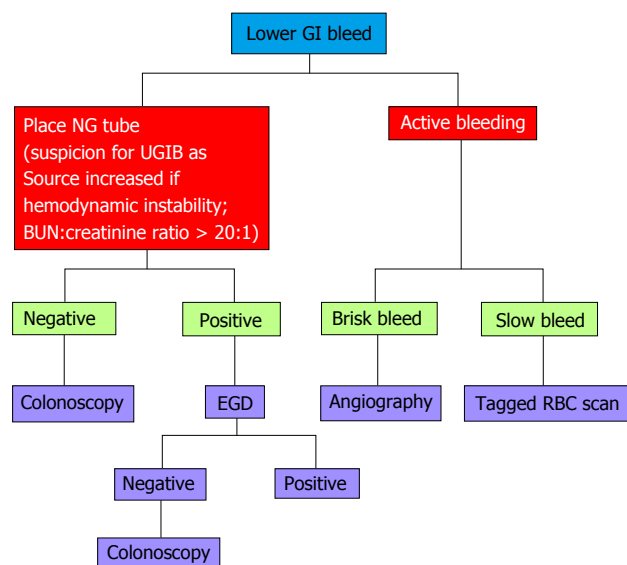


Figure 6 Algorithmic approach to lower gastrointestinal bleeding. EGD: Esophagoduodenoscopy; GI: Gastrointestinal; NG: Nasogastric; BUN: Blood urea nitrogen; RBC: Red blood cell; UGIB: Upper gastrointestinal bleeding.

the 10 patients, hemostasis was achieved in 8 patients and in 6 the clip was essential in identifying the bleeding vessel. Another recent prospective study evaluated the utility of rotational angiography after endoscopic marking in patients with acute bleeding ulcers with similar results^[58].

The bottom line

Endoscopic markings facilitate interventional radiologic procedure by helping to accurately localize the bleeding focus thus enhancing the possibility that the correct vessel is embolized. This can minimize the risk of recurrent bleeding after embolization and preclude further unnecessary procedures.

CONCLUSION

Recently, there have been many radiologic advances in the diagnostic and therapeutic approach to GIB. In order to optimize patient care and practice cost effective medicine, it is essential for the clinician to familiarize themselves with advantages and limitations of these new modalities. Since the approach to UGIB differs greatly from LGIB, it is necessary to have an algorithmic approach to a patient presenting with each such as those we have suggested. The recommendations and the strengths and weaknesses of the modalities discussed in this review are based on the best evidence available at this time.

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Protein kinases are potential targets to treat inflammatory bowel disease

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Abstract

Protein kinases play a crucial role in the pathogenesis of inflammatory bowel disease (IBD), the two main forms of which are ulcerative colitis and Crohn's disease. In this article, we will review the mechanisms of involvement of protein kinases in the pathogenesis of and intervention against IBD, in terms of their effects on genetics, microbiota, mucous layer and tight junction, and the potential of protein kinases as therapeutic targets against IBD.

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Key words: Inflammatory bowel disease; Protein kinase; Barrier function; Microbiota; Genetics

Core tip: The roles of protein kinases in the pathogenesis and intervention of inflammatory bowel diseases (IBD) are emerging. In this article, we will review the specific roles of different protein kinases in the pathogenesis of IBD, classify these protein kinases into different categories based on their fundamental functions in IBD, and describe substantial new mechanistic insights into the pathogenesis of IBD, highlighting protein kinases as potential intervention targets against IBD.

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INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD), two main forms of inflammatory bowel disease (IBD), are relapsing, idiopathic intestinal inflammatory conditions, caused by inappropriate and continuing immunologic responses to aberrant intestinal microorganisms in genetically susceptible individuals under certain environmental conditions^[1].

UC and CD differ^[2] with each other dramatically in different respects. UC is confined to the superficial area of the intestinal wall, whereas CD is transmurally distributed throughout the entire digestive tract but in a discontinuous way. The lesion is patchy with "lead pipe sign" in UC, but many polyps with "string sign" are often observed in CD. UC displays a Th2-like immune response, while CD shows a Th1 dominant response. Antineutrophil cytoplasmic antibodies were found in 65% of UC cases and 5%-10% of CD cases, and antibodies to yeast *S. cerevisiae* were found in 60%-70% of CD cases and 10%-15% of UC cases^[3]. Meanwhile, UC and CD share many similarities, such as neutrophil infiltration and epithelial barrier dysfunction. Despite the fact that there is no cure for IBD thus far, enormous progress about the pathogenic mechanisms of this inflammatory disorder has been around the corner in different aspects, such as genetics, regulatory immunology and microbiome.

The signaling pathways mediated by protein kinases have drawn much attention for connecting external stimuli including hostile environmental stresses with internal biological responses, such as intestinal inflammation. Protein kinases can be defined as enzymes which add phosphate

Table 1 Protein kinases related to inflammatory bowel disease genetics

Kinase	IBD	Ref.
ERK1	CD	[8]
p38	CD and UC	[9]
TYK2	CD and UC	[10]
JAK2	CD and UC	[11]
GCKR	CD	[12]
CDKAL1	CD	[13]
LRRK2	CD	[15]

ERK1: Extracellular signal-regulated Kinase; TYK2: Tyrosine kinase 2; JAK2: Janus kinase 2; GCKR: Glucokinase regulator; CDKAL1: Cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like; LRRK2: Leucine-rich repeat kinase 2; IBD: Inflammatory bowel diseases; UC: Ulcerative colitis; CD: Crohn's disease.

(called phosphorylation) to the side chain of serine, threonine or tyrosine of substrate molecules. This modification alters the biological function of the substrate, such as changing enzyme activity, cellular distribution, and even causing diseases^[4,5]. In this review, we will shed light on the roles of protein kinases in the pathogenesis of intestinal inflammation and describe some new mechanistic insights into the intervention of IBD, which targets at protein kinases.

PROTEIN KINASES AND GENETIC FACTORS

Genome-wide association studies demonstrated that genetic factors are very crucial in the individual susceptibility to IBD, for example, relatives of UC patients including twins display almost ten times greater risk of UC than non-relatives^[6,7]. As shown in Table 1, major IBD susceptibility regions on chromosomes 16 and 6 contain some genes encoding protein kinases like extracellular signals-regulated kinase 1 (ERK1)^[8] and p38^[9]. Several single-nucleotide polymorphisms in tyrosine kinase 2^[10] and Janus kinase 2^[11] were identified in IBD patients. Glucokinase regulator has also been associated with the risk of CD^[12]. The cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like plays an important role in susceptibility to CD, psoriasis and type II diabetes^[13,14]; leucine-rich repeat kinase 2 is identified to be related to the pathogenesis of CD^[15].

PROTEIN KINASES AND MICROBIOTA

Up to 10^{14} individual bacteria in the human gastrointestinal (GI) tract^[16], together with the mucous layer where the microbiome lives in, constitute the first line of defense in host against hostile external environment, modulating GI tract development, maintaining immune homeostasis, and regulating host metabolism rate. The bacterial abnormality plays a dominant role in the onset and development of IBD.

Commensal bacteria and host innate immune system

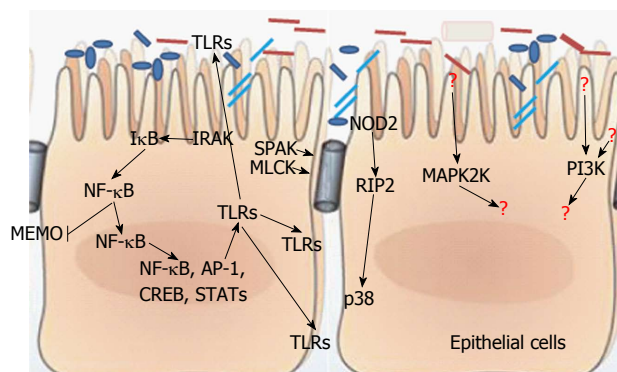


Figure 1 Intestinal epithelial cells use a variety of different molecules including protein kinases to monitor the presence of microbial pathogens, commensal bacteria, or host-generated products. Pathogen-recognition receptors, including TLRs, NOD2, and NLRs, are located on and within the cell where they recognize different threats. Recognition results in NF-κB activation, leading to the production of cytoprotective factors when stimulated by commensal bacteria and proinflammatory products when stimulated by potential pathogens, or blocks the activity of NEMO. Some other undefined factors can stimulate protein kinases such as PI3K or MAPK2K to regulate the process of intestinal inflammation. TLR: Toll like receptor; IRAK: Interleukin 1 receptor associated kinase; IκB: Inhibitor kappa B; NF-κB: Nuclear factor kappa B; SPAK: Ste20 like proline/alanine rich kinase; NEMO: NF-kappa-B essential modulator; MLCK: Myosin light chain kinase; CREB: cAMP response element binding protein; STAT: Signal transducer and activator of transcription; NOD2: Nucleotide-binding oligomerization domain-containing protein 2; NLRs: NOD-like receptors; RIP2: Receptor-interacting protein kinase 2; PI3K: Phosphoinositide 3 kinase; MAPK2K: Mitogen-activated protein kinases 2 kinase; AP-1: Activator protein 1.

evolve together and thus maintain mucosal immune homeostasis by balancing inflammatory responses and regulating a variety of bacteria-triggering signal transduction pathways^[17], such as uncoupling nuclear factor (NF)-κB or mitogen activated protein kinase (MAPK) dependent target genes in a negative feedback manner^[18,19]. The host's innate immune system is poised to be triggered by signs of bacterial challenge, specially, some pathogen-associated molecules such as flagellin, peptidoglycan, lipoteichoic acid, or lipopolysaccharide, together called pathogen-associated molecular patterns which can wake up the host innate immune system^[20,21] and be further sensed by pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) or the nucleotide-binding oligomerization domain containing protein (NOD)-like receptors^[22] (Figure 1). These PRRs would then induce the activation of signaling cascades, mostly MAPK and NF-κB pathways. In terms of MAPK pathways, it follows MAP4K-MAP3K-MAP2K-MAPK pattern, and then, the activated MAPK undergoes translocation to the nucleus to activate molecules required for gene transcription, including inflammatory molecules^[23,24]. For example, anthrax toxin can induce macrophage death by inhibiting the p38 signaling pathway^[25,26], and MAPK-activated protein kinase 2 plays an important role in the pathogenesis of *Clostridium difficile*-associated intestinal inflammation^[27]. For the NF-κB pathway, after being activated by IκB kinase complex, it phosphorylates α subunit of IκB, the inhibitor of NF-κB. Phosphorylation of IκB, accompanied by its ubiquitination and proteolytic degrada-

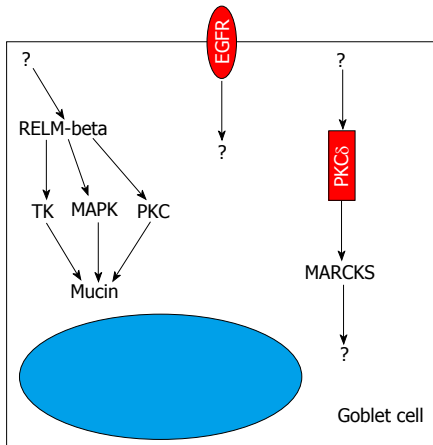


Figure 2 Intestinal Goblet cells employ different mechanisms including protein kinase related pathways to modulate the secretion of mucus, such as pathways related to tyrosine kinase, protein kinase C delta, myristoylated alanine-rich C-kinase substrate or receptors with tyrosine kinase activity such as epidermal growth factor receptor. MARCKS: Myristoylated alanine-rich C-kinase substrate; EGFR: Epidermal growth factor receptor; TK: tyrosine kinase; RELM-beta: Resistin-like molecule beta; PKCδ: Protein kinase C delta; MAPK: Mitogen activated protein kinase.

tion, results in exposure of the nuclear localization signal (NLS) on the now unbound NF- κ B^[28], which will further facilitate nuclear translocation of NF- κ B and be followed by transcriptional activation of many genes. In addition, even being regarded as an molecule which can promote inflammatory responses, an anti-inflammatory effect of NF- κ B was noticed; absence of NF- κ B essential modulator kinase causes spontaneous severe colitis, but commensal bacteria can stimulate the NF- κ B pathway to protect the host from exacerbating consequence^[29]. Blockage of epithelial NF- κ B pathway will deteriorate this colitis by increasing the translocation of bacterial to the mucosa^[30]. Besides the MAPK and NF- κ B pathways, some other signaling pathways are also very important, for example, after recognition of *Salmonella enterica* serovar *Typhimurium* curli fibrils in the gut, the TLR2-phosphatidylinositol 3 (PI3)-kinase pathway will be stimulated to tight the epithelial barrier^[31]. However, PI3 kinase signaling promotes *Campylobacter jejuni*-induced colitis through neutrophil recruitment in mice^[32]. RIP2 tyrosine kinase activity is required for NOD2-dependent autophagy process, but plays a dual role in this process. RIP2 sends a positive autophagy signal through activation of p38 MAPK and further relieves repression of autophagy mediated by the phosphatase PP2A^[33]. Not like NOD2 whose signaling induces cryptidins, MyD88-mediated TLR signaling induces RegIIIg and α -defensins, and more importantly, regulates bacterial infection-related mucosal immunity^[34-36]. In parallel, protein kinase C (PKC) can mediate the function of MyD88 adaptor-like (Mal) molecule in the maintenance of epithelial barrier integrity^[37].

PROTEIN KINASES AND BARRIER DYSFUNCTION

Basically, IBD is characterized by passive leaky diarrhea

and compromised intestinal barrier function. Except for the fact that commensal bacteria function as primary line of defense, protein kinases are also important in regulating the intestinal barrier function.

Mucus layer

The luminal side of the intestine is covered by a mucus layer which provides protection to the mucosa from mechanical damage and invasion of pathogens, and, together with commensal bacteria, constitutes a physical barrier between the epithelium and luminal contents including pathogenic bacteria, viruses, and parasites^[38,39]. This gel-like mucus layer can be divided by two distinguished layers-the outer and inner layers. The vast majority of intestinal bacteria, viruses and even parasites live in the flowing outer mucus layer; the inner layer is, however, an unstirred and relatively sterile layer adjacent to epithelial surface. The sterility of the inner layer accredits to the preservation of huge amounts of defensins, cathelicidins, and cryptidins with important function of anti-intestinal pathogens. Mucin coding gene *muc2*^{-/-} mice demonstrated spontaneous colitis because of increased transepithelial permeability^[40], in which bacteria can stick to the surface of the intestinal mucosa, which facilitates the translocation of bacteria into lower crypts and epithelial cells, thus triggering an inflammatory response^[39,41]. Protein kinases are involved in the integrity and maintenance of these mucus layers (Figure 2). Epidermal growth factor receptor (EGFR), harboring tyrosine kinase (TK) activity, has critical functions in development, growth, differentiation, proliferation and repair of epithelial cells^[42,43]. After stimulation by EGFR ligands such as transforming growth factor- α and epidermal growth factor, epithelial cells can develop into a mucous phenotype^[44,45]. However, inhibition of EGFR tyrosine kinase activity can abolish the effects of EGFR ligands on mucus production both *in vivo* and *in vitro*. PKC δ stimulates the secretion of mucin in the epithelium *via* regulation of myristoylated alanine-rich protein kinase C substrate pathway^[46]. Treatment of epithelial cells with PD98059 (MEK inhibitor) can inhibit MAPK activity and block the expression of terminal differentiation markers, such as sucrase-isomaltase, ITF, and MUC2, thereby interfering with the production of mucin^[47]. Some kinases like ERKs, TK, and PKC^[48] can regulate the production of mucin by mediating the activity of resistin and resistin-like molecule-beta; cathelicidin up-regulates MUC1 and MUC2 expression through MAPK pathway to modulate mucus synthesis^[49].

Protein kinase and epithelial junctions

The intestinal monolayer is characterized by polarization of apical and basolateral sides. The apical membrane is generally impermeable to hydrophilic solutes and contributes predominantly to mucosal barrier^[41]. Among the most important structures to determine paracellular permeability of the intestinal barrier are the epithelial tight junctions (TJs), which are made up of multiple proteins such as occludin and claudins^[50]. Occludin as the first

identified TJ^[51], plays an important role in epithelial/endothelial barrier integrity, and disruption of occludin regulation is an important aspect of a number of diseases^[52-54]. The claudins, as a group of TJ proteins with approximately 24 members, interact with numbers of other cell structures and affects junctional function^[55-58]. Claudins are expressed in a tissue-specific manner and may show distinct functions, for example, in the colon are expressed the claudins-1, 2, 3, 4, 5, 7, and 8; the claudin-2 is a pore-forming TJ protein, but claudins-1 and 4 are barrier tightening proteins^[59-63]. 12-O-tetradecanoylphorbol-13-acetate can increase transepithelial electrical resistance by activating different isoforms of PKC and enhancing the expression of TJ proteins ZO-1, 2, occludin and claudin-1^[64,65]. Ca²⁺/calmodulin-dependent protein kinase II can compromise endothelial barrier function^[66]. Ras-transfected epithelial cells demonstrated compromised barrier function; however, inhibition of the MAPK signaling pathway can restore the morphology of epithelial cells and the TJ assembly. Further, the phosphorylation of tyrosine residues in occludin and ZO-1 may be crucial for the formation of TJ^[67]. cAMP-dependent protein kinases regulate epithelial barrier function by phosphorylation of claudin-3^[68,69].

Generally, at least two relatively independently routes known thus far are responsible for communication between host and external environment through paracellular pathway, both of which can be regulated by protein kinases^[70-72]. The size-selectivity related paracellular pathway is one of the two routes, which facilitates transepithelial passage of different size of molecules, such as lipopolysaccharides^[71,72], and can be regulated by protein kinases, such as MAPKs, Ste20 like proline/alanine rich kinase (SPAK)^[73], PKC^[64,65] and myosin light chain kinase (MLCK)^[74]. Another route, also called charge-selectivity route, is composed of pore-forming proteins claudins^[75-77]. Dysfunction of these two routes, either size-dependent or charge-dependent pathway, may result in the abnormality of overall epithelial TJ, which provides an even more leaky gut. This situation will facilitate the contact of intestinal microorganisms including bacteria, viruses and parasites with the host's immune system, resulting in altered production of inflammatory mediators that contribute to the compromised barrier function.

Mucosal permeability is influenced by many different factors in there distinct ways. Except the mucus layer, microbiota and epithelial cells themselves mentioned above, genetic factors play crucial roles in the regulation of intestinal barrier function^[6]; innate and adaptive immune systems can interfere with epithelial permeability in a dramatic manner^[78]; autonomic nerves, like enteric glial nerve ablation, can perish epithelial permeability to develop fulminant jejunoileitis^[79]. However, barrier dysfunction itself, like in MLCK^[74] and SPAK^[73] gene modified mice, does not necessarily mean that the mice are destined to develop intestinal inflammation, implying formidable compensation in host.

PROTEIN KINASES AND PATHOGENESIS OF IBD

MAPKs

Notably, protein kinases play very crucial roles in many aspects of pathogenesis of IBD, highlighting their emerging roles as potential therapeutic targets against IBD. Besides the NF- κ B pathway, the MAPK signaling pathway is another highlighted pathway involved in many different diseases including IBD^[80]. The activation of MAPK-ERK1/2 phosphorylates the downstream proinflammatory proteins such as cytosolic phospholipase A2 and some transcription factors such as activated proteins, Ets-1, Elk and c-myc. Interestingly, ERK1/2, by a study using an ERK1/2 inhibitor, was found to play an important role in the function of immune cells and other cell types during IBD, by regulating some pro-inflammatory mediators [such as interleukin-1 (IL-1)] related signaling transduction^[81,82], evidenced by their enhanced expression and phosphorylation status during IBD^[83,84]. Furthermore, the "tightening" junction protein claudin-4, which plays an important role in epithelial barrier function, is regulated by protein kinase ERK^[85]. By inducing Akt but blocking p38 signaling, *Lactobacillus* GG prevents cytokine-induced apoptosis of intestinal epithelial cells, indicating p38 and Akt as key mediators of epithelial barrier function^[86,87]. p38 activity is increased significantly in tissues from IBD patients and in mouse models of colitis^[83,84,88], in which inhibition of p38 lowers KC (IL-8) and IL-6 production. A similar result was reported that *heat-killed Lactobacillus brevis* phosphorylates p38 kinase to regulate the expression of proinflammatory cytokines such as TNF- α , and to improve intestinal integrity^[89]. JNK1/2 kinase activity was enhanced in IBD inflamed tissue and blockage of JNK1/2 in experimental colitis reduced the production of proinflammatory cytokines^[84,90,91].

Serine and threonine kinases

SPAK: SPAK is a serine/threonine kinase containing an N-terminal series of proline and alanine repeats (PAPA box) followed by a kinase domain, an NLS, a consensus caspase cleavage motif, and a C-terminal regulatory region^[92]. Colonic SPAK presents as a unique isoform that lacks the PAPA box and F-helix loop in the N-terminus^[93]. The diversity of domains in SPAK might be associated with a variety of biological roles. For example, SPAK was reported to play roles in cell differentiation, transformation and proliferation, and regulation of chloride transport^[94,95]. More importantly, a linkage has been established between SPAK and inflammation. SPAK as an upstream kinase to Na⁺-K⁺-2Cl-co-transporter 1 (NKCC1), can phosphorylate NKCC1 and play an important role in inflammation^[96]. Further, we have demonstrated that SPAK can activate the p38 pathway^[93]. Decreased expression of SPAK contributes to enhanced intestinal barrier, and thus SPAK knockout mice were more tolerant to experimental colitis induced by dextran sodium sulphate (DSS) with

decreased intestinal microorganism translocation into the mucosa and inhibition of the production of inflammatory mediators^[97].

MLCK: MLCK is named after its phosphorylation of MLC to induce contraction of the perijunctional actomyosin ring, and it is indispensable for tumor necrosis factor (TNF) related barrier dysfunction. In turn, TNF can induce the phosphorylation and transcription of MLCK^[98,99]. Constitutive MLCK activation in the intestinal epithelium increases intestinal paracellular permeability and aggravates the severity of colitis in mouse models. However, blockage of MLCK activation can increase significantly the intestinal barrier function and ameliorate DSS-induced colitis^[100].

PKC: PKC has a variety of isoforms that are involved in the pathogenesis of IBD by their effect on the mucus layer^[101], microbiota^[34-37], cell junction^[64,65] and immune system. Specially, PKC θ plays an important role in T cell receptor activation and signaling^[102], and PKC δ is crucial for B cell tolerance^[103,104]. PKC η can control CTLA-4-mediated regulatory T cell (Treg) function^[105]; however, PKC- θ inhibits Treg function, implying its blocking of Treg-mediated suppression. Inhibition of PKC- θ stimulates Treg, resumes compromised Treg function in rheumatoid arthritis patients, and enhances protection against experimental colitis in mice. As a result, PKC- θ mediates negative feedback on Treg cell function^[106].

CONCLUSION

Protein kinases and the related signaling transduction pathways are involved in many physiological and pathological processes such as development, inflammation (for example, intestinal inflammation) and tumorigenesis. In this review, we shed some light on the roles of protein kinases in terms of their effect on IBD-related genetic factors, microbiota, mucus layer, epithelial cell and the tight junction. Further studies are needed to explore the feasibility and application of these signaling pathways in the control of IBD.

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