Original Article

The Correlation between The Images from Confocal Endomicroscopy and The Change of Gastric Mucosa

Pittayanon R[1]
Wisedopas N[2]
Rerknimitr R[1]

ABSTRACT

Background: Gastric intestinal metaplasia (GIM) is a premalignant lesion for gastric cancer. Although magnifying Fujinon intelligence chromoendoscopy (FICE) or narrow band imaging (NBI) provides a higher sensitivity over white light endoscopy to diagnose GIM, its sensitivity is still suboptimal. Recently, probe-based confocal laser endomicroscope (pCLE) has been introduced as a highly magnified imaging (x1,000) that may be comparable to standard histology. However, only few reports on the results of pCLE to diagnose GIM are available.

Objective: To evaluate the correlation between the image from pCLE and the change of gastric mucosa.

Methods: Fifty patients, previously diagnosed GIM, underwent EGD with magnified FICE plus pCLE by single endoscopist. Standard and magnified (x100) FICE were used as a screening tool to target the GIM lesion for pCLE. Biopsies were taken from both GIM and non-GIM suspicious epithelium in each patient. Our goal standard was histology that read by a clinically-blinded GI pathologist. The results were assessed by agreement (kappa) between images from pCLE and pathological reports.

Results: Of those 43 patients with suspected GIM by pCLE, 38 were confirmed GIM by histology. The kappa is 0.81 which is the almost perfect agreement of both diagnostic tools. There was no gastric cancer detected in any patients.

Conclusion: There is a very good correlation between the image from pCLE and the change of gastric mucosa, especially GIM.

Key words : Confocal laser endomicroscope, gastric intestinal metaplasia

[Thai J Gastroenterol 2012; 13(3):140-146.]

[1]*Division of Gastroenterology, Department of Medicine, Faculty of Medicine, King Chulalongkorn Memorial Hospital, Chulalongkorn University, Bangkok 10330, Thailand.*
[2]*Department of Pathology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.*
Address for Correspondence: *Rungsun Rerknimitr, M.D., Division of Gastroenterology, Department of Medicine, Faculty of Medicine, King Chulalongkorn Memorial Hospital, Chulalongkorn University, Bangkok 10330, Thailand.*

Introduction

Gastric cancer remains the second leading cause of cancer related death in the world from recently data with the mortality rate of 16.3 per 100,000 in men and 7.9 per 100,000 in women[1]. The incidence and mortality rate are predominant in East Asia[1] and in the fourth rank in Thailand[2]. Usually, gastric cancer is asymptomatic in early stage; therefore, most patients are in the advanced stage and incurable at diagnosis. The pathogenesis of intestinal type gastric cancer is sequential and multistep pathway. Moreover, the direction of pathway can be reversible unless carcinoma[3]. (Figure 1) The strategies which can detect preneoplastic and neoplastic transformations are very beneficial because only early gastric cancer can potentially be cured by endoscopic treatment.

The recent study, Imraporn and colleagues[4,5], showed that narrow-banded imaging with magnification (NBI-ME) had a better sensitivity for gastric intestinal metaplasia (GIM) detection than a standard white light endoscopy (73% VS. 13%) and could detect two early gastric cancers from tissue pathology in 1-year follow-up cohort study. However, NBI-ME cannot differentiate among GIM, dysplasia and gastric cancer.

Confocal laser endomicroscope (CLE) is a novel endoscopic device which is available in 2005[6]. CLE is a powerful instrument for performing high-resolution (x1,000 time) imaging (Figure 2) to enable real-time histology by displaying video imaging and/or optical biopsy at the time of endoscopic examination (in vivo histology)[7,8]. There is two types of CLE; endoscopic-based CLE (eCLE) and probe-based CLE (pCLE)[9]. The eCLE is a fluorescein-based CLE that integrated a confocal fluorescence microscope into the distal tip of a conventional 12.8-mm diameter flexible endoscope (Pentax, Tokyo, Japan) (Figure 3). On the other hand, Mauna Kea Technologies (Paris, France) designed a 2.5-mm catheter probe with a semiconductor laser that oscillates at 685 nm with a scanning field of 30,000 pixels, known as pCLE[10] (Figure 4). However, eCLE imaging system provides superior resolution confocal imaging using an incident 488-nm wavelength laser, and enables the detection of fluorescence of 505-585 nm wavelength. In addition, it provides Z-axis which makes it possible to scan at different depths and visualize different histologic structures, together

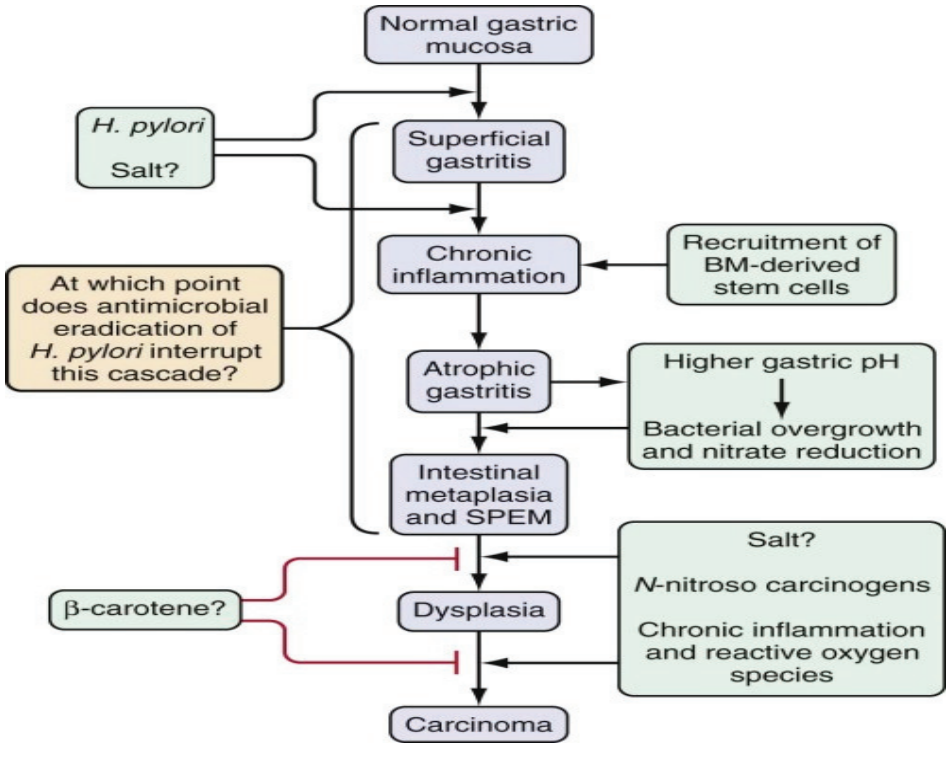

Figure 1. Multistep pathway in the pathogenesis of intestinal-type gastric cancer (Correa pathway)[3] (BM, bone marrow; SPEM, spasmolytic polypeptide-expressing metaplasia).

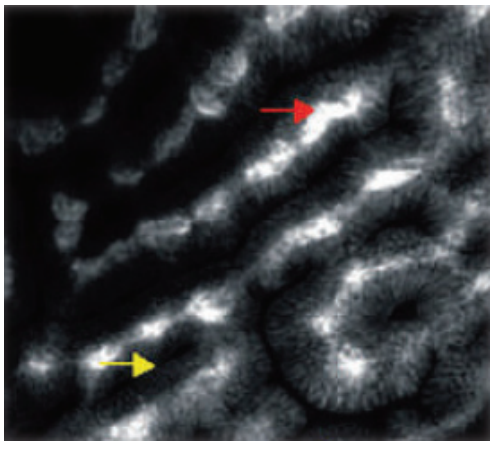

Figure 2. The pCLE revealed normal gastric mucosa.

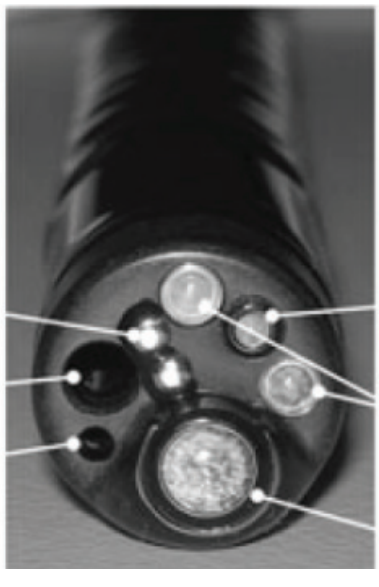

Figure 3. The eCLE probe.

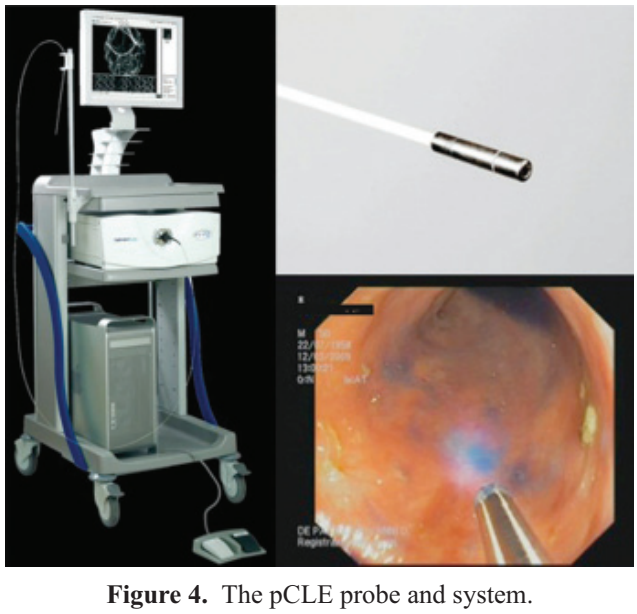

Figure 4. The pCLE probe and system.

with a 0.7 μm lateral resolution whereas pCLE has a lateral resolution of 1 μm[10, 11]. The eCLE has a field of view of 475 x 475 μm with a variable imaging plane depth of up to 250 μm. Although, the pCLE system has a fixed imaging plane, 200 μm maximum depth, its use is more versatile as it can be used with any endoscope. Moreover, the temporal resolution of the pCLE system is higher (12 images/second) compared with the eCLE (± 1 image/second)[12] (Table 1).

Fluorescein, is a slightly acidic and hydrophilic dye, will be used as staining substance via IV administration. The fluorescein established a stable distribution throughout surface epithelial cells, which is regular columnar epithelium with round gland openings and cobblestone pattern; the connective tissue matrix of lamina proria; blood vessels, which are regular shape visible in the deeper mucosa; and red blood cells[8,13]. It is not highly miscible with mucin; hence, mucin in globlet cells, which indicated GIM, will appear dark[8].

Because GIM is characterized by the diminutive lesions or microscopic abnormalities amidst large fields of diffuse disease, biopsy targeting and adequate sampling can be difficult and time consuming. Therefore, the instant resection with confocal laser microendoscope is not possible. Consequently, the patient should be diagnosed GIM via a magnifying Fujinon intelligence chromoendoscopy (FICE) or NBI-ME first. And then applying pCLE at the suspected lesion for taking an optical biopsy seems to be the smart technique.

Nowadays, a point-scanning fiber-optic fluorescein confocal endomicroscope for evaluate cellular morphology of the upper- and the lower-GI tract is possible. However, the improvement of diagnostic yield is evaluated in only Barrett's esophagus[13,14], colorectal cancer[6-8], detecting *Helicobacter pylori*[8]. There is only one study about CLE related detection of GIM

Table 1. Comparison data between eCLE and pCLE[10-12].

	eCLE	pCLE
Lateral resolution	0.7 μm	1 μm
Field of view	475x475 μm	240x240 μm
Z-axis	Yes	No
Versatility	No	Yes
Imaging plane depth	Vary up to 250 μm	Fixed, maximum 200 μm
Image/ second	± 1	12

Table 2. pCLE criteria for diagnosis GIM and gastric cancer[12,15].

Type of gastric tissue	Vascular pattern	Cellular pattern
Normal	Regular subepithelial capillary network	Regular columnar epithelial with round gland openings and cobblestone pattern
GIM	Regular subepithelial capillary network	Globet cell, villiform shape of foveolar epithelial, columnar absorptive cell
Gastric cancer	Irregular capillary, vessel leakage, bright lamina propria	Black cells with irregular boarders, disorganized cell

which use eCLE as a diagnostic instrument[15]. They use the Updated Sydney System Recommendation[16] for obtaining the gastric tissue which is not the true abnormality area. On the other hand, their strategy is still random biopsy. No literature in pCLE plus chromoendoscopy (FICE) for GIM-targeted biopsy was reported.

In this study, we choose pCLE as a diagnostic tool because it can be adaptable in all endoscopes and used in the real life. This pCLE, called Cellvizo, provided 1 μm resolution, 240 μm field of view, and 60 μm depth of focus[17]. We apply the criteria for diagnosis GIM and carcinoma from the previous publications[12,15] (Table 2). The aim of this study is to evaluate the correlation between the image from pCLE and the change of gastric mucosa.

MATERIALS AND METHODS

Study groups

The patients who were diagnosed gastric intestinal metaplasia (GIM) between January 2008 and December 2009 at King Chulalongkorn Memorial Hospital (KCMH) were recruited. Those who met the following criteria were enrolled in the study. The inclusion criteria were 1) willingness to give the written informed consent, 2) age 18-80 years, and 3) previously diagnosed GIM. The exclusion criteria were 1) previous gastric surgery including gastrectomy and bypass surgery, 2) bleeding tendency including decompensated cirrhosis, chronic kidney disease and long-term antiplatelets or anticoagulants, 3) pregnancy, and 4) history of fluorescein allergy.

Study design

A total of 50 patients were eligible to the study protocol. All patients had informed the consent. The demographic data, clinical history, and physical examination were recorded. The appointment for esophagogastro-duodenoscopy (EGD) with pCLE was on schedule. The magnified FICE plus pCLE was performed by one endoscopist (RP). During the procedure, conscious sedation was achieved for each patient by using 2.5-5 milligrams of intravenous midazolam and 25-50 milligrams of intravenous meperidine. The vital signs were monitored during the entire procedure. All patients were administered 10 milligrams of hyoscine intravenously to decrease bowel movement for obtaining the satisfied visualization. Simethicone solution was rinsed to reduce mucous and gas bubble in the stomach. Then, standard and magnified (x100) FICE (station 8; 415 nm and 540 nm) (Fujinon, Tokyo, Japan) was used as a screening tool. All suspicious lesions detected with magnified FICE were carefully examined by pCLE (Mauna kea technologies, Paris, France) along with 2.5 ml 10% Fluorescein sodium (Novartis Pharmaceutical Co., Bangkok, Thailand) intravenously. Each patient had a biopsy taken from both GIM and non-GIM suspicious epithelium. We recorded the video clip of pCLE imaging in order to compare with our goal standard which was histology that read by a clinically-blinded GI pathologist (NW). We also recorded the duration and complication of the procedure. At the end, we reported the result to the patient by phone.

Statistical analysis

All the variables collected were subjected to a descriptive analysis. For numerical variables, the results were express as a mean ± SD. Quantitative variables were shown in percentage. For the correlation between pCLE and histology, kappa (κ) was used to represent the agreement of these two instruments. The value of kappa (κ) for agreement evaluation are 0.01

to 0.20 indicating poor agreement, 0.21 to 0.40 fair agreement, 0.41 to 0.60 moderate agreement, 0.61 to 0.80 substantial agreement, and 0.81 to 1.00 almost perfect. We calculated kappa (κ) to assess the interobserver agreement among 5 experienced readers in pCLE images. The SPSS version 17.0 for windows was selected for the statistical analysis.

RESULTS

There were 43 patients (85%) who finally diagnosed GIM from pCLE and of these, 38 were confirmed as GIM by histology. Baseline characteristics including gender, age, underlying disease, history of *H. pylori* infection, history of smoking, alcohol drinking, and family history of gastric cancer were recorded and showed in Table 3.

Interobserver agreement, evaluated by Fleiss's kappa, is almost perfect (κ=0.83). The correlation between pCLE and histology is the almost perfect kappa is (κ=0.81). False positive and false negative is 16.2 and 3.5 percent, respectively. The overall data showed in Table 4. There was no gastric cancer detected. Additionally, the mean duration in each patient is 29.40±7.76 minutes (range 20-60 minutes).

Table 3. Baseline characteristics in GIM patient enrolled in this study.

Baseline characteristics	Patient (%)
Male	29 (58.0)
Age (mean ± SD; yrs.)	63.1±13.5
Underlying disease	36 (72)
History of *H.pylori* infection	25 (50)
History of smoking	37 (74)
History of alcohol drinking	8 (16)
Family history of gastric cancer	3 (6)
Total	**50 (100)**

Table 4. Comparison data between pCLE and histology.

pCLE	Histology		Total
	GIM	Normal	
GIM	36	7	43
Normal	2	55	57
Total	**38**	**62**	**100**

DISCUSSION

GIM is defined as replacement of gastric mucosa by intestinal epithelium which carried a malignant tendency[18]. Globet cell is a very strong histological diagnostic clue for GIM[19]. Because CLE can detect globet cells easily, the result of this study revealed almost perfect of agreement in diagnostic reliability between pCLE images and histological findings.

From the present study, false positive rate seemed to be moderately high. However, eighty percent of the mistakes occurred in the first-half period of the study which was a learning period of the investigator. In addition, the endoscopist needed to withdraw the probe before taking a targeted biopsy. We tried to pressure the pCLE probe at the lesion to make a "mark" before withdrawal the probe; sometimes it could not identify well causing sampling error. Double-channel endoscope might be the solution for this problem.

This study revealed almost perfect of agreement in diagnostic reliability in pCLE and pathology (κ=0.83). This satisfied result might be from our practical and easy-learning diagnostic criteria in GIM using pCLE which were; 1) villous-like gastric epithelium, and 2) dark (no fluorescein uptake) goblet cells in gastric columnar epithelium. Both criteria made 88.3 percent accuracy for diagnosis GIM.

The mean duration in each procedure was 29 minutes which was not much different in previous studies[15,20]. In contrast, both of the previous studies used random biopsy strategy from Updated Sydney System Recommendation[16] whereas present study performed magnified FICE as a screening tool followed by pCLE. Our study provided a more reliable and practical technique than previously report.

Base on pathology, GIM has been classified as complete type (type I) and incomplete type (type II or III) (Table 5)[21], and complete metaplasia is believed to carry no risk of gastric cancer, whereas incomplete types of metaplasia have been closely linked to adenocarcinoma[22]. Therefore, classification of GIM is necessary, in order to assess the risk of gastric cancer and provide appropriate follow-up for GIM patients[23]. However, this study did not mentioned about type of GIM.

There were some limitations in this study. First, pCLE cannot classify type of GIM because of the quality of pCLE images. Even though eCLE provided better resolution than pCLE, Guo Y-T and colleagues[15]

Table 5. Definition of each GIM type

Definition	Histologic description
Gastric intestinal metaplasia (GIM)	**Type I** Closely resembles the morphology of the small intestine, with absorptive enterocytes, well-defined brush borders, and well-formed goblet cells.
	Type II Incomplete metaplasia with irregular mucous vacuoles, absence of brush borders, and difficult-to-identify absorptive enterocytes. Cells secrete mainly sialomucins.
	Type III Same as type II except cells secrete mainly sulfomucins.

showed that eCLE cannot distinguish very well between incomplete and complete GIM, with sensitivities for the diagnosis of complete and incomplete GIM are only 68.03% and 68.42%, respectively. Second, CLE provided only gray-color images which cannot identify the detail in histology such as nuclear-cytoplasm ratio, nuclear pleomorphism and hyperchromatism leading to easily misdiagnose[20]. Third, this novel, real-time histological device is very expensive. And finally, the diagnostic accuracy was also influenced by the quality of the CLE images. With the development of the pCLE quality, development of the technique and operating skill individually, these problems can be overcome. Now, we still believe that smart biopsy by pCLE is enough to improve the accuracy rate of biopsy. Further histological examination for confirmation and evaluation in detail is still needed.

In conclusion, our study showed that pCLE may useful for the diagnosis GIM with the almost perfect agreement in diagnostic reliability between pCLE images and histological reports. Moreover, learning in interpretation of pCLE images is not too difficult to make it familiar. Currently this modality might not be sufficient to replace histological examination because of various limitations. However, further study in high-risk patient with no previously EGD reports is essential for fulfill the protocol as a diagnostic study. If we know sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likely hood ratio (LR) of this equipment, we might be apply pCLE as a screening test for high-risk gastric cancer population in the future.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, *et al*. Global Cancer Statistics 2002. CA Cancer J Clin 2005;55:74-108.
2. Thong-Ngam D, Tangkijvanich P, Mahachai V, *et al*. Current status of gastric cancer in Thai patients. J Med Assoc Thai 2001; 84:475-82.
3. Fox JG, Wang TC. Inflammation, atrophy, and gastric cancer. J Clin Invest 2007; 117:60-9.
4. Imraporn B, Jutaghokiat S, Wisedopas N, *et al*. Validity of Magnify NBI for Gastric Intestinal Metaplasia Targeted Biopsy. Gastrointest Endosc 2008;67:AB 280.
5. Imraporn B, Jutaghokiat S, Wisedopas N, *et al*. Validity of Magnify NBI for Gastric Intestinal Metaplasia Targeted Biopsy. Gastrointest Endosc 2009;69:AB 182.
6. Kiesslich R, Burg J, Vieth M, et al. Confocal laser endoscopy for diagnosing intraepithelial neoplasias and colorectal cancer in vivo. Gastroenterology 2004; 127:706-13.
7. Pitris C, Jesser C, Boppart SA, *et al*. Feasibility of optical coherence tomography for high-resolution imaging of human gastrointestinal tract malignancies. J Gastroenterol 2000; 35:87-92.
8. Polglase AL, McLaren WJ, Skinner SA, *et al*. A fluorescence confocal endomicroscope for in vivo microscopy of the upper- and the lower-GI tract. Gastrointest Endosc 2005; 62:686-95.
9. Meining A. Confocal endomicroscopy. Gastrointest Endosc Clin N Am 2009;19:629-35.
10. Nguyen NQ, Leong RW. Current application of confocal endomicrosocpy in gastrointestinal disorders. J gastroenterol Hepatol 2008;23:1483-91.
11. Wallace MB, Fockens P. Probe-based confocal laser endomicroscopy. Gastroenterology 2009; 136:1509-13.
12. Bisschops R, Bergman J. Probe-based confocal laser endomicroscopy: scientific toy or clinical tool? Endoscopy 2010; 42:487-9.
13. Dunbar KB, Okolo P, Montgomery E, Canto MI. Confocal laser endomicroscopy in Barrett's esophagus and endoscopically inapparent Barrett's neoplasia: a prospective, randomized, double-blind, controlled, crossover trial. Gastrointest Endosc 2009;70(4):645-54.
14. Kiesslich R, Gossner L, Goetz M, *et al*. In vivo histology of Barrett's esophagus and associated neoplasia by confocal laser endomicroscopy. Clin Gastroenterol Hepatol 2006;4:979-87.
15. Guo YT, Li YQ, Yu T, *et al*. Diagnosis of gastric intestinal metaplasia with confocal laser endomicroscopy in vivo: a prospective study. Endoscopy 2008;40:547-53.
16. Dixon MF, Genta RM, Yardley JH, *et al*. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol 1996;20:1161-81.
17. Evans JA, Nishioka NS. Endoscopic confocal microscopy. Curr Opin Gastroenterol. 2005;21:578-84.
18. Kyrlagkitsis I, Karamanolis DG. Premalignant lesions and conditions for gastric adenocarcinoma: diagnosis, management and surveillance guidelines. Hepatogastroenterology 2003;

50:592-600.

19. Morson BC, Dawson IMP. Morson and Dawson's gastrointestinal pathology 3rd edition. Oxford (Oxfordshire): Blackwell Scientific; 1990; p221 & 446.
20. Li Z, Yu T, Zuo XL, *et al*. Confocal laser endomicroscopy for in vivo diagnosis of gastric intraepithelial neoplasia: a feasibility study. Gastrointest Endosc 2010;72:1146-53.
21. Rugge M, Correa P, Dixon MF, *et al*. Gastric dysplasia: The Padova international classification. Am J Surg Pathol 2000; 24:167-76.
22. Rokkas T, Filipe MI, Sladen GE. Detection of an increased incidence of early gastric cancer in patients with intestinal metaplasia type III who are closely followed up. Gut 1991; 32:1110-13.
23. Correa P, Piazuelo MB, Wilson KT. Pathology of gastric intestinal metaplasia: clinical implications. Am J Gastroenterol 2010;105:493-8.