Confocal laser endomicroscopy for in vivo detection of gastric intestinal metaplasia: a randomized controlled trial

Authors Zhen Li[1], Xiu-Li Zuo[1], Tao Yu[1], Xiao-Meng Gu[1], Cheng-Jun Zhou[2], Chang-Qing Li[1], Rui Ji[1], Yan-Qing Li[1]

Institutions
[1] Department of Gastroenterology, Qilu Hospital, Shandong University, Jinan, China
[2] Department of Pathology, the Second Affiliated Hospital, Shandong University, Jinan, China

submitted 14. June 2013
accepted after revision 19. November 2013

Bibliography
DOI http://dx.doi.org/10.1055/s-0033-1359215
Published online: 2014
Endoscopy

ISSN 0013-726X

Corresponding author
Yan-Qing Li, MD/PhD
No. 107, Wenhuaxi Road
Jinan, 250012
China
Fax: +86-531-82169236
liyanqing@sdu.edu.cn

Background and study aims: Gastric intestinal metaplasia (GIM) is associated with a risk for development of intestinal-type gastric cancer. This study aimed to compare the diagnostic yield of GIM from confocal laser endomicroscopy (CLE) and white light endoscopy (WLE).

Patients and methods: In a prospective, double-blind, randomized study, patients were randomly assigned to receive either CLE with targeted biopsies (group A) or WLE with a standard biopsy protocol (group B).

Results: A total of 168 patients were finally analyzed (group A 85, group B 83). On a per-patient analysis, the diagnostic yields of GIM (including GIM with gastric intraepithelial neoplasia [GIN]) for groups A and B were 44.71% and 31.33%, respectively ($P=0.074$). On a per-biopsy analysis, CLE-targeted biopsy gave a significantly higher diagnostic yield of GIM compared with WLE and standard biopsy, at 65.70% (113/172 biopsies) versus 15.73% (81/515 biopsies) ($P<0.001$). Moreover, the diagnostic yield of the operative link on gastric intestinal metaplasia (OLGIM) assessment stages III and IV was higher at 20.93% (36/172 biopsies) in group A versus 4.08% (21/515 biopsies) in group B ($P<0.001$). In addition, use of CLE-guided biopsy significantly decreased by 68% ($P<0.001$) the mean number of biopsies required per patient.

Conclusions: CLE with targeted biopsies is superior to WLE with standard biopsies for the detection and surveillance of GIM. The number of biopsies needed to confirm GIM is about one third of that needed with WLE with standard biopsies.

Introduction

Gastric intestinal metaplasia (GIM) is a well-known premalignant lesion for intestinal-type gastric cancer [1,2]. Current diagnosis of GIM, which has a high rate of interobserver variation based on conventional white-light endoscopy (WLE) findings, still relies on histological assessment of nontargeted endoscopic biopsy specimens [3,4].

The updated Sydney System is the most widely accepted guideline to date for the classification and grading of gastritis, combining topographical, morphological, and etiological information in a reporting schema [5]. It recommends that five gastric biopsies are obtained, two from the antrum (3 cm from the pylorus, greater/lesser curvature), two from the corpus (one from the lesser curvature, 4 cm proximal to the incisura, and one from the middle of the greater curvature), and one from the incisura). However, using conventional white-light endoscopy (WLE), the recommended multiple biopsy protocol shows an unsatisfactory yield regarding the detection and surveillance of GIM. In addition, El-Zimaity & Graham have shown that when the updated Sydney recommendations were applied, GIM was missed in more than 50% of those with confirmed intestinal metaplasia [6].

Recently, several novel endoscopic techniques, such as magnification endoscopy, chromoendoscopy and narrowband imaging (NBI), have been developed to overcome the limitations of conventional white-light endoscopy [7–10]. Dinis-Ribeiro et al. first showed that gastric pit patterns observed by magnifying endoscopy and methylene blue chromoendoscopy were valid and reproducible for the diagnosis of GIM [7]. Studies also revealed that NBI with magnifying endoscopy enabled a highly accurate prediction of histological intestinal metaplasia [9,10]. Nevertheless, all the above technologies are limited to macroscopic views, and furthermore, recent research has revealed that the well-known dye agent methylene blue can induce oxidative damage to DNA when photosensitized by white light [11].

Confocal laser endomicroscopy (CLE) is a newly developed optical endoscopic imaging modality

which can visualize living tissue at cellular and subcellular levels [12]. A confocal laser scanning microscope is integrated at the distal tip of a flexible endoscope, enabling ×1000 magnification for real-time in vivo histological assessment or "virtual biopsy" of the gastrointestinal tract mucosa within 250 µm beneath the surface. It is based on tissue illumination with a low-power laser with subsequent capture of the fluorescence light reflected from the tissue through a pinhole. An image of the scanned area can thus be generated as greyscale values after electronic processing and encoding of the captured emitted light from successive points. Previous clinical investigations have reported that as CLE identifies endomicroscopic features, including goblet cells, columnar absorptive cells, and villiform foveolar epithelium, it can diagnose GIM with a sensitivity of 98.13% and a specificity of 95.33%; these are much higher than the corresponding values for conventional endoscopy within the same group of patients, that is, sensitivity 36.88%, specificity 91.59%) [13]. Another study from a different endoscopic center also found that CLE can enable accurate diagnosis of GIM, and that greater accuracy would be achieved with increased experience in CLE [14]. However, these studies had the limitations of small sample sizes and a cohort design. Until now, for the identification of GIM, no investigation has validated, in a prospective, randomized controlled fashion, the value of CLE with in vivo optical biopsy as compared with a routine endoscopic biopsy protocol.

Therefore, the primary objective of this study was to investigate the diagnostic yield of GIM using CLE-guided targeted biopsies compared with WLE with a standard biopsy protocol. For the secondary objective, the number of biopsies needed per patient for the detection of GIM was analyzed and compared between the two groups.

Patients and methods

Patients

Consecutive patients scheduled for CLE examination were enrolled in this study at Qilu Hospital, Shandong University, according to the inclusion and exclusion criteria. Inclusion criteria were: (i) having dyspeptic symptoms and aged 40 years or older; or (ii) having *Helicobacter pylori* infection, or histologically verified GIM or atrophic gastritis. Exclusion criteria were: (i) presence of gastrectomy, acute gastrointestinal bleeding, or known gastric neoplasia; (ii) presence of conditions unsuitable for performance of CLE including coagulopathy (prothrombin time < 50% of control [normal value], partial thromboplastin time > 50 s), impaired renal function (creatinine level > 1.2 mg/dL), pregnancy or breastfeeding, or known allergy to fluorescein sodium; (iii) inability to provide informed consent.

Sample size calculation

Based on previously published data for standard upper endoscopy using the revised Sydney System biopsy protocol, we estimated a GIM detection rate of 25% using WLE with standard biopsies [6]. According to our previously published GIM diagnostic criteria, we hypothesized that CLE would increase the diagnostic yield of GIM to 50% in this specific patient population. Similar sample size estimation examples could be found in previous researches [15, 16]. A calculated sample size indicated that 152 patients (76 per group) would yield an overall power for the study of 0.9 at an α value of 0.05. We proposed recruiting 170 eligible patients (85 per group) to allow an attrition rate of 10%.

Randomization procedure and endoscopic technique

Enrolled patients were randomly allocated using computer-generated codes at a 1:1 ratio to undergo either CLE with targeted biopsies (group A) or WLE with the standard biopsy protocol (group B). An independent nurse, who was blinded to the study design, revealed the randomization codes before the gastroscopy was started.

All endoscopic procedures were performed by one experienced endoscopist (X. L. Z.), who had more than 10 years' endoscopic experience and had performed more than 300 CLE examinations before embarking on the present study. Although the endoscopist was aware that the study population was enriched and included patients older than 40 years with dyspeptic symptoms and patients undergoing surveillance endoscopy, she was blinded to any previous endoscopic and pathologic diagnoses before the present endoscopy. All endoscopic procedures were conducted under the supervision of a study coordinator (R. J.) [17].

The Pentax EC3870K endoscope was used for all procedures. Patients were prepared for routine gastroscopy, and 20000 U α-chymotrypsin and 80 mg dimethylpolysiloxane were taken orally 15 – 20 min before endoscopy. The total duration of the procedure was noted, timed from the passage of the endoscope beyond the larynx to withdrawal into the proximal esophagus.

Group A: Confocal laser endomicroscopy with targeted biopsies

Endoscopic procedures

After intravenous administration of 5 ml of fluorescein sodium (10% solution), macroscopic lesions and five standardized locations were carefully examined using the CLE system. The five standard regions included two from the distal antrum (within 2 – 3 cm from the pylorus, greater/lesser curvature), one from the incisura and two from the mid corpus (greater/lesser curvature) [5]. Focal lesions were recorded with regard to their locations and morphology according to the Paris classification [18].

Multiple endomicroscopic images were acquired from the surface down to 250 µm depth by placing the distal tip of the endomicroscope in direct contact with the target tissue site. Confocal images were then displayed at a scan rate of 1.6 frames per second, resulting in a resolution of 1024×512 pixels. Finally, if the CLE-scanned areas were diagnosed as GIM or neoplasia, targeted biopsies would be performed 5 mm immediately to the left of the "polyp" created by suction.

Assessment of CLE images

In vivo diagnosis of endomicroscopic images was done by the operating endoscopist (X.L.Z.) during the CLE examinations, according to previously published criteria for GIM and neoplasia [13, 19, 20]. CLE images obtained from different lesions and from the standardized regions were stored separately to allow for further post-procedure assessment.

Biopsy protocol in group A

At extubation, biopsies were taken in a targeted fashion after careful examination using CLE. Targeted biopsies were only taken at the scanning sites where CLE images had revealed GIM or neoplasia. In addition, all macroscopic lesions were biopsied before withdrawal of the endoscope.

Interobserver and intraobserver assessment of CLE images

After all the procedures in group A, separately stored CLE images of macroscopic lesions were reviewed in randomized order by three independent CLE investigators (T. Y., X. M. G., and C. Q. L.) who were blinded to the WLE and the real-time endomicroscopic diagnoses and to the corresponding histopathologic findings for these scanned areas. Each endoscopist stated whether GIM was present in each lesion according to the appearance of the endomicroscopic images. In addition, after a 4-week interval, all CLE images were reassessed by the three CLE investigators to evaluate the intraobserver agreement.

Group B: White-light endoscopy (WLE) with standard biopsy protocol

Patients received standard WLE examinations using the white-light function of the endomicroscope. All the preparations before performance of the endoscopy were the same as those in group A.

After successful insertion of the endoscope, all the stomach was carefully examined using WLE. At extubation, biopsies were first taken from endoscopically macroscopic lesions in the stomach, and then from the five standard biopsy sites following the updated Sydney System (described in group A). Focal lesions were also noted according to the Paris classification [18].

Histological examination

The biopsy specimens were each processed in an individual formalin pot with exact documentation of the source location. Biopsy samples were subsequently sectioned at 4-µm intervals following paraffin embedment, and sectioned in both horizontal and vertical planes to facilitate comparison with confocal images. Afterwards, the serial sections were stained with hematoxylin & eosin (H & E) and periodic acid-Schiff and Alcian blue (PAS – AB) for identification of goblet cells.

One experienced gastrointestinal pathologist (C. J. Z.) who was blinded to the endoscopic and endomicroscopic diagnosis examined all specimens. The histological diagnosis was reported according to the updated Sydney Classification of chronic gastritis and the modified Vienna criteria for neoplasia [5,21].

Statistical analysis

Demographic data of patients were analyzed using descriptive statistics and compared by use of the independent sample *t* test. The chi-squared test and the Fisher exact test were applied for the comparison of categorical variables. The Mann – Whitney *U* test was used to analyze non-normally distributed quantitative variables. A *P* value of less than 0.05 (two-tailed) and α (probability for error) of 0.05 were considered to be statistically significant.

Interobserver and intraobserver agreements were expressed using the kappa coefficient. Kappa value <0.20 indicates poor agreement, 0.20 to 0.40 fair, 0.41 to 0.60 moderate, 0.61 to 0.80 good, and 0.81 to 1.0 excellent.

All calculations were done using the SPSS 13.0 statistical software package (SPSS, Chicago, Illinois, USA). The study was presented according to the Consolidated Standards of Reporting Trials (CONSORT) [22].

Ethical considerations

This study was approved by the local ethics committee of Qilu Hospital, Shandong University (clinicaltrials.gov NCT01024621), and was conducted in accordance with the revised declaration of Helsinki (1989). Written informed consent was obtained from all participants before the procedures.

Results

From December 2009 to June 2010, a total of 354 patients was screened for possible inclusion in the present study, with 170 patients being finally enrolled according to the predetermined inclusion and exclusion criteria (**Fig. 1**). Indications included: (i) dyspeptic symptoms and aged 40 years and older (group A, 61; group B, 60); or (ii) H. *pylori* infection (group A, 6; group B, 9), or histologically verified GIM (group A, 8; group B, 8) or atrophic gastritis (group A, 10; group B, 6). Two patients in group B were excluded during the procedure because of severe stenosis. Thus 168 patients (85 in group A and 83 in group B) were finally analyzed.

Patients' characteristics are presented in **Table 1**, and also the characteristics of those lesions that were macroscopically visible. There were no significant differences between groups A and B regarding patients' sex, age, or number and location of macroscopic lesions. The mean procedure durations in groups A and B were 19 minutes (range 13 – 37 minutes) and 18 minutes (range, 12 – 27 minutes), respectively; there was no statistically significant difference between the two groups for duration of examination ($P = 0.095$).

Primary outcome analysis

Final histopathological findings were as follows: 52 patients had GIM only (group A, 30; group B, 22); 12 patients had gastric intraepithelial neoplasia (GIN) with GIM (group A, 8; group B, 4), and 4 patients had GIN without GIM (group A, 2; group B, 2). On a per-patient analysis, the diagnostic yields for GIM (including GIN with GIM) for groups A and B were 44.71% and 31.33%, respectively ($P = 0.074$).

Regarding macroscopic lesions, a total of 131 were detected in 92 patients (group A, 48 patients ; group B, 44 patients). Histological diagnoses for these lesions are shown in **Table 1**.

In group A, a total of 492 areas (425 standardized locations and 67 macroscopic lesions) were imaged by CLE. A total of 172 targeted biopsies were obtained, from standardized locations with in

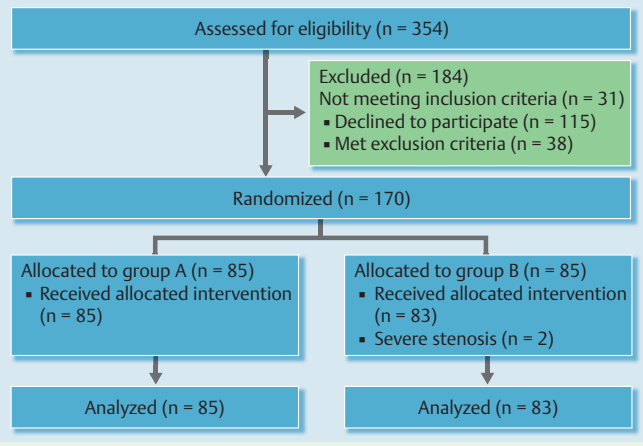

Fig. 1 Comparison of confocal laser endomicroscopy (CLE) with targeted biopsies with white-light endoscopy (WLE) with standard biopsy protocol for detection of gastric intestinal metaplasia (GIM): enrollment and allocation of study participants.

	Group A	Group B	*P* value
Patients, n	85	83	
Male/female, n/n	45/40	46/37	0.747
Age, mean (range), years	55 (31–76)	54 (30–77)	0.574
Macroscopic lesions, n	67 (in 48 patients)	64 (in 44 patients)	
Histological diagnosis			
Gastritis	31	45	
GIM without GIN	24	12	
GIM + GIN	9	5	
GIN without GIM	3	2	
Patients with GIM with/without GIN as percent of all patients with macroscopic lesions			0.042
%	47.92	27.27	
95 %CI	34.47–61.67	16.35–41.85	
Locations			
Upper third of the stomach	8	6	
Middle third of the stomach	19	20	
Lower third of the stomach	40	38	

GIN, gastric intraepithelial neoplasia; 95 %CI, 95 % confidence interval

Table 1 Endoscopic detection of gastric intestinal metaplasia (GIM): patient and macroscopic lesion characteristics. Group A underwent confocal laser endomicroscopy with targeted biopsies and group B had white-light endoscopy (WLE) with a standard biopsy protocol.

	Group A	Group B	*P* value
Total number of biopsies, n	172 (from 85 patients)	515 (from 83 patients	
From standardized locations	59	415	
From macroscopic lesions	113 (from 67 lesions)	100 (from 64 lesions)	
Biopsies with GIM only or and GIM + GIN, n	113 (from 38 patients)	81 (from 26 patients)	0.224
From standardized locations	57	48	
From macroscopic lesions	56 (from 33 lesions)	33 (from 17 lesions)	
Biopsies with GIN only	6	7	
Biopsies with OLGIM I/II stage, n	58	49	0.330
From standardized locations	35	34	
From macroscopic lesions	23	15	
Biopsies with OLGIM I/II as percent of total	33.72 %	9.51 %	< 0.001
(95 %CI)	27.08 %–41.07 %	7.27 %–12.36 %	
Biopsies with OLGIM III/IV stage, n	36	21	0.675
From standardized locations	22	14	
From macroscopic lesions	14	7	
Biopsies with OLGIM III/IV as percent of total	20.93 %	4.08 %	< 0.001
(95 %CI)	15.52 %–27.61 %	2.68 %–6.15 %	

OLGIM, operative link on gastric intestinal metaplasia.

Table 2 Yield of gastric intestinal metaplasia (GIM) and gastric intraepithelial neoplasia (GIN). Group A underwent confocal laser endomicroscopy (CLE) with targeted biopsies and group B had white-light endoscopy (WLE) with standard biopsies.

vivo endomicroscopic diagnosis of GIM or neoplasia (59 biopsies), and from all of the macroscopic lesions (113 biopsies). Finally, 90 locations, in 38 patients, were histologically diagnosed as having GIM (81 GIM only and 9 GIM with GIN); these included 33 macroscopic lesions (56 biopsies) and 57 from the standardized locations (57 biopsies). Clinical examples are given in **Fig. 2–4**.

In group B, a total of 515 biopsies were obtained from 415 standardized locations (415 biopsies) and 64 macroscopic lesions (100 biopsies). Among these areas, GIM (GIM only or GIM with GIN was detected in 48 standardized locations (48 biopsies) and in 17 macroscopic lesions (33 biopsies), in 26 patients.

Therefore, on a per-biopsy analysis, CLE with targeted biopsies had a significantly greater diagnostic yield for GIM compared with WLE with standard biopsies, being 65.70 % (113/172) versus 15.73 % (81/515) ($P<0.001$). Moreover, the diagnostic yield on the basis of the operative link on gastric intestinal metaplasia assessment (OLGIM) stages III and IV was significantly higher at 20.93 % (36/172) in group A versus 4.08 % (21/515) in group B ($P<0.001$). These data are summarized in **Table 2**. In addition, on a per-macroscopic lesion analysis, endomicroscopy-targeted biopsies gave a higher diagnostic yield for GIM, of 49.25 % (33/67) compared with that of WLE with standard biopsies, of 26.56 % (17/64) ($P=0.008$).

Secondary outcome analysis

The mean numbers of biopsies per patient obtained in group A and group B were 2.0 (172/85; range 0–6) and 6.2 (515/83; range 5–10) respectively. Thus CLE targeting of biopsies led to a significant decrease of 68 % ($P<0.001$) in the number of biopsies per patient compared with WLE using the standard biopsy protocol (**Table 2**).

In addition, if only endomicroscopically diagnosed GIM or GIN lesions as well as standardized locations had been biopsied, the biopsy number would have been further decreased to 119 in group A with a mean of 1.4 per patient (range 0–6). However, the histopathology results showed that there was 1 lesion with low-grade intraepithelial neoplasia (LGIN) and 2 lesions with

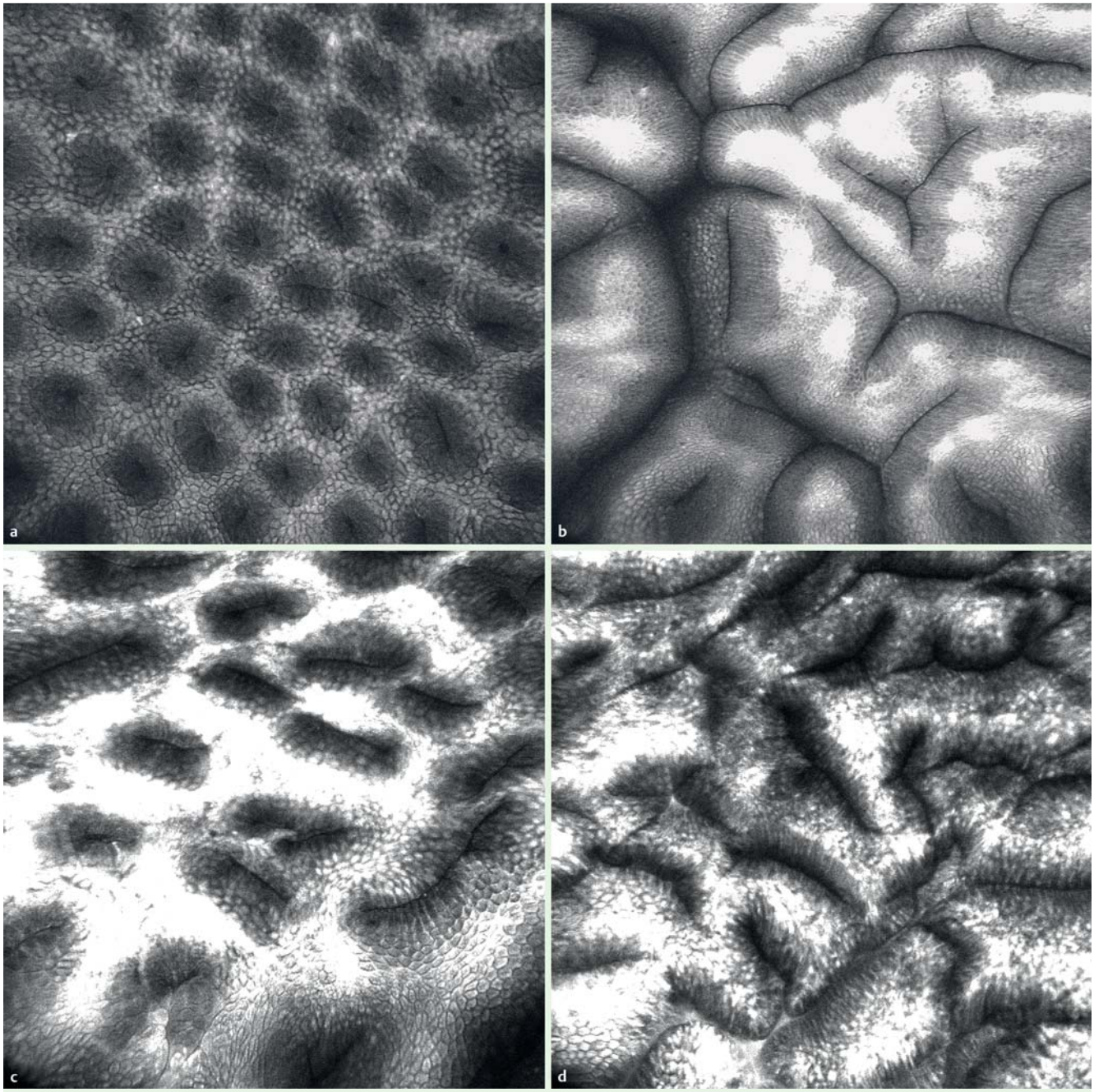

Fig. 2 Confocal laser endomicroscopy (CLE) appearances of normal and inflamed gastric mucosa. **a** Normal gastric mucosa with fundic glands; round gastric pits with round openings. **b** Normal gastric mucosa with pyloric glands; continuous short rod-like pits with slit-like openings. **c** Corporal mucosa with chronic inflammation; noncontinuous short rod-like pits with short thread-like openings. **d** Antral mucosa with chronic inflammation; elongated and tortuous branch-like pits.

GIM that displayed only inflammatory changes during real-time endomicroscopic imaging (**Table 3**).

Analysis of endomicroscopic imaging

A total of 17 901 confocal images from 492 different sites were analyzed during ongoing endoscopy, among which 3485 confocal images from 90 different sites revealed intestinal metaplasia. According to previously published CLE criteria [13, 17, 18], GIM and GIN lesions could be diagnosed with a sensitivity of 91.67% (95% confidence interval [95%CI] 78.17%–97.13%), specificity 96.77% (95%CI 83.81%–99.43%), positive likelihood ratio 28.42, and negative likelihood ratio 0.086 (**Table 3**).

One lesion in a patient with histologically verified GIM was misdiagnosed as gastritis under CLE, and was proved to be intestinal metaplasia after biopsy. Therefore, for patients with already known GIM in group A (n=8), GIM and GIN lesions were diagnosed with a sensitivity of 90% (95%CI, 59.58%–98.21%), and a specificity of 100% (95%CI, 51.01%–100%). And for the remaining patients in group A (n=77), GIM and GIN lesions were diagnosed with a sensitivity of 94.44% (95% CI 81.86%–98.46%), and a specificity of 94.12% (95%CI 73.02%–98.95%). Fisher's exact test showed that there was no statistical difference for the diagnostic accuracy between these two groups of patients (92.86% vs. 94.34%; $P=1.000$).

	CLE diagnosis				Total
	Gastritis	GIM without GIN	GIM with GIN	GIN	
Histopathological diagnosis					
Gastritis	30	1	0	0	31
GIM without GIN	2	22	0	0	24
GIM with GIN	0	0	9	0	9
GIN	1	0	0	2	3
Total	33	23	9	2	67

GIM, gastric intestinal metaplasia; GIN, gastric intraepithelial neoplasia.

Table 3 Comparisons of real-time confocal laser endomicroscopy (CLE) diagnosis and final histopathological diagnosis for the 67 macroscopic lesions identified in group A (CLE with targeted biopsies).

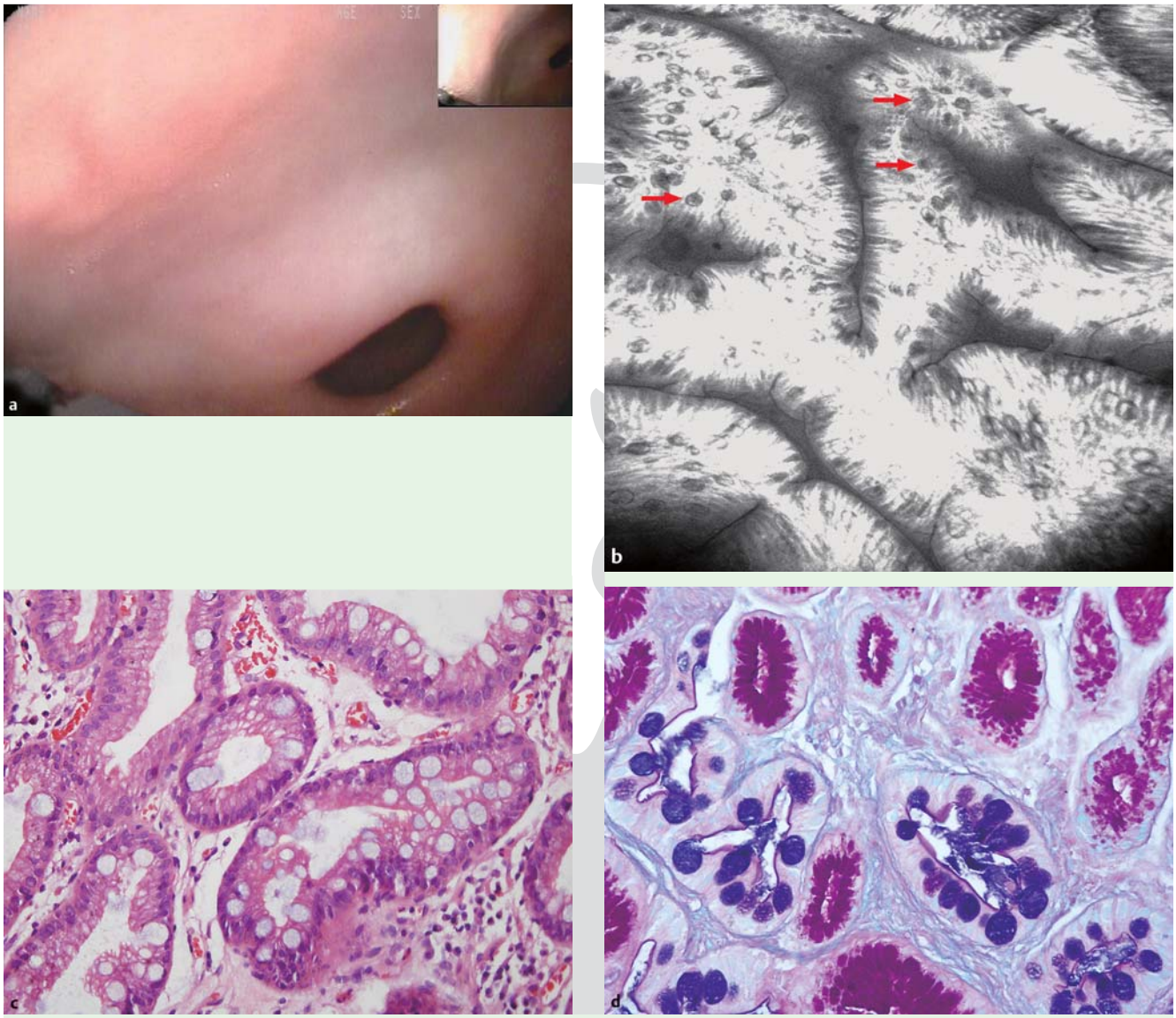

Fig. 3 Gastric intestinal metaplasia (GIM). **a** White-light endoscopy (WLE) showing focal erythema and edema within the anterior wall of the gastric antrum. **b** Confocal laser endomicroscopy (CLE) of this focal area showed dark goblet cells (arrows) within the columnar epithelium. **c** Corresponding histological results confirmed GIM of the mucosa. **d** Periodic acid-Schiff–Alcian blue (PAS – AB) staining showed blue goblet cells and purple columnar epithelial cells.

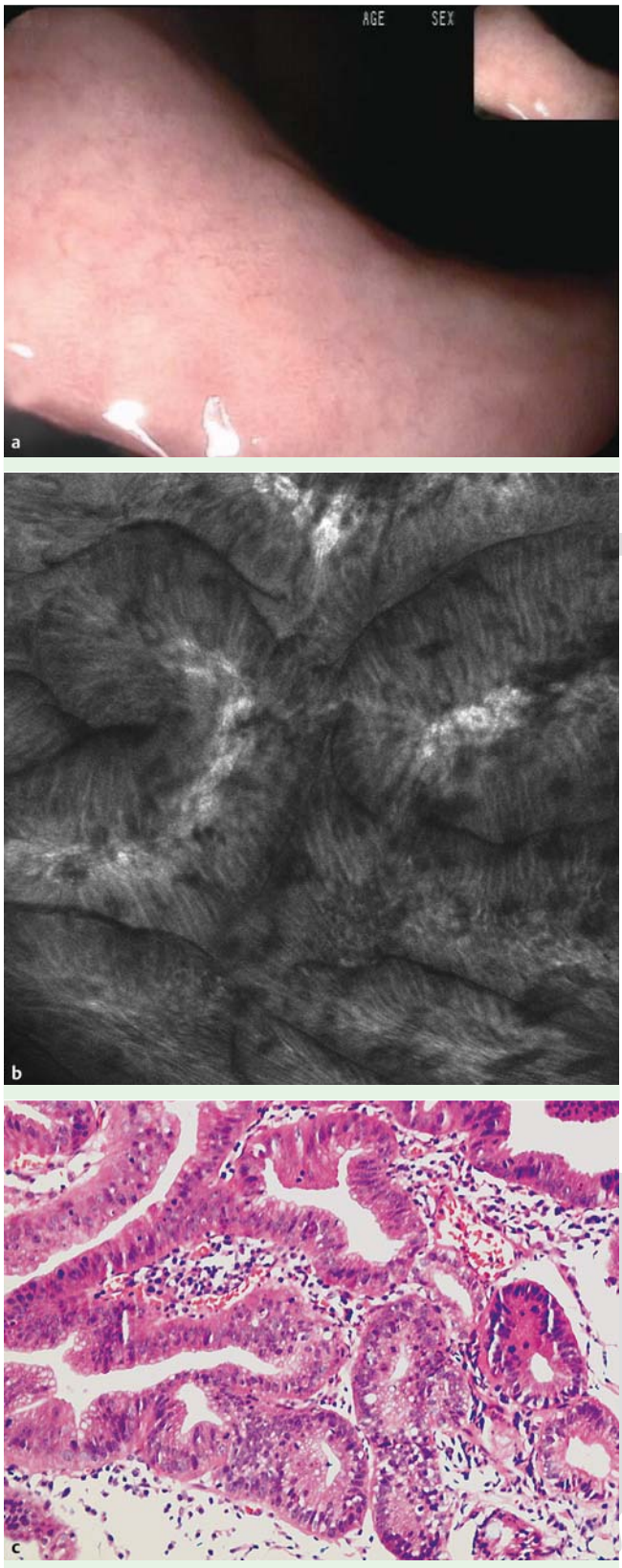

Fig. 4 Gastric intraepithelial neoplasia (GIN) with intestinal metaplasia. **a** White-light endoscopy (WLE) image of the angularis incisura. **b** Confocal laser endomicroscopy (CLE) view of incisura shows variably sized glands with slight unevenness of the glandular epithelium, and slight increase in gland density; large, black goblet cells are interspersed among the columnar epithelium. **c** Histological image showing low grade intraepithelial neoplasia with intestinal metaplasia.

In addition, the post-procedure assessment of CLE images of all macroscopic lesions (n=67) showed excellent agreement among the three CLE investigators, with a mean kappa value of 0.899. The intraobserver agreement was also graded as excellent with a mean kappa value of 0.909 (0.908, 0.910 and 0.909).

Discussion

Gastric adenocarcinoma still represents a lethal malignancy in the gastrointestinal tract despite its declining incidence. GIM is a part of the pathologic spectrum of gastric mucosal atrophy [23], and is acknowledged to be an important risk factor for the intestinal type of gastric cancer [1,2]. Surveillance of patients with GIM may therefore lead to earlier detection of advanced precursor lesions and gastric cancer [24,25].

The updated Sydney System for the classification of gastritis described the multifocal distribution of glandular loss with intestinalization of the gastric mucosa [5]. Following the recently proposed operative link on gastritis assessment (OLGA) system [26], Capelle et al. replaced atrophic gastritis by intestinal metaplasia in the staging of gastritis, in the OLGIM system, and showed that using the OLGIM system considerably increased interobserver agreement, while the correlation with the severity of gastritis remained at least as strong [27]. According to new guidelines [28], because of the multifocal nature of intestinal metaplasia in the stomach, OLGIM stages III/IV may be useful for identifying patients with increased risks of progression to gastric cancer. However, current guidelines also note that conventional white light endoscopy cannot accurately differentiate between and diagnose preneoplastic gastric lesions. Therefore, magnification chromoendoscopy or NBI may be offered in these cases as they improve diagnosis of such lesions. In this study, CLE with targeted biopsies showed a higher diagnostic yield for OLGIM III/IV, at 20.93% compared with 4.08% ($P<0.001$) from WLE with standard biopsies, proving that CLE may be helpful in selecting a smaller population with intestinal metaplasia for whom surveillance would need to be considered.

Moreover, Cassaro et al. have demonstrated that GIM was distributed in four distinct topographic patterns, with type C ("magenstrasse") and type D ("diffuse") being significantly associated with increased cancer risk [29]. Consequently, both the early detection of GIM and an accurate evaluation of the topographic distribution of these lesions play important roles in patients' prognoses. However, the diagnostic yield for GIM from conventional WLE with multiple biopsies is far from satisfactory [3].

CLE allows in vivo microscopic visualization of the gastrointestinal mucosa, allowing real-time endomicroscopic diagnosis of the scanned area and enabling targeted biopsies [12]. CLE can reliably enable the diagnosis of GIM from the presence of goblet cells (large black cells with mucin), columnar absorptive cells (more slender and brighter than normal columnar cells of gastric mucosa), and distinctive villous-like architecture [13,14,30]. Our previous study compared the diagnostic sensitivity and specificity of CLE with those of WLE, but it included only 53 patients and was conducted as a cohort study [13]. Hence, the true benefit of endomicroscopy compared with conventional WLE alone remained uncertain.

To the best of our knowledge, this is the first prospective, randomized, controlled, double-blind study to evaluate the real value of CLE in the detection of GIM in comparison with conventional WLE. By using real-time endomicroscopic imaging with targe-

ted mucosal biopsies (group A), a greater diagnostic yield of GIM was achieved with significantly fewer biopsies (68 % less per patient), compared with WLE with standard biopsies (group B) In addition, CLE with targeted biopsies did not significantly prolong the procedure duration compared with WLE with standard biopsies. Our study also demonstrated that the interobserver and intraobserver agreements for the interpretation of endomicroscopic images of macroscopic lesions were all excellent (mean kappa values 0.899 and 0.909, respectively).

Since the primary aim of this study was to evaluate the diagnostic yield for GIM using CLE with targeted biopsies compared with WLE with standard biopsies, we excluded at recruitment patients with histologically confirmed neoplasia including GIN and gastric cancer. Comparable numbers of macroscopic lesions were identified in the two groups (group A, n = 67; group B, n = 64; $P > 0.05$). But significantly more lesions with GIM (GIM-only and GIN with GIM) were detected in group A (49.25 %) compared with group B (26.56 %). In addition, the detection rate for GIM in standardized locations was similar in both groups (group A, 57 from 425 locations examined; group B, 48 from 415 locations examined) despite the fact that only 59 standardized locations in group A were biopsied according to the endomicroscopic diagnosis. However, since targeted biopsies were only performed at endomicroscopically diagnosed GIM or GIN for standardized locations in group A, we can only perform a diagnostic assessment of CLE imaging for the macroscopic lesions. And the results showed that CLE was highly accurate for in vivo diagnosis of GIM with a sensitivity of 91.67 % and a specificity of 96.77 %; these were in accordance with other published reports [13, 14].

There was no statistically significant difference between the two groups in this study for the duration of examination ($P = 0.095$). Possible explanations of this result are as follows. Firstly, for the purpose of the study protocol, the WLE procedure was longer because biopsies with forceps take much more time compared with "optical biopsies" with CLE [15]. Secondly, the endomicroscopist (X. L. Z.) had performed more than 300 CLE examinations before embarking on the present study. This may shorten the time required for obtaining satisfactory "optical biopsies" using CLE, although statistical data are needed to further validate this assumption. Finally, it has been noted that "Endoscopists who are more experienced with confocal image interpretation may set the scanning rate to "faster" or 1.6 frames/s (1024 × 512 pixels) for more efficient optical biopsy" [31]. Thus in this study, confocal images were displayed at a scan rate of 1.6 frames per second.

There were several limitations to our present study. Firstly, this study was carried out in a prospective randomized controlled fashion, thus a potential bias may exist since different equipment and different biopsy protocols were used in groups A and B. However, we tried to overcome this potential bias by standardizing the randomization protocol and having a single endoscopist performing all the procedures and using the same equipment. Further studies with a randomized crossover design would be desirable to avoid this potential bias.

Secondly, standard WLE examinations in group B were performed using the white-light function of the endomicroscope. Similar examples of this issue can be found in previous studies [16, 20]. Although this use of equipment as in the present study can eliminate possible bias arising from white-light image resolutions and the specifications of endoscopes, the white-light gastroscope with standard definitions most widely used in clinical practice is still to be investigated.

Thirdly, this study focused on the diagnostic yield of GIM and the reduction in numbers of biopsies required. Thus locations with a final histological diagnosis of GIN were not included in the statistical assessment of outcomes. However, previous studies have shown that targeted biopsies using CLE also enabled in vivo discrimination of GIN lesions. Therefore, we will evaluate the diagnostic yield of premalignant gastric lesions (both GIM and GIN) in our future studies.

As a fourth limitation, there was only a clear trend toward the identification of more patients with GIM (including GIN with GIM) in group A ($P = 0.074$). However, considering the number of detected GIM lesions, endomicroscopically targeted biopsies almost doubled the diagnostic yield for GIM compared with WLE with standard biopsies. Moreover, the number of biopsies needed for detection can be another option for the sample size calculation. In fact, fewer patients are needed if we set the "number of biopsies needed for detection" as the primary outcome in this study (15.37 % vs. 65.70 %). Chi-squared analysis demonstrated highly significant differences between the two groups on the diagnostic yield of GIM on a per-biopsy analysis, and revealed that significantly fewer biopsies were needed. Nevertheless, further studies with a larger sample size are warranted to validate the concept that CLE with target biopsies, compared with WLE, may identify more patients with GIM.

Finally the comparatively higher cost of CLE might be another limitation for its wide application in clinical practice, and there is potential for improved results with conventional endoscopy using high definition or narrowband imaging. Thus future studies are needed to accurately estimate the cost – effectiveness of CLE in this field.

In conclusion, this prospective, double-blind, randomized trial demonstrated that CLE with targeted biopsies provided a greater diagnostic yield for GIM compared with WLE with standardized biopsies, and that use of CLE allows a significant reduction in the number of biopsies required. Using predefined endomicroscopic diagnostic criteria, CLE enables accurate and reliable in vivo diagnosis of GIM. These data suggest that CLE with targeted biopsies may serve as a superior alternative to the standard white-light endoscopic technique for the detection and surveillance of GIM, and eventually contribute to the prevention of gastric cancer.

Competing interests: None

Acknowledgments

This investigation was funded by a program from the Clinical Projects of the Ministry of Health of China (2010), the Taishan Scholar program of Shandong Province.

References

1 *Correa P.* The biological model of gastric carcinogenesis. IARC Sci Publ 2004: 301 – 310
2 *Walker MM.* Is intestinal metaplasia of the stomach reversible? Gut 2003; 52: 1 – 4
3 *Sauerbruch T, Schreiber MA, Schüssler P* et al. Endoscopy in the diagnosis of gastritis: diagnostic value of endoscopic criteria in relation to histological diagnosis. Endoscopy 1984; 16: 101 – 104
4 *Redeen S, Petersson F, Jonsson KA* et al. Relationship of gastroscopic features to histological findings in gastritis and Helicobacter pylori infection in a general population sample. Endoscopy 2003; 35: 946 – 950
5 *Dixon MF, Genta RM, Yardley JH* et al. Classification and grading of gastritis. The updated Sydney System. International Workshop on the His-

topathology of Gastritis, Houston 1994. Am J Surg Pathol 1996; 20: 1161 – 1181
6 *El-Zimaity HM*, *Graham DY*. Evaluation of gastric mucosal biopsy site and number for identification of Helicobacter pylori or intestinal metaplasia: role of the Sydney System. Hum Pathol 1999; 30: 72 – 77
7 *Dinis-Ribeiro M*, *da Costa-Pereira A*, *Lopes C* et al. Magnification chromoendoscopy for the diagnosis of gastric intestinal metaplasia and dysplasia. Gastrointest Endosc 2003; 57: 498 – 504
8 *Areia M*, *Amaro P*, *Dinis-Ribeiro M* et al. External validation of a classification for methylene blue magnification chromoendoscopy in premalignant gastric lesions. Gastrointest Endosc 2008; 67: 1011 – 1018
9 *Uedo N*, *Ishihara R*, *Iishi H* et al. A new method of diagnosing gastric intestinal metaplasia: narrow-band imaging with magnifying endoscopy. Endoscopy 2006; 38: 819 – 824
10 *Capelle LG*, *Haringsma J*, *de Vries AC* et al. Narrow band imaging for the detection of gastric intestinal metaplasia and dysplasia during surveillance endoscopy. Dig Dis Sci 2010; 55: 3442 – 3448
11 *Olliver JR*, *Wild CP*, *Sahay P* et al. Chromoendoscopy with methylene blue and associated DNA damage in Barrett's oesophagus. Lancet 2003; 362: 373 – 374
12 *Kiesslich R*, *Goetz M*, *Vieth M* et al. Confocal laser endomicroscopy. Gastrointest Endosc Clin North Am 2005; 15: 715 – 731
13 *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 – 553
14 *Lim LG*, *Yeoh KG*, *Salto-Tellez M* et al. Experienced versus inexperienced confocal endoscopists in the diagnosis of gastric adenocarcinoma and intestinal metaplasia on confocal images. Gastrointest Endosc 2011; 73: 1141 – 1147
15 *Dunbar KB*, *Okolo P3rd*, *Montgomery E* et al. 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: 645 – 654
16 *Hurlstone DP*, *Kiesslich R*, *Thomson M* et al. Confocal chromoscopic endomicroscopy is superior to chromoscopy alone for the detection and characterisation of intraepithelial neoplasia in chronic ulcerative colitis. Gut 2008; 57: 196 – 204
17 *Kato M*, *Kaise M*, *Yonezawa J* et al. Autofluorescence endoscopy versus conventional white light endoscopy for the detection of superficial gastric neoplasia: a prospective comparative study. Endoscopy 2007; 39: 937 – 941
18 The Paris endoscopic classification of superficial neoplastic lesions: esophagus, stomach, and colon: November 30 to December 1, 2002. Gastrointest Endosc 2003; 58: 3 – 43
19 *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 – 1153
20 *Li WB*, *Zuo XL*, *Li CQ* et al. Diagnostic value of confocal laser endomicroscopy for gastric superficial cancerous lesions. Gut 2011; 60: 299 – 306
21 *Dixon MF*. Gastrointestinal epithelial neoplasia: Vienna revisited. Gut 2002; 51: 130 – 131
22 *Moher D*, *Schulz KF*, *Altman D*. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. JAMA 2001; 285: 1987 – 1991
23 *Rugge M*, *Correa P*, *Dixon MF* et al. Gastric mucosal atrophy: interobserver consistency using new criteria for classification and grading. Aliment Pharmacol Ther 2002; 16: 1249 – 1259
24 *de Vries AC*, *van Grieken NC*, *Looman CW* et al. Gastric cancer risk in patients with premalignant gastric lesions: a nationwide cohort study in the Netherlands. Gastroenterology 2008; 134: 945 – 952
25 *Whiting JL*, *Sigurdsson A*, *Rowlands DC* et al. The long term results of endoscopic surveillance of premalignant gastric lesions. Gut 2002; 50: 378 – 381
26 *Rugge M*, *Genta RM*. Staging gastritis: an international proposal. Gastroenterology 2005; 129: 1807 – 1808
27 *Capelle LG*, *de Vries AC*, *Haringsma J* et al. The staging of gastritis with the OLGA system by using intestinal metaplasia as an accurate alternative for atrophic gastritis. Gastrointest Endosc 2010; 71: 1150 – 1158
28 *Dinis-Ribeiro M*, *Areia M*, *de Vries AC* et al. Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointest Endosc (ESGE), European Helicobacter Study Group (EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). Endoscopy 2012; 44: 74 – 94
29 *Cassaro M*, *Rugge M*, *Gutierrez O* et al. Topographic patterns of intestinal metaplasia and gastric cancer. Am J Gastroenterol 2000; 95: 1431 – 1438
30 *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 – 987
31 *Goetz M*, *Dunbar KB*, *Canto MI*. Examination technique of confocal laser endomicroscopy. In: Kiesslich R, Galle PR, Neurath MF eds. Atlas of endomicroscopy. Berlin: Springer-Heidelberg; 2008: 34