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Diagnostic value of confocal laser endomicroscopy for gastric superficial cancerous lesions

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ABSTRACT

Background The identification of gastric superficial cancerous lesions based on conventional white-light endoscopy (WLE) is challenging, and histological analysis remains the 'gold standard' for the final diagnosis. Confocal laser endomicroscopy (CLE) can provide in vivo histological observation without the need for biopsy.

Objective To develop and evaluate CLE imaging criteria for gastric superficial cancerous lesions and to compare the diagnostic value of real-time integrated CLE (iCLE) and WLE alone in distinguishing gastric superficial cancerous lesions.

Design Prospective study.

Setting Qilu Hospital, Shandong University, Jinan, China.

Patients A total of 182 patients were enrolled into phase I and 1786 patients were enrolled into phase II.

Interventions CLE images were blindly evaluated after endoscopy in phase I, and real-time iCLE diagnosis during endoscopy was compared with WLE diagnosis by using histopathology as a gold standard in phase II.

Main Outcome Measurements The validity and reliability of the CLE diagnosis for identifying gastric superficial cancerous lesions.

Results Off-line CLE diagnosis for early gastric cancers had a high sensitivity (88.1%) and specificity (98.6%). When the two-tiered CLE classification of non-cancerous lesions and cancer/high-grade intraepithelial neoplasia (HGIN) lesions was introduced, CLE diagnosis led to a higher sensitivity (90.2%) and specificity (98.5%) (phase I). Real-time iCLE diagnosis had a higher sensitivity (88.9%), specificity (99.3%) and accuracy (98.8%) for gastric superficial cancer/HGIN lesions than WLE diagnosis (sensitivity, 72.2%; specificity, 95.1%; and accuracy, 94.1%) ($p<0.05$) (phase II).

Limitations This was a single-centre study.

Conclusions CLE can be used to identify gastric superficial cancer/HGIN lesions with high validity and reliability.

Significance of this study

What is already known about this subject?
- Confocal laser endomicroscopy (CLE) provides real-time in-vivo histological evaluation for gastrointestinal lesions.
- Gastric pit patterns identified by CLE were established.
- Gastric cancerous mucosa could be differentiated from normal gastric mucosa by CLE in some preliminary studies.

What are the new findings?
- The CLE diagnostic criteria for gastric superficial cancerous lesions were developed and evaluated prospectively.
- Gastric cancer/high-grade intraepithelial neoplasia lesions could be distinguished by CLE from non-cancerous lesions.
- Integrated CLE (iCLE) diagnosis had a higher sensitivity, specificity and accuracy for gastric superficial cancerous lesions than white-light endoscopy (WLE) diagnosis.

How might it impact on clinical practice in the foreseeable future?
- The CLE diagnostic criteria developed for gastric superficial cancerous lesions were simple and easy to apply in clinical practice.
- It might be helpful for the early detection of gastric cancers, and screening and surveillance for high-risk populations of gastric cancers.

INTRODUCTION

According to the Paris endoscopic classification, a neoplastic lesion of the stomach is called 'superficial' at endoscopy when its endoscopic appearance suggests that the depth of penetration in the gastric wall is not more than into the submucosa.[1] To some extent, gastric superficial cancerous lesions correspond to early gastric cancers (EGC). Detecting gastric cancer at an early stage is vitally important because EGC may be curable, with reported 5-year survival rates of more than 90%, whereas the prognosis of advanced gastric cancer is poor.[2 3]

Although early detection may be enhanced by advanced endoscopic instruments and techniques, distinguishing EGC from benign lesions is still challenging. During endoscopy, routine biopsies are often taken for suspicious lesions because identifying malignant or benign lesions on endoscopy is unreliable, and histological analysis remains the 'gold standard' for the final diagnosis. Undoubtedly, redundant biopsies are taken for many lesions that are subsequently determined to be not malignant. Thus, pathologists must bear a heavy burden of the work, and patients incur increased costs and associated risks (such as bleeding). Moreover, because of local fibrosis after biopsy, endoscopic resection is difficult; adequate lifting of the lesion by submucosal injection is precluded.[4 5] Hence, in vivo histological imaging without the need for physical sectioning is desirable.

Confocal laser endomicroscopy (CLE), producing both conventional white-light endoscopic (WLE)

and confocal microscopy images, can provide a direct histological observation of the in vivo tissue without the need for biopsy. Recently, CLE has shown its value for gastrointestinal diseases.[6 7] However, few CLE studies on gastric cancer have been reported.[8 9] Kakeji *et al* examined ex vivo normal and malignant tissues of 27 gastric cancers using CLE,[8] and Kitabatake *et al* obtained in vivo CLE images from normal mucosa and cancerous lesions in 27 patients with EGC.[9] These preliminary results showed that gastric cancerous mucosa could be differentiated from normal mucosa by CLE with a sensitivity of 81.8–92.6%, specificity of 97.6–100%, and accuracy of 94.2–96.3% as compared with histology findings.[8 9] Our previous study showed similar results.[10] A limitation of these studies was the small sample size and that only normal and cancerous mucosa was as the study object. In clinical practice, various pathological disorders except for normal and cancerous mucosa, such as inflammation, atrophy, intestinal metaplasia, and dysplasia often appear during endoscopy. Until now, it has not been clear whether cancerous lesions can be distinguished from other abnormal gastric mucosal lesions by using CLE.

Current CLE techniques include probe-based CLE (pCLE) and integrated CLE (iCLE).[11] In this study, we investigated the diagnostic value of iCLE for gastric superficial cancerous lesions. Since real-time iCLE involved endomicroscopic observation combined with white-light imaging, iCLE was termed as meaning WLE plus CLE, in contrast to off-line CLE. Before the beginning of this study, we first developed the initial CLE imaging criteria for gastric mucosal lesions by reviewing our previous data in an unblinded manner. Then, the initial criteria were evaluated afterwards in a phase I study by three endoscopists blinded to the corresponding histopathology findings. The primary endpoint in phase I was to develop the CLE diagnostic criteria for gastric superficial cancerous lesions. Finally, in phase II, a prospective study was carried out to evaluate the real-time iCLE diagnosis for identifying gastric superficial cancerous lesions by using histopathology findings as a gold standard. The primary endpoint in phase II was to evaluate, prospectively, the CLE diagnostic criteria for gastric superficial cancerous lesions, and the secondary endpoint was to compare the diagnostic value of iCLE and WLE with histopathology as a gold standard for identifying gastric superficial cancerous lesions.

PATIENTS AND METHODS

Patients

In phase I, between January 2007 and August 2008, patients with gastric definite or suspected inflammatory or superficial neoplastic lesions were recruited for CLE at Qilu Hospital, Shandong University. Inclusion criteria were definite or suspected EGC, gastric intraepithelial neoplasia, intestinal metaplasia, or gastritis. Exclusion criteria were advanced gastric cancer or any other malignancy; or with conditions unsuitable for performing CLE such as coagulopathy, liver cirrhosis, impaired renal function, pregnancy, breastfeeding, known allergy to fluorescein sodium, or inability to provide informed consent. Targeted biopsy was taken at the site examined by CLE in a point-to-point way. The clinical, endoscopic and histological findings were recorded and stored in a database. High-quality WLE images were captured and stored by the Medical Imaging System (Medicon Digital EngineeringCo., Qingdao, China). CLE images were stored in computer folders corresponding to each patient on compact disks.

In phase II, between August 2008 and July 2009, consecutive patients were enrolled for CLE at Qilu Hospital, Shandong University. Inclusion criteria were: (1) patients with gastric definite or suspected superficial cancerous lesions; (2) patients with gastric precancerous lesions for surveillance endoscopy (including atrophic gastritis, intestinal metaplasia and intraepithelial neoplasia); or (3) patients with dyspeptic symptoms and with 45 years old ≤ age <80 years old. Exclusion criteria were: (1) patients with known advanced cancer, submucosal neoplasm or gastrectomy; (2) patients with alarm symptoms such as anaemia, dysphagia, marked weight loss, etc; or (3) patients under conditions unsuitable for performing CLE such as coagulopathy, liver cirrhosis, impaired renal function, pregnancy, breastfeeding, known allergy to fluorescein sodium, inability to provide informed consent, and other situations.

Written informed consent was obtained from all patients before the procedure.

All the participating patients were prepared for routine gastroscopy. In total, 20 mg scopolamine butylbromide was given by intramuscular injection, and 50 ml of saline solution containing 1 g bicarbonate sodium and 20 000 units α-chymotrypsin was taken orally 15–20 min before endoscopy.[9 12]

Estimation of sample size

The significance level of α was set at 0.05, and the allowable error of δ was set at 0.1. From previous research, gastric cancerous lesions could be diagnosed by CLE with an estimated sensitivity of 90% and specificity of 95% as compared with the gold standard of histological findings.[8 9] According to the sample size formula, the minimum sample size of cases with gastric cancerous lesion was 35, and non-cancerous cases should be not less than 18 in phase I and phase II. Because patients were recruited consecutively in phase II, most of those with dyspeptic symptoms would be non-cancerous cases. Then, many more patients would be enrolled in phase II than in phase I.

Initial CLE imaging criteria

Our research group carried out a series of projects on CLE between June and December 2006.[10–14] CLE images of gastric mucosal lesions were reviewed and compared with corresponding histological findings from targeted biopsy specimens in an unblinded manner. Data for 198 patients with gastritis (68), intestinal metaplasia (54), ulcer (eight), low-grade intraepithelial neoplasia (LGIN, 19), high-grade intraepithelial neoplasia (HGIN, six), adonocarcinoma (26) and healthy volunteers (17) were reviewed. Then we developed the initial CLE imaging criteria for gastric mucosal lesions (table 1). We used information from histopathology and previously published research [8–10 13–16] to help formulate the initial CLE imaging criteria.

Endomicroscopy procedure

CLE involved the use of a Pentax EC3870K endomicroscope (Pentax, Tokyo, Japan) at a scanning speed of 1.6 frames/s, which produced confocal images with a field of view of 475 μm×475 μm (1024×512 pixels), and a contrast agent (5–10 ml of 10% fluorescein sodium; Baiyunshan Mingxing Pharmaceutical Co., Guangzhou, China).

WLE was first performed with the white-light function of the endomicroscope. Gastric mucosal lesions, especially subtle mucosal abnormalities such as elevated, depressed, discoloured, hyperaemic or uneven lesions, were focussed. Then, a total of 5 ml of 10% fluorescein sodium was administered intravenously.[15 16] CLE was performed when the distal end of the endomicroscope was placed in gentle contact with the lesion. Advanced cancers appearing on WLE were not examined by CLE. For patients with endoscopic normal-looking mucosa found on WLE, CLE was performed in a standardised fashion at the following positions:

Table 1 Initial confocal laser endomicroscopy (CLE) imaging criteria for gastric mucosal lesions

	Architecture	Cells	Microvessels
Not IM, not IN	Regularly ranged glands with good polarity; homogeneous in size and epithelial heights	Homogeneous epithelial cells with normal polarity	Honey-comb like (gastric body) or coil-shaped (gastric antrum)
IM	Villous appearance	Large black 'goblet cells'; slender tall, and bright 'absorptive' cells	Normal calibre, honey-comb like or coil-shaped
IN	Impaired gland polarity; irregular in size and epithelial heights	Abnormal cell polarity; irregularity of cellular arrangement; hyperdense epithelial cells with increased stratification	Dilated and distorted appearance
Cancer	Loss of gland polarity: disorganised or destroyed	Loss of cell polarity: irregular and variable in size, disordered appearance	Increased calibre and irregular in size and shape

IM, intestinal metaplasia; IN, intraepithelial neoplasia.

antrum, angulus, antrum—corpus border, body or cardia. In short, the endoscopist switched between WLE and CLE for any particular lesion identified by WLE, and for any standard location in those patients in whom no lesions were seen with WLE. After CLE observation, the endoscopist made a marker by the suction port adjacent to the CLE imaging window, and then biopsied the site 5 mm to the left of the marker. Routine biopsies were also taken for advanced cancers shown on WLE.

Phase I study

The images obtained for each patient selected for analysis were pooled even if they were taken at different areas of the stomach. The CLE images for each patient were coded, randomised, and examined by three endoscopists familiar with CLE. Each endoscopist, blinded to the histological findings, made a diagnosis independently on the basis of the CLE images according to the initial CLE imaging criteria for gastric mucosal lesions (table 1). Each endoscopist was allowed to review the images repeatedly and was asked to explain which of the CLE imaging criteria the diagnosis was based on. The same three endoscopists re-examined the images at 4 week internals.

Phase II study

Each patient was examined by experienced endoscopists, who were blinded to the patient's clinicopathological data before the endoscopy procedure. Endoscopic findings were described according to the updated Sydney System of gastritis,[17] the Paris endoscopic classification of gastric superficial neoplastic lesions,[1] and the Borrmann classification of gastric cancer.[18] WLE scores were recorded prior to CLE observation and could not be changed any more. Each patient received a real-time iCLE diagnosis according to the two-tiered CLE imaging criteria developed in phase I (table 2). Then, each WLE or iCLE diagnosis was compared with the gold standard of histological diagnosis, respectively.

Histological assessment

All the specimens were fixed with 10% formalin, sectioned into 4-mm thick serials, and stained with H&E for histological examination. The slices were evaluated by two experienced pathologists blinded to the endoscopic diagnoses. The histological diagnostic criteria were based on the Updated Sydney System for the classification and grading of gastritis[17] and the WHO classification of tumours (digestive system).[19 20] Gastric adenocarcinoma was further subdivided into differentiated and undifferentiated carcinoma according to the Nakamura classification.[21] The histological analysis after surgical or endoscopic resection was accepted as the final diagnosis.

Diagnosis of EGC

EGC is defined as carcinoma confined to the mucosa or submucosa, regardless of lymph node metastatic status. Chest radiography, CT and/or ultrasound examination were performed to exclude metastatic disease. Surgical or endoscopic ablation was recommended for neoplastic lesions. Endoscopic resection was selected for HGIN or mucosal carcinoma. Endoscopic ultrasonography (EUS) was used to assess the invasion depth of lesions before endoscopic resection. Surgery was used for incomplete endoscopic resection.

Statistical analysis

The statistical analysis was performed by using the statistical software package SPSS 13.0 (SPSS). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated, respectively. The Pearson χ^2 test was used to examine the significance of the association between two variables in a contingency table. A p value of 0.05 (two-sided) was considered statistically significant. Agreement was regarded as poor with κ values below 0.4, good with κ values between 0.4 and 0.75, and excellent with κ values over 0.75. Data are means with ranges and 95% CIs.

RESULTS

Clinicopathological features

In phase I, data for 182 patients were eligible for study analysis (figure 1). The most severe abnormality was used as each patient's diagnosis. For example, intestinal metaplasia associated with atrophic gastritis was considered as intestinal metaplasia. In total, 42 patients had EGC, nine had HGIN, 30 had LGIN, 49 had intestinal metaplasia, and 52 had chronic gastritis. Patients with EGC or intraepithelial neoplasia had a solitary lesion. Among 101 patients with gastritis or intestinal metaplasia, 75 were men (74%), and the mean age was 51.3 years (range,

Table 2 Two-tiered CLE classification for gastric superficial lesions

Feature	Non-cancerous lesions	Cancer/HGIN lesions
Architecture	Orderly ranged glands with regular pit patterns, or mildly heterogeneous in arrangement and distribution	Irregularity in glandular size and shape; disorganised or destroyed pits and glands
Cells	Regular in shape and size; mildly increase in epithelial stratification; normal cell polarity	Irregular cells with disordered appearance; severe stratification; loss of cell polarity
Microvessels	Normal calibre, honey-comb like or coil-shaped	Irregular in shape and calibre

HGIN, high-grade intraepithelial neoplasia.

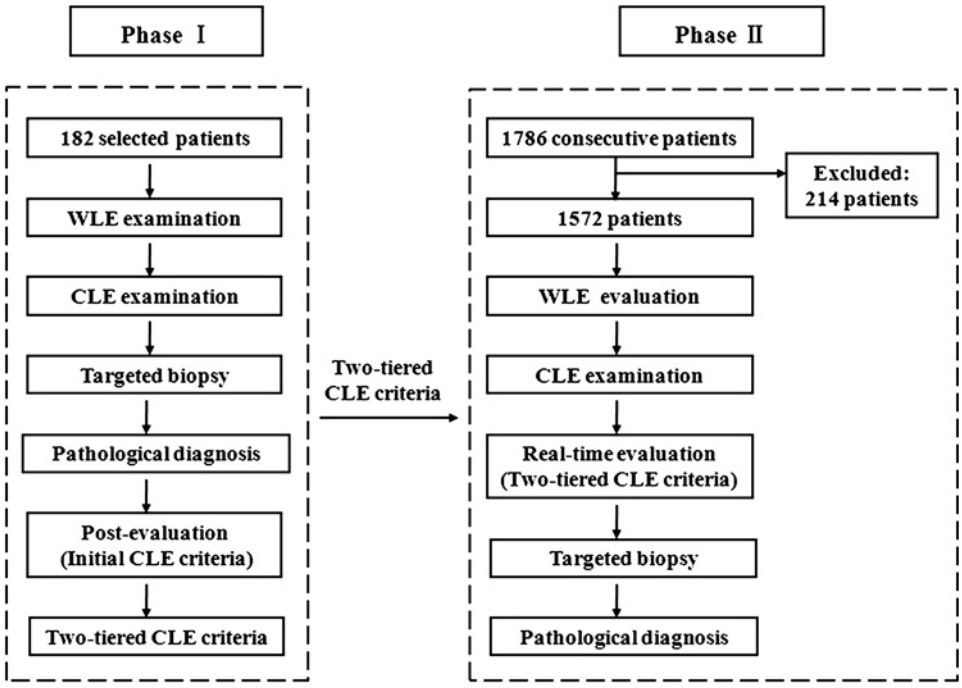

Figure 1 A flow diagram for the study.

32–78 years). Among 81 patients with EGC or intraepithelial neoplasia, 58 were men (72%), and the mean age was 61.8 years (range, 30–79 years). The clinicopathological features of the EGC lesions are summarised in table 3.

In phase II, a total of 1786 patients were enrolled into the study. As far as the reason for endoscopy was considered, 52 patients had gastric ulcer, 39 had gastric polyp, 62 had gastritis, 17 had gastric suspicious lesion, 1598 had dyspeptic symptoms, and the others was 18 cases. In the end, 172 patients who had not undergone CLE were excluded from final analysis, including 70 cases with advanced gastric cancer, 13 cases with duodenal neoplasia, 79 cases with oesophageal neoplasia, and 10 cases with oesophageal varices. In addition, 42 cases had not undertaken CLE for other reasons. Therefore, a total of 1572 patients had completed CLE procedure and were eligible for study analysis. Of these, 1038 were men (66.0%), and the mean age was 58.1 years (range, 36–79 years). In terms of the final histological diagnosis, 57 patients had gastric malignant neoplasms, 15 HGIN, 109 LGIN, 454 intestinal metaplasia, and 937 other indications. Of 57 malignant cases, 14 showed advanced cancer, 40 EGC, one lymphoma, one malignant stromal tumour, and one carcinoid. The clinicopathological features of the EGC lesions are summarised in table 3.

Off-line CLE diagnosis in phase I

The mean number of CLE images obtained for each patient was 127 frames (range, 78–361 frames). Of all images, 26.7% were removed because of poor quality.

Diagnoses were determined on the basis of off-line CLE images alone. The CLE diagnosis was compared with the final histology diagnosis. As shown in table 4, early gastric cancers could be determined by CLE with a high sensitivity (88.1%) and specificity (98.6%); and gastric neoplastic lesions (EGC + intraepithelial neoplasia) could be identified by CLE with 84.0% sensitivity and 92.1% specificity; however, gastric intraepithelial neoplasias were identified by CLE with a low sensitivity (66.7%) and high specificity (92.3%).

As shown in our study as well as in other studies,[7–10 13–15] the characteristics of CLE images varied by disease: inflammation, intestinal metaplasia, intraepithelial neoplasia or cancer (figures 2 and 3). However, just as shown in this study, the distinction between LGIN and gastritis was still unsatisfactory; HGIN was easily confused with cancer; and well-differentiated adenocarcinoma was also sometimes misdiagnosed as intestinal metaplasia by CLE. Therefore, we proposed a simplified two-tiered CLE classification for gastric mucosal lesions: non-cancerous lesions and cancer/HGIN lesions. The former were composed of LGIN and other benign lesions. The distinction was based on irregular and abnormal signs of architecture, cells, or microvessels on CLE images. Abnormal architecture was characterised by loss of regular surface patterns and appearance of atypical glands or disorganised patterns; atypical cells were often dark, irregular in shape and size, and disordered; abnormal microvessels were often rigid or irregular, with increased calibre and unusual shape.

Table 3 Patients' demographics and clinicopathological features of all early gastric cancers in the study

	Phase I	Phase II
Patients	182	1572
Sex (male/female)	133/49	1038/534
Mean age, years (range))	56.0 (30–79)	58.1 (36–79)
Early gastric cancers	42	40
Tumour size, cm (mean, range)	2.1 (0.6–4.0)	1.4 (0.5–3.5)
Macroscopic type		
0–I	2	4
0–II	28	31
0–III	12	5
Histological type		
well-differentiated	14	17
moderately differentiated	11	12
poorly differentiated	12	6
signet-ring cell carcinoma	5	5
Infiltration depth		
intramucosal invasion	13	24
submucosal invasion	29	16

Table 4 Assessment of diagnosis for gastric mucosal lesions based on off-line CLE images in phase I

Lesions	Sensitivity (%, (CI))	Specificity (%, (CI))	PPV (%, (CI))	NPV (%, (CI))	Accuracy (%, (CI))
Not IM, not IN	86.5 (77.3 to 95.8)	91.5 (86.8 to 96.3)	80.3 (70.0 to 90.8)	94.4 (90.4 to 98.4)	90.1 (85.8 to 94.4)
IM	93.9 (83.1 to 98.7)	97.0 (92.5 to 99.2)	92.0 (80.8 to 97.8)	97.7 (93.5 to 99.5)	96.2 (93.4 to 98.9)
IN	66.7 (51.9 to 81.5)	92.3 (87.9 to 96.7)	70.3 (55.5 to 85.0)	91.0 (86.4 to 95.7)	86.8 (81.9 to 91.7)
Cancer	88.1 (78.3 to 97.9)	98.6 (96.6 to 100)	94.9 (82.7 to 99.4)	96.5 (93.5 to 99.5)	96.2 (93.4 to 98.9)
IN + cancer	84.0 (76.0 to 91.9)	92.1 (86.8 to 97.3)	89.5 (82.6 to 92.4)	87.7 (81.5 to 94.0)	88.5 (83.8 to 93.1)

CLE, Confocal laser endomicroscopy; IM, intestinal metaplasia; IN, intraepithelial neoplasia; PPV, positive predictive value; NPV, negative predictive value.

When the two-tiered CLE classification was introduced (table 2), EGC/HGIN lesions could be distinguished from non-cancerous lesions by CLE with a high sensitivity (90.2%; 95% CI: 82.0% to 98.4%), specificity (98.5%; 94.6% to 99.8%), PPV (95.8%; 85.7% to 99.55%), NPV (96.3%; 93.1% to 99.5%), and accuracy (96.2%; 93.4% to 98.9%). Intra- and inter-observer agreement was 0.827 (95% CI: 0.759 to 0.891) and 0.783 (95% CI: 0.712 to 0.874), respectively, for differentiating non-cancerous lesions and EGC/HGIN lesions.

Real-time iCLE diagnosis in phase II

A total of 1572 patients completed the CLE procedure. Each WLE or CLE diagnosis was compared with the histological diagnosis, respectively (table 5). The real-time iCLE diagnosis had a higher accuracy for gastric superficial cancer/HGIN lesions than did the WLE diagnosis ($p<0.05$) (table 6). It seemed that undifferentiated carcinomas were sometimes missed as non-cancerous lesions, and differentiated carcinomas were easily confused with intestinal metaplasia lesions by CLE. In addition, among the patients with the 'endoscopic normal looking mucosa' appearing on WLE, 189 with intestinal metaplasia and 15 with LGIN, but none with HGIN or cancer, were detected in phase II.

DISCUSSION

Gastric superficial cancerous lesions often have subtle changes in gastric mucosa on endoscopy, such as changes in colour or texture, mild elevations or shallow depressions, and areas of uneven surface, which are not specific enough to be identified. In general, biopsy is indispensable for accurate diagnosis. With the development of new endoscopic instruments, direct microscopy observation of the in vivo gastrointestinal mucosa during endoscopy is possible without the need for biopsy.[22] These 'virtual histology' technologies may have a great role in the diagnosis and treatment of EGC.

CLE, a new diagnostic modality, one of the virtual histology technologies, permits real-time in-vivo histological evaluation of gastrointestinal mucosa during on-going endoscopy without

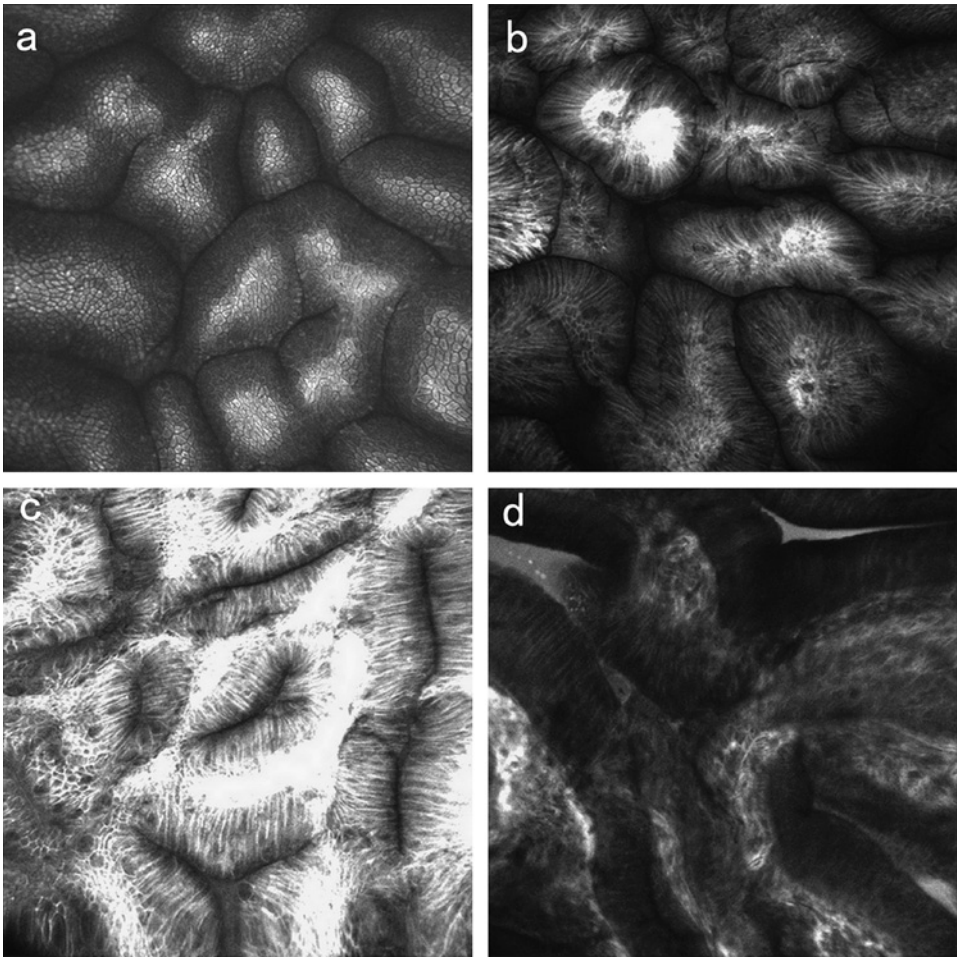

Figure 2 Confocal laser endoscopic images with normal mucosa, intestinal metaplasia (IM), low-grade intraepithelial neoplasia (LGIN) and high-grade intraepithelial neoplasia (HGIN) after intravenous fluorescein injection. (A) Normal mucosa with pyloric glands. Cobble-like appearance with regular columnar cells. (B) Intestinal metaplasia: villous-like appearance with goblet cells. (C) Low-grade intraepithelial neoplasia: black atypical cells, variably sized glands with mild unevenness of the epithelium, and increased fluorescein leakage. (D) High-grade intraepithelial neoplasia: obviously abnormal glands with black atypical cells, disorganised polarity, irregular in epithelial heights, and distorted micro-vessels.

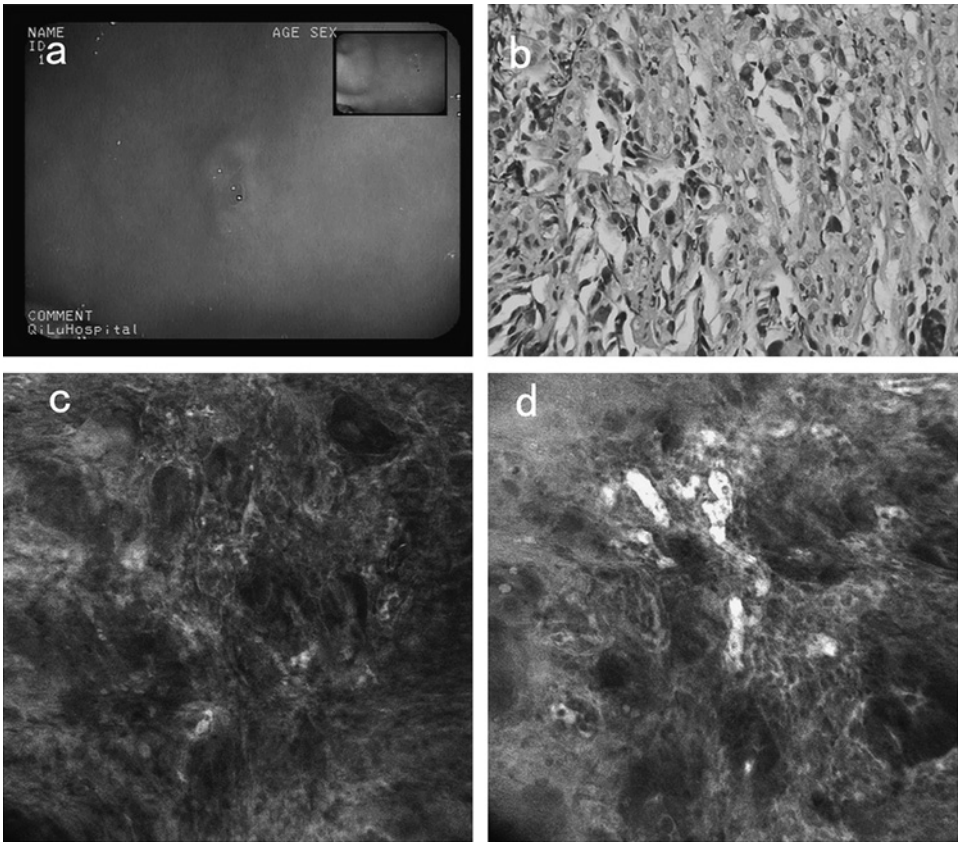

Figure 3 Endoscopic, histological and confocal laser endomicroscopy (CLE) images of an early gastric cancer. White light endoscopy (WLE) view of an antral Paris 0—Ic lesion (A). Corresponding histology specimen showed a poorly differentiated adenocarcinoma (H&E; original magnification, ×400) (B). CLE images after intravenous fluorescein injection (C,D). Irregular black cells with poorly formed glands (C); irregular, short-branched microvessels (D).

taking an actual biopsy.[6 7] The present study aimed to evaluate the Pentax CLE technology in the diagnosis of gastric superficial cancerous lesions in vivo. We first developed the initial CLE imaging criteria for gastric mucosal lesions. Then, we developed a simplified two-tiered CLE classification of non-cancerous lesions and EGC/HGIN lesions. Finally, the two-tiered CLE classification was evaluated prospectively, and we compared the diagnostic value of iCLE and WLE in identifying gastric superficial cancerous lesions. Our study showed that CLE was of a great value in identifying gastric superficial cancer/HGIN lesions and iCLE diagnosis had a higher sensitivity, specificity, PPV, NPV, and accuracy than WLE diagnosis ($p<0.05$).

We proposed a two-tiered CLE classification of non-cancerous lesions and cancer/HGIN lesions for gastric mucosal lesions. This classification was based on several reasons. First, the repeatability or reproducibility of CLE diagnosis for gastric mucosal lesions would be nice. The distinction between LGIN and inflammation is especially difficult and the reported inter-observer variation in the diagnosis of LGIN is large.[20] In addition, HGIN is recognised more reproducibly, but it is difficult to differentiate it from cancer on biopsy.[20 23 24] Second, this classification could provide meaningful risk stratification and management guidelines. LGIN lesions seldom progress to cancer, but HGIN lesions readily evolve to cancer.[23 24] Lesions with HGIN or cancer are often recommended for resection, whereas follow-up is usually recommended for non-neoplastic or LGIN lesions.[20]

As with histopathology observations,[19 20 25 26] on CLE imaging, atypical cells combined with irregular architecture or microvessels are crucial for distinguishing non-cancerous lesions and cancer/HGIN lesions. Regular or normal signs are: (1) cells that are homogeneous in shape and size; there is reserved cell polarity. (2) architecture that has a consistent appearance of pit patterns or crypt openings; there is homogeneous or symmetrical in arrangement and distribution. (3) microvessels that are coil-shaped at the antrum and honeycomb-like at the gastric body; are uniform in shape and calibre; and are consistent in arrangement and distribution. Irregular or abnormal signs are: (1) cells that are often dark; heterogeneous in shape and size; exhibit a loss of cell polarity and severe cell stratification. (2) architecture that is heterogeneous or abnormal; there are disorganised or destroyed pits and glands. (3) microvessels that are varied in shape and calibre; are tortuous or branched; and are heterogeneous in distribution. On the basis of these parameters, we found good intra- and inter-observer agreements for differentiating gastric EGC/HGIN and non-cancerous lesions (phase I).

Gastric cancer is believed to arise from a series of pre-malignant lesions: atrophic gastritis, intestinal metaplasia and dysplasia.[27] Numerous studies have also demonstrated that gastric cancerous lesions are associated with a number of different non-cancerous lesions. Thus, lesions with gastritis, intestinal metaplasia or

Table 5 Comparative data of the diagnosis of WLE and integrated CLE (iCLE) with histopathology for gastric superficial lesions in phase II

	Histopathology				
			Malignant		
Diagnosis	Benign	HGIN	EGC	AGC	Other
WLE					
Non-cancerous lesion	1427	7	11	0	2
Cancer/HGIN lesion	73	8	29	14	1
iCLE					
Non-cancerous lesion	1489	3	4	0	1
Cancer/HGIN lesion	11	12	36	14	2

AGC, advanced gastric cancer; CLE, confocal laser microscopy; EGC, early gastric cancer; HGIN, high-grade intraepithelial neoplasia; iCLE, integrated CLE; WLE, white-light endoscopy.

Table 6 Comparison of the diagnostic value of WLE and integrated CLE (iCLE) for gastric superficial lesions in phase II

Diagnosis	Sensitivity (%, (CI))	Specificity (%, (CI))	PPV (%, (CI))	NPV (%, (CI))	Accuracy (%, (CI))
WLE	72.2 (61.9 to 82.6)	95.1 (94.0 to 96.2)	41.6 (33.0 to 50.2)	98.6 (98.0 to 99.2)	94.1 (92.9 to 95.3)
iCLE	88.9 (81.6 to 96.1)	99.3 (98.8 to 99.7)	85.3 (77.3 to 93.3)	99.5 (99.1 to 99.8)	98.8 (98.3 to 99.3)
p Value	0.012	0.000	0.000	0.018	0.000

CLE, confocal laser microscopy; iCLE, integrated CLE; NPV, negative predictive value; PPV, positive predictive value; WLE, white-light endoscopy.

intraepithelial neoplasia were selected as confounding factors in our phase I study. Normal gastric mucosa and advanced gastric cancer were not included because they are easily recognised on CLE or WLE.[9] The phase I study showed that CLE had a high ability to diagnose intestinal metaplasia or cancer, but a low ability to diagnose intraepithelial neoplasia. LGIN and gastritis, HGIN and cancer, were easily confused by CLE, respectively. With use of the two-tiered CLE classification, gastric superficial cancer/HGIN lesions could be identified by iCLE with a sensitivity of 88.9%, a specificity of 99.3%, a PPV of 85.3%, a NPV of 99.5% and an accuracy of 98.8% in the phase II study.

In our phase II study, we compared the diagnostic value of iCLE and WLE for gastric superficial cancerous lesions using histopathology as a gold standard. The results showed that iCLE diagnosis had a higher accuracy than WLE diagnosis ($p<0.05$). Moreover, we detected 189 patients with intestinal metaplasia and 15 with LGIN by iCLE from the patients with the 'endoscopic normal looking mucosa' appearing on WLE. Our previous study has proved that the sensitivity and specificity for the diagnosis of intestinal metaplasia with WLE were significantly lower than those with CLE (sensitivity: 36.88% vs 98.13%; specificity: 91.59% vs 95.33%).[13] In this study, 189 patients with intestinal metaplasia were identified by random CLE imaging among 1572 patients with the 'endoscopic normal-looking mucosa' appearing on WLE. These results indicate that iCLE may become an important screening and surveillance modality for intestinal metaplasia. However, there were fewer cases of LGIN identified by random CLE imaging (15/1572), which suggests that iCLE screening and surveillance for gastric LGIN is currently limited to WLE-identified lesions.

Evidence suggests that the association of endoscopic and histological findings of gastric superficial neoplastic lesions is not always perfect. In general, judging the nature of suspicious gastric mucosal lesions and whether a biopsy is needed on endoscopy depends on the endoscopist's skill and experience. Impersonal diagnostic criteria could be established by using CLE, which would contribute to the diagnosis and management of suspicious lesions. CLE could enable targeted biopsy, thereby avoiding unnecessary biopsies and mistaken diagnosis. In addition, for subtle lesions, the initial biopsy is important for the accurate diagnosis because bleeding in the target area may prevent further biopsies. CLE would be helpful to select the proper targeted points, and increase the accuracy of biopsies.

There were several limitations in this study. The first was the patient selection. Most patients enrolled in phase I had a definite diagnosis, and were cancer-enriched populations. In addition, although patients were enrolled consecutively into phase II, some patients with a definite diagnosis were still included. Moreover, patients with advanced cancers appearing on WLE were excluded from CLE examination. As a result, bias would be induced by the patient selection. Second, although iCLE was compared with WLE on the diagnostic accuracy for gastric superficial cancerous lesions in phase II, the detection rate was not investigated. In addition, because the iCLE results were based on the information of both WLE and CLE, iCLE may be favoured by the process. More comparative studies, especially randomised controlled trails, should be designed to evaluate the value of CLE for gastrointestinal diseases in the future.

In conclusion, we showed that gastric superficial cancer/HGIN lesions could be determined with high validity and reliability by CLE. Our two-tiered CLE classification of non-cancerous lesions and cancer/HGIN lesions for gastric mucosal lesions is rational. In addition, real-time iCLE diagnosis may have a higher accuracy than WLE diagnosis alone in this setting. These results suggest that CLE might be a useful means for the screening and surveillance of patients at high risk of gastric cancers. With the development of instruments and new contrast agents, real-time in vivo CLE might have an important role in the management of gastrointestinal diseases.[28]

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Competing interests None

Ethics approval This study was conducted with the approval of the clinical research ethics committee of Shandong University's Qilu Hospital, and according to the Declaration of Helsinki.

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