Confocal laser endomicroscopy for in vivo diagnosis of gastric intraepithelial neoplasia: a feasibility study

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Background: Confocal laser endomicroscopy (CLE) is a novel endoscopic modality that allows subsurface analysis of the gastric mucosa during ongoing endoscopy. Several studies have reported that this technique is of value in the diagnosis of premalignant lesions in the GI tract, but as yet no investigations have reported its application in the analysis of gastric intraepithelial neoplasia (GIN).

Objective: To assess the feasibility of CLE for the identification and grading of GIN.

Design: Prospective double-blind feasibility study.

Setting: Qilu Hospital, Shandong University, Jinan, China.

Patients: CLE images of 33 patients were first evaluated to establish the diagnostic criteria for gastric lesions. Eligible patients were then prospectively investigated by CLE using the newly established criteria.

Interventions: All endoscopically suspicious lesions were examined by CLE, and CLE diagnoses were compared with corresponding histopathologic results.

Main Outcome Measurements: Sensitivity, specificity, and positive and negative likelihood ratios of CLE diagnosis of biopsy-proven intraepithelial neoplasia by per-lesion analysis.

Results: The sensitivity, specificity, and positive and negative likelihood ratios of CLE diagnosis of GIN were 77.8%, 81.8%, 4.28, and 0.27, respectively. The mean κ value for interobserver agreement for the diagnosis of GIN was 0.70 among endoscopists and 0.71 between endoscopist and GI pathologist. Intraepithelial neoplasia score $\geq$5 differentiated high-grade from low-grade intraepithelial neoplasia with a sensitivity of 66.7% and a specificity of 88.0%.

Limitations: Nonrandomized single-center study, limited number of patients.

Conclusions: CLE is an acceptable and potentially useful technology for the identification and grading of GIN in vivo. The diagnostic accuracy needs to be improved. (Gastrointest Endosc 2010;72:1146-53.)

Gastric cancer remains the world's second leading cause of cancer-related deaths, with a mortality rate of 16.3 per 100,000 in men and 7.9 per 100,000 in women.[1] Strategies to improve prognosis essentially depend on earlier detection of preneoplastic and neoplastic transformations because only intraepithelial neoplasia and early gastric cancers can potentially be cured by endoscopic treatment.

Abbreviations: CI, confidence interval; CLE, confocal laser endomicroscopy; GIN, gastric intraepithelial neoplasia; HGIN, high-grade intraepithelial neoplasia; INS, intraepithelial neoplasia score; LGIN, low-grade intraepithelial neoplasia; ROC, receiver operating characteristic.

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Gastric intraepithelial neoplasia (GIN) is widely regarded as a precancerous lesion that should be closely followed or treated endoscopically.[2-5] The diagnosis of these lesions, which present fairly inconspicuous endoscopic features, is currently based on pathologic assessment of endoscopic biopsy specimens. Modern endoscopic devices, such as chromoendoscopy, magnifying endoscopy, narrow-band imaging, and trimodal imaging endoscopy, have demonstrated significant value for the detection of early gastric neoplasia.[6-8] However, earlier studies mainly focused on the recognition and characterization of early gastric carcinoma, and the endoscopic detection of GIN was less mentioned and investigated.[6,8] Considering the higher incidence of GIN compared with early gastric carcinoma, especially in high-risk areas such as China, it is desirable to explore a novel endoscopic device with the primary purpose of identifying GIN in vivo.[9] Furthermore, given the different progression risk of GIN, it is essential to differentiate high-grade intraepithelial neoplasia (HGIN) from low-grade intraepithelial neoplasia (LGIN) in screening and surveillance populations.

Confocal laser endomicroscopy (CLE) is a novel endoscopic device that can provide real-time microscopic visualization of the mucosal layer with a high resolution (lateral resolution 0.7 μm) while at the same time displaying standard video imaging. The endomicroscopic imaging is generated by a confocal laser microscope that is integrated into the distal tip of a conventional videoendoscope. Several studies have reported that this technique is of value in the diagnosis of premalignant lesions in the upper and lower GI tract.[10-15] Gastric pit patterns defined by CLE have been found to be predictive of the histology of gastric atrophy, and the diagnostic criteria for the identification of gastric intestinal metaplasia have been validated with a high diagnostic accuracy.[10,11] There are several studies of CLE in patients with Barrett's esophagus–associated neoplasia.[13] CLE has also been evaluated as useful for the diagnosis of intraepithelial neoplasias in the colon.[14,15] However, no investigation has yet reported its application in the analysis of GIN. Therefore, the aim of the present study was to evaluate the feasibility of CLE for identifying and grading GIN.

Take-home Message

- Distinct characteristics on gland architecture, cell morphology, and vasculature in endomicroscopic images can enable the identification of gastric intraepithelial neoplastic (GIN) changes in vivo. Of note, the interobserver and intraobserver agreements for endomicroscopic diagnosis of GIN were all substantial.
- The results of this feasibility study need to be validated in a larger population with routine application of broad-field techniques such as chromoendoscopy.

METHODS

Derivative study

To establish the endomicroscopic classification of gastric lesions, 33 subjects with histologically confirmed normal mucosa (n = 4) and nonneoplastic (n = 12) and neoplastic (n = 17; including 12 LGINs and 5 HGINs) lesions were selected from outpatients at Qilu Hospital. Clinical indications for endoscopic examination for these 33 patients included upper abdominal symptoms (8 subjects) and surveillance endoscopy (25 subjects). All subjects received the same endoscopic procedure as described in the validation study, and targeted biopsy specimens were sectioned in both vertical and transverse planes to facilitate comparison of histology and CLE images. Confocal images and corresponding histopathologic pictures from nonneoplastic and GIN lesions were openly analyzed by 5 experienced endoscopists (Y.-Q.L., K.-Y.H., X.-L.Z., T.Y., and X.-M.G.) and 1 reference GI pathologist (C.-J.Z.). Endomicroscopic classification for nonneoplastic and GIN lesions was developed based on the comparison between in vivo and conventional ex vivo histology, pathologic criteria used for diagnosing GIN, and previous published research (Table 1).[10,11,16-19] In an attempt to differentiate LGIN from HGIN, distinguishing endomicroscopic features between LGIN and HGIN were identified. These features are based on changes in gland architecture, cell morphology, and vessel architecture. Each parameter was graded 0, 1, or 2 according to the severity of GIN changes as imaged with CLE (Table 2). The sum of scores was proposed as an intraepithelial neoplasia score (INS). The newly derived endomicroscopic criteria were subsequently validated in the prospective study.

Validation study

From July 2009 to January 2010, consecutive patients with long-standing upper abdominal symptoms (≥15 years) or who were undergoing surveillance endoscopy (for known GIN, atrophic gastritis, or history of GIN) from outpatients at Qilu Hospital were informed about the purpose of this study. The exclusion criteria were advanced gastric carcinoma or any other malignancy in the GI tract, active gastric ulcer, acute upper GI bleeding, coagulopathy, known allergy to fluorescein, impaired renal function, pregnancy, or lactation. Suitable patients were recruited only if they were willing to sign the written informed consent of the study.

This study was approved by the Institutional Ethics Committee of Qilu Hospital and was conducted in accordance with the revised Declaration of Helsinki (1989).

Endoscopic procedure

Before the endomicroscopic examination, 20,000 U α-chymotrypsin and 80 mg dimethylpolysiloxane were given orally to remove gastric mucus. Then 1 mL 2% fluorescein sodium (Baiyunshan Mingxing Pharmaceutical Co, Guangzhou, China) was administered intravenously for allergy test. Conscious sedation was achieved for each patient by using propofol and fentanyl, and vital signs

TABLE 1. Endomicroscopic classification of gastric lesions

	Gland architecture	Cell morphology	Vessel architecture
Normal architecture	Regularly ranged glands, with round (fundic glands) or continuous short rod-like (pyloric glands) pits	Homogeneous epithelial cells with normal polarity	Honeycomb-like (gastric body) or coil-shaped (gastric antrum)
Nonneoplastic lesion	Good polarity with elongated pits Homogeneous in size and epithelial heights	Good cell polarity: regularly ranged epithelial cells, uniform in size and shape	Honeycomb-like or coil-shaped, no or mild increase in the capillaries number
Lesion with intraepithelial neoplasia	Impaired gland polarity: crowded glands with variable degrees of intraluminal folding, glandular budding and branching Irregular in size and epithelial heights	Abnormal cell polarity: mild to severe irregularity of cellular arrangement Hyperdense epithelial cells with increased stratification	Increased capillaries with dilated and distorted appearance

TABLE 2. Endomicroscopic scoring system for GIN

Parameters	Illustrations of the scoring grade
Gland architecture	0: normal 1: preserved polarity and maturation, variably sized glands with mild unevenness of the glandular epithelium; mild to moderate increase in gland density 2: lack of maturation for most glands, prominent irregularity in glandular size and morphology; obviously crowded glands with complex budding and branching
Cell morphology	0: normal 1: preserved cell polarity, mild to moderate increase in epithelial stratification 2: disorganized polarity, severe increase in epithelial stratification
Vessel architecture	0: normal 1: mild to moderate irregularity with increased capillaries number 2: severely dilated and distorted appearance with increase in capillaries number

TABLE 3. Patient demographics and clinical features of gin lesions in this study

	Derivative study	Validation study
Patients, no.	33	75
Gender (male/female), no.	23/10	53/22
Mean age, y (range)	56 (38-78)	57 (31-79)
GIN lesions, no.	17	47
Mean size, mm (range)	8 (3-20)	7 (3-20)
Macroscopic type, no.		
0-I	1	5
0-IIa	5	18
0-IIb	1	4
0-IIc	9	19
0-III	1	1
Location		
Upper third of the stomach	1	4
Middle third of the stomach	3	9
Lower third of the stomach	13	34

GIN, gastric intraepithelial neoplasia.

were monitored during the entire procedure. All patients received standard white-light endoscopic and endomicroscopic examination by using a Pentax EC-3870K confocal laser endomicroscope (Pentax, Tokyo, Japan). Each CLE examination was performed by 1 of the 3 senior endoscopists (X.-L.Z., T.Y., and X.-M.G.), who each had >10 years' endoscopic experience performing >10,000 EGDs. More than 300 CLE procedures had been performed by each of the endoscopists before embarking on the present study. After successful intubation of the endoscope into the duodenum, 5 to 10 mL fluorescein sodium solution was applied intravenously as a contrast dye.

All suspicious lesions detected with white-light endoscopic observation were carefully examined by CLE. The definitions of suspicious lesions are changes in color, ruggedness, elevation, and depression of the gastric mucosa. Ten to 15 mL 0.2% indigo carmine was applied topically in selective cases to facilitate lesion demarcation and detailed observation. Additionally, if a single lesion

had more than one site examined, the CLE diagnosis for this lesion was made according to the most severe abnormalities. The morphology of lesions was characterized according to the Paris classification.[20] Finally, targeted biopsy of the examined site was performed 5 mm immediately to the left of the "polyp" created by suction.

Histopathologic evaluation

The biopsy specimens obtained from each examined lesion were immediately fixed in 10% formalin, embedded in paraffin, and serial sections at 4-μm intervals stained with hematoxylin-eosin for histopathologic analysis. Two experienced GI pathologists (C.-J.Z. and T.-G.Z.) who were unaware of the patients' clinical or endoscopic information examined all of the specimens independently. The diagnosis and graduation of gastric epithelial neoplasia were made according to the Vienna classification.[21]

Design of confocal image assessment

A preliminary CLE diagnosis of the observed lesion was made by 1 of the 3 endoscopists during the procedure. Although the endoscopists were informed that the study population was enriched and included patients with long-lasting upper abdominal symptoms and patients undergoing surveillance endoscopy, they had no access to any clinical information before endoscopy. All endoscopic procedures were performed under the supervision of a study coordinator.[8]

CLE images of each lesion were stored in a specific folder, and were reevaluated after the endoscopy by another CLE investigator (R.J.) blinded to the patients' clinical history and endoscopic information. The final CLE diagnosis regarding GIN was made according to the newly developed gastric lesions classification as described in Table 1.

A post hoc assessment of interobserver and intraobserver agreements for the CLE findings was performed according to the following protocol. A data set containing 50 confocal images of medium depth from 50 enrolled subjects were randomly selected and displayed to 3 independent endoscopists (Y.-Q.L., T.Y., and R.J.) and 1 GI pathologist (C.-J.Z.) in a blinded fashion. The selection criteria of certain confocal images were based on the presence of interpretable epithelial images as determined by one experienced CLE investigator (X.-M.G.). Each image was evaluated by using the newly developed GIN diagnostic criteria. Interobserver agreement was calculated between endoscopists and between endoscopist and GI pathologist. To evaluate the intraobserver agreement, one investigator (R.J.) reassessed these 50 pictures after a 7-day interval.

Statistical analyses

The SPSS 13.0 statistical software package (SPSS, Chicago, Ill) was used for data analysis. Besides the diagnostic yield description, such as sensitivity and specificity, we also assessed the likelihood ratios to calculate the probability of abnormality and their 95% confidence intervals (CIs).[22] Chi-square test was used for comparisons. A P value of $<.05$ (two-tailed) was considered to be statistically significant. Receiver operating characteristic (ROC) curve was computed to explore the optimal cutoff value of INS that could differentiate between LGIN and HGIN. The interobserver and intraobserver agreements were estimated by κ value, values of 0.01 to 0.20 indicating poor agreement, 0.21 to 0.40 fair, 0.41 to 0.60 moderate, 0.61 to 0.80 substantial, and 0.81 to 1.00 almost perfect. The study report was in accordance with the standards for reporting studies of diagnostic accuracy.[23]

RESULTS

The demographic characteristics of patients in the derivative study and the validation study as well as the clinical features of GIN lesions identified in this study are summarized in Table 3. All of the cases in the derivative study were not included in the feasibility study.

Validation study

Indications included long-standing upper abdominal symptoms (21 patients) or surveillance endoscopy (54 patients). All of the participants completed the study protocol, and no severe side effects were observed during the entire procedure. The mean duration of the examination was 24 minutes (range, 16-45 minutes). In total, 15,093 confocal images were obtained from 91 macroscopic lesions in the 75 patients. The examples of normal gastric mucosa and nonneoplastic and intraepithelial neoplastic gastric lesions by CLE and corresponding histopathology are illustrated in Figure 1.

Histopathology showed 47 neoplastic lesions obtained from 45 patients, of which 36 were LGIN, 9 HGIN, 1 intramucosal carcinoma, and 1 small cell carcinoma. Additionally, 44 nonneoplastic (16 inflammatory, 1 hyperplastic, 11 atrophic, and 16 intestinal metaplastic) lesions were evaluated. The two lesions with the final diagnosis of carcinoma were excluded from further data analysis.

Comparison of CLE and histopathologic diagnosis for GIN

By using the earlier-described CLE diagnostic criteria for gastric lesions (Table 1), the preliminary and final CLE diagnoses were compared with histopathologic results (Table 4). The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio of final CLE diagnosis of GIN were 77.8% (95% CI, 63.7%-87.5%), 81.8% (95% CI, 68.0%-90.5%), 81.4% (95% CI, 67.4%-90.3%), 78.3% (95% CI, 64.4%-87.7%), 4.28 (95% CI, 2.24-8.16), and 0.27 (95% CI, 0.16-0.48), respectively. There was no statistical difference between preliminary and final CLE diagnostic accuracy for GIN ($P = .549$).

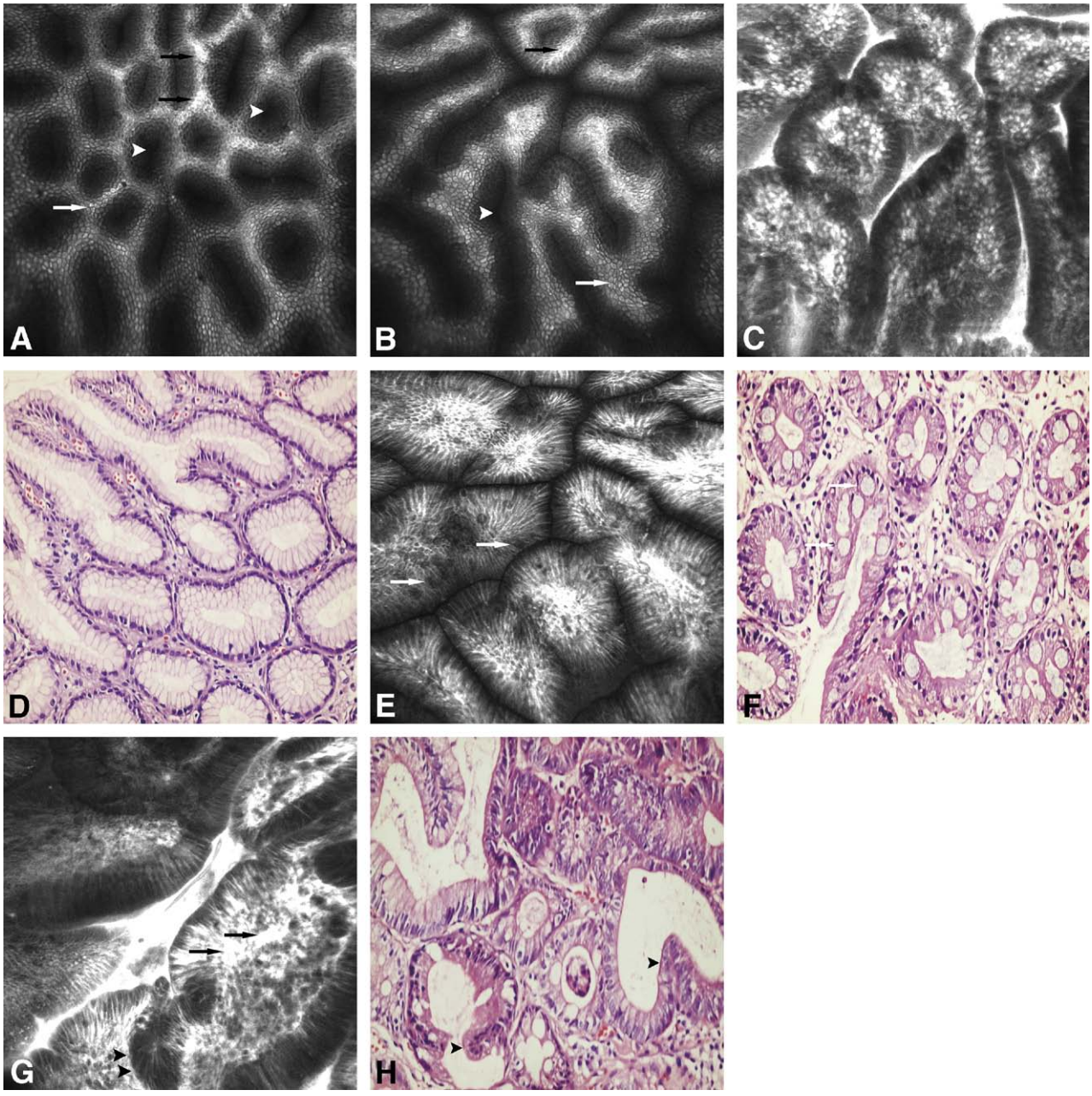

Figure 1. A, The confocal image of normal gastric mucosa with fundic glands shows round pits (*arrowhead*). Homogeneous columnar cells (*white arrow*) and honeycomb-like subepithelial capillary network (*black arrow*) are readily identifiable. **B,** The confocal image of normal gastric mucosa with pyloric glands shows continuous short rod-like pits (*arrowhead*). Regular columnar cells (*white arrow*) and coil-shaped subepithelial capillary network are also present (*black arrow*). **C,** Endomicroscopy shows elongated and tortuous branch-like pits with good polarity and mild fluorescein leakage caused by inflammation. **D,** Corresponding histopathology shows inflammatory antral mucosa (H&E, ×400). **E,** CLE shows villous-like foveolar epithelium with goblet cells (*white arrows*), and the glandular architecture retains its normal polarity. **F,** Corresponding histopathology confirms intestinal metaplasia of the antral mucosa without GIN (H&E, ×400). **G,** Confocal image obtained from this lesion shows irregular gland architecture, hyperdense epithelium with focal budding (*arrowheads*) and increased fluorescein leakage (*black arrows*). **H,** Corresponding histological specimens confirms LGIN (H&E, ×400).

Interobserver and intraobserver agreements

The interobserver agreement was substantial for the diagnosis of GIN among 3 endoscopists (mean κ, 0.70). We also investigated the interobserver agreement between endoscopist and GI pathologist, and the mean κ value was 0.71. Intraobserver agreement was also graded as substantial (κ, 0.78).

Classification of GIN and ROC curve analysis

To assess the feasibility of CLE for the differentiation between LGIN and HGIN, one confocal image of medium depth from each CLE-diagnosed GIN lesion was further evaluated by an investigator (R.J.) according to the predefined scoring system in a blinded fashion (Table 2). Histopathologic results confirmed 35 CLE-diagnosed GIN

TABLE 4. Comparison of CLE and histopathology for diagnosing GIN

		Primary CLE		Final CLE	
Histopathology	n	GIN (+)	GIN (−)	GIN (+)	GIN (−)
GIN (+)	45	36	9	35	10
GIN (−)	44	12	32	8	36
Total	89	48	41	43	46

CLE, confocal laser endomicroscopy; *GIN,* gastric intraepithelial neoplasia.

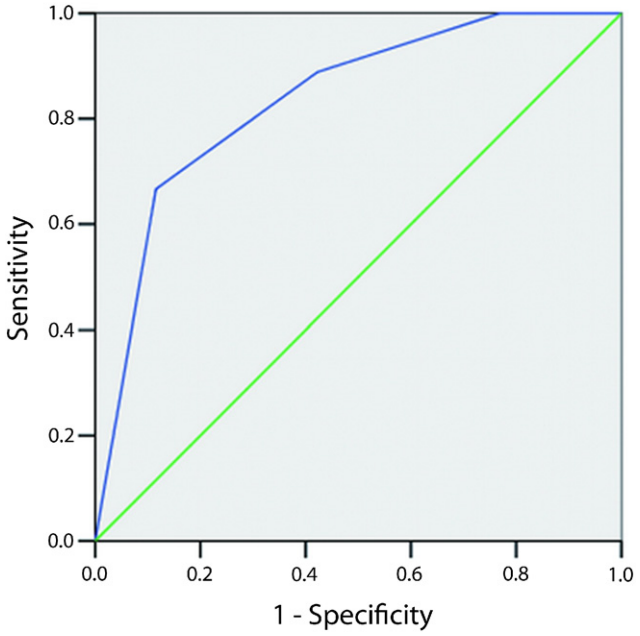

Figure 2. ROC curve analysis for INS in gastric HGIN and LGIN lesions. Sensitivity = 66.7%; specificity = 88.5%; cut off score = 5; area under the ROC curve = 0.835 (95% CI, 0.686-0.985).

lesions (26 LGIN and 9 HGIN), and the scoring outcomes of 35 confocal images from these lesions were plotted into ROC curves (Fig. 2). The optimal cutoff value between LGIN and HGIN was evaluated as 5. INS ≥5 had a sensitivity of 66.7% and a specificity of 88.5% in discriminating HGIN from LGIN. Severity changes of corresponding lesions are depicted in Figure 3.

DISCUSSION

It is generally accepted that GIN, previously known as gastric dysplasia, is a precancerous lesion that requires either long-term follow-up or endoscopic treatment, depending on the severity of histologic changes. Compared with gastric atrophy and intestinal metaplasia, the clinical importance of GIN surveillance is further strengthened because GIN is believed to be the penultimate stage of gastric carcinogenesis.[2] CLE is a novel endoscopic device that allows in vivo microscopic imaging 250 μm below the surface layer. It may provide endoscopists a new option with the ability to organize appropriate follow-up schemes or make immediate decisions on endoscopic resection of gastric lesions detected during endoscopy. Although the clinical values of CLE in diagnosing gastric cancer and intestinal metaplasia have been reported,[11,12,18,19] clearly defined criteria for confocal diagnosis for GIN are still lacking. Results of the present study showed that GIN can be identified in confocal images by studying the associated cellular and tissue changes.

Similarly to the case with histopathologic features, distinct cellular abnormalities (including changes in the orientation, size, and shape of the cells), organizational derangements (including impairment of polarity, glandular budding, and branching), and microvascular alterations (including vessel dilation, distortion, and increased capillary number and fluorescein leakage) were identifiable for the diagnosis of GIN during confocal imaging with fluorescein. After comparing the in vivo confocal images with the histopathologic results and using the newly established CLE diagnostic criteria for gastric lesions, we observed that CLE could diagnose the presence of GIN with a sensitivity of 77.8%, a specificity of 81.8%, a positive likelihood ratio of 4.28, and a negative likelihood ratio of 0.27. Of note, the interobserver and intraobserver agreements for the diagnosis of GIN were all substantial, and these further supported the reliability of our CLE criteria for diagnosing GIN.

In the present study, there were 10 false-negative and 8 false-positive diagnoses by the CLE criteria on a per-lesion assessment. Histologic examination of the false-negative cases showed mostly columnar cells with hyperchromatic and pencillate nuclei, whereas the general architecture was only slightly altered. The 8 false-positive cases were diagnosed mainly by features of obvious reactive changes such as foveolar hyperplasia or complex intestinal metaplasia. The diagnosis of these kinds of histopathologic changes, however, also challenges many general pathologists.[3] Furthermore, the main reason for the impairment of diagnostic specificity was that we empirically excluded apparent benign lesions (such as erosions and hyperemia) from the present study because this study targeted only gastric lesions that were suspected of GIN with white-light endoscopy.

Based on the degree of endomicroscopic GIN changes, we proposed that CLE could differentiate LGIN from HGIN. We used the ROC curve analysis, which revealed that an INS score ≥5 could discriminate HGIN from LGIN with a sensitivity of 66.7% and a specificity of 88.0%. The high specificity supports the concept that more severe endomicroscopic GIN changes do correlate with higher grade of epithelial neoplasia. In practical terms, this is useful because it is the higher grade of epithelial neoplasia

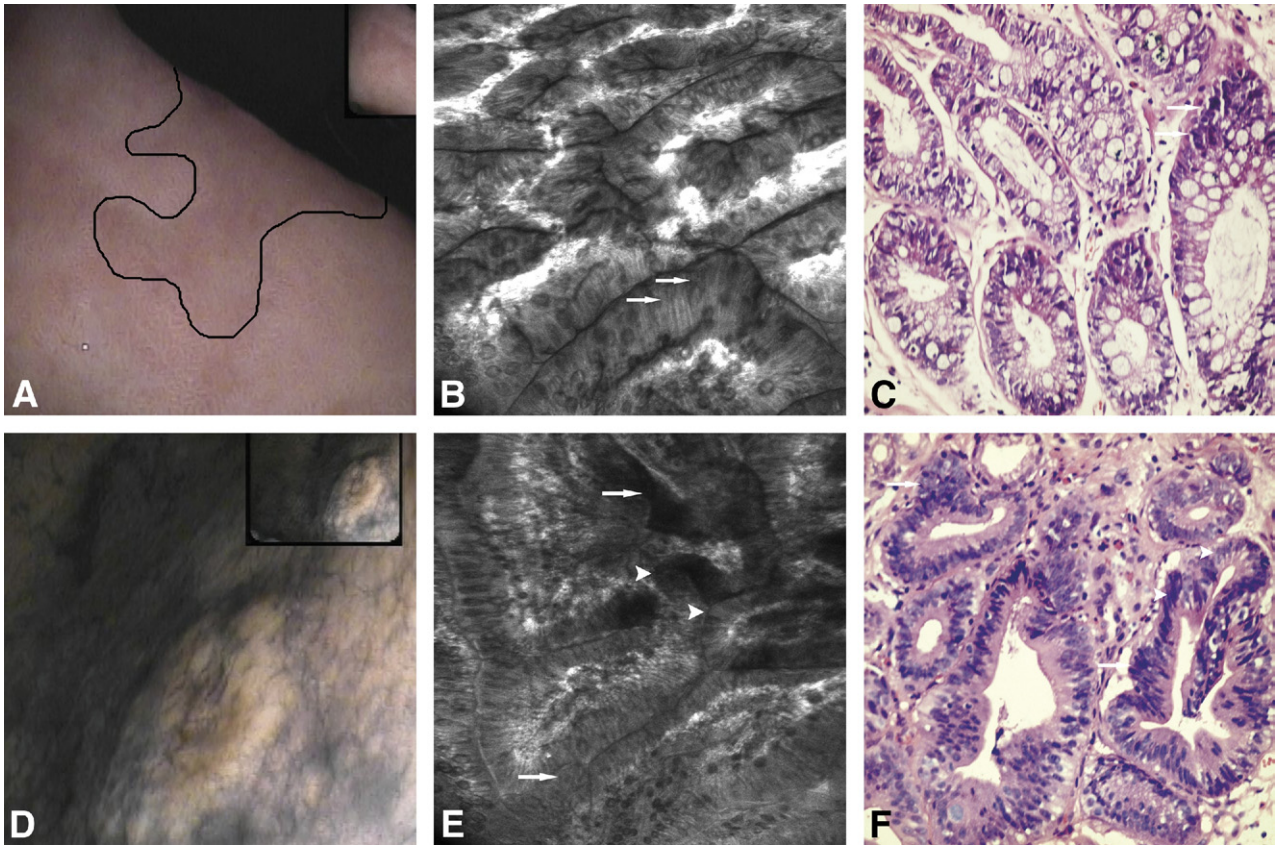

Figure 3. A, White-light endoscopic view of a flat gastric lesion (type 0-IIb). **B,** Confocal image obtained from this lesion shows variably sized glands with mild unevenness of the epithelium, moderate increase in gland density and mild increase in epithelial stratification (*white arrows*). **C,** Corresponding histopathology confirms LGIN (H&E, ×400). **D,** Endoscopic view of a superficial elevated lesion (type 0-IIa) after spraying indigo carmine. **E,** Confocal image obtained from this lesion shows severe crowding glands with complex budding and branching (*arrowheads*) and prominent stratified epithelial cells with disorganized polarity (*white arrows*). **F,** Corresponding histopathology shows similar findings (H&E, ×400).

that demands immediate diagnosis for the endoscopist to make a spot-decision regarding whether to perform endoscopic resection or otherwise. The lower sensitivity in distinguishing between LGIN and HGIN can be explained on the basis of the inability of CLE using fluorescein per se to accurately distinguish the nuclear-cytoplasmic ratio, nuclear pleomorphism, and hyperchromatism, pathologic features that are important in grading the degree of neoplasia.

Here, we chose fluorescein sodium as the only contrast agent for CLE imaging rather than the combined use of acriflavine hydrochloride for the following reasons: First, acriflavine spraying merely stains the superficial 50-μm cell layers, and given that scanning at the superficial layers may induce compression artifacts, confocal images obtained from such limited scanning depth are generally not used for the diagnosis of GIN; second, and more importantly, acriflavine carries potential risk for mutagenicity, as has been suggested in experimental data,[24] and therefore cannot be practically advocated as a contrast agent in many countries. However, we do acknowledge that the lack of direct nuclei definition does hamper the precise CLE diagnosis of GIN, resulting in a reduced diagnostic accuracy of 79.8%. Future studies should explore the use of other nuclei-enhancing agents that could overcome these drawbacks.[25]

There were several limitations of this feasibility study. First, chromoendoscopy was only applied in selected cases (10 lesions in the validation study) when the demarcation of a lesion was difficult to recognize. Gastric lesions may be missed in cases without chromoendoscopy, because CLE is not well suited to examining large areas of tissue and should ideally be combined with broad-field techniques. Further studies using CLE for GIN surveillance should be combined with routine application of chromoendoscopy. Second, considering the primary outcome of this feasibility study was the accuracy of CLE for diagnosing GIN lesions rather than the detection rate of GIN lesions, we only included patients with a higher risk of developing GIN, such as patients with long-lasting (≥15 years) upper GI symptoms and patients undergoing surveillance endoscopy. However, these selection criteria may have some effects on the results. Therefore, the results of this feasibility study need to be validated in future studies including patients with varying indications. Third, LGIN took up a larger proportion of the whole sample (36

LGIN, 9 HGIN). Because LGIN is difficult to be differentiated accurately from atypical reactive/regenerative epithelial changes,[26] it may decrease the accuracy of CLE for diagnosing GIN. To make this issue clear, we plan to investigate the respective diagnostic ability of CLE for LGIN and HGIN in future studies. Finally, the overall number of patients in the validation study was limited. We will further validate the initial findings of this feasibility study in future research with a larger population.

In conclusion, by using the newly developed endomicroscopic criteria, GIN can be differentiated from nonneoplastic lesions with an acceptable accuracy and substantial interobserver and intraobserver agreement. The use of INS is a promising tool for the grading of GIN during CLE imaging. We recommend that these preliminary findings be validated in a larger number of patients with a systematic red-flag technique and biopsy protocol at different centers. With continued improvement and refinement of the scoring system and the technique, we believe that CLE will be an efficient surveillance tool for GIN and lead to better management of these lesions.

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