Usefulness and Future Prospects of Confocal Laser Endomicroscopy for Gastric Premalignant and Malignant Lesions

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Confocal laser endomicroscopy (CLE) is a new technology enabling endoscopists to visualize tissue at the cellular level. CLE has the fundamental potential to provide a histologic diagnosis, and may theoretically replace or reduce the need for performing biopsy for histology. The clinical benefits of CLE are more obvious in esophageal disease, including Barrett's esophagus. Currently, this technology has been adapted to the diagnosis and surveillance of Barrett's esophagus and related neoplasia. Standard white light endoscopy is the primary tool for gastric cancer screening. Currently, the only method available to precisely diagnose these lesions is upper endoscopy with an appropriate biopsy. A recent study showed that CLE could characterize dysplasia or cancer and identify the risk factors for gastric cancer, such as intestinal metaplasia and the presence of *Helicobacter pylori in vivo*, although fewer studies on CLE were performed on the stomach than on Barrett's esophagus and other esophageal diseases. However, the application of CLE to routine clinical endoscopy continues to be refined. This review focused on the usefulness and future prospects of CLE for gastric premalignant and malignant lesions. **Clin Endosc 2015;48:511-515**

Key Words: Confocal laser endomicroscopy; Stomach neoplasms; Gastric intestinal metaplasia

INTRODUCTION

Endoscopy is an essential procedure for the diagnosis of a gastrointestinal (GI) disease. Biopsy and histology were the gold standards for final diagnosis. However, detection of premalignant and early malignant lesions in the inflamed stomach and intestine remains difficult. In recent years, a range of innovative techniques have entered the endoscopic arena due to their ability to enhance contrast and magnify diseased tissue regions at the cellular level, beyond the ability of standard white-light endoscopy (WLE) equipment. Confocal laser endomicroscopy (CLE) is a technology that

enables microscopic views (×1,000) of the mucosa in real time during endoscopy. The technology can be used either via a single endoscope-based system (eCLE; OptiScan, Notting Hill, Australia) or via probe-based CLE (pCLE, Cellvizio; Mauna Kea Technologies, Paris, France).[1] CLE is one of the newest advancements in diagnostic endoscopy, and is a highly promising technique for investigating the mucosal surface and the immediate subsurface areas. Cell structures and tissue morphological characteristics can be visualized to a maximum depth of 250 μm.[1] This technique aims to achieve few or no biopsies in regions of interest via multiple optical biopsies. The usefulness of CLE is evaluated to shift the focus from random to targeted biopsies.

Gastric cancer is known to develop either *de novo* or from precancerous lesions. *Helicobacter pylori* infection progresses from non-atrophic gastritis to chronic atrophic gastritis and from intestinal metaplasia to dysplasia and intestinal non-cardia adenocarcinoma.[2] To the best of our knowledge, Japan and Korea are the only countries with ongoing nationwide organized gastric cancer screening programs. Standard WLE is the primary tool for screening. Currently, the only method

available to precisely diagnose these lesions is upper endoscopy with an appropriate biopsy. In Asian countries including Korea and Japan, early gastric cancer (EGC) is detected by WLE; however, identification of EGC is difficult because some lesions are too small to recognize and characterize. Metaplastic lesions in the background make EGC difficult to find. In this review, we will discuss the role and future prospects of CLE that enable characterization of neoplastic and preneoplastic lesions better than conventional endoscopy.

TYPES OF CLE AND THEIR CHARACTERISTICS

CLE is currently performed with two systems, eCLE and pCLE. eCLE was launched earlier than pCLE, but is no longer marketed. Both systems use blue laser light (488 nm) for excitation, and require contrast. Fluorescein is mostly used as the intravenous (IV) contrast agent. Compared to eCLE, pCLE can pass through any type of conventional endoscope, including state-of-the-art high definition endoscopes.[3] eCLE offers a wider field of view, the ability to vary the depth of imaging from 0 to 250 µm, and slightly better lateral resolution. Advantages of pCLE are the versatility of probe system that can be passed via any endoscope, or even needles, and more rapid image acquisition (12 frames/sec vs. 0.8 to 1.6 frames/sec), which allows imaging of the blood flow *in vivo*.[4]

Both systems are basically similar, but differ in details such as image resolution, field of view, and depth of imaging. Because researchers and clinicians use only one of these systems, the opportunity to compare the two is rare. To our knowledge, close comparison of the two systems in clinical practice is not reported to date; therefore, usability of the same diagnostic classification for specific diseases by both systems is unclear. Moreover, CLE is classified according to two criteria: the Mainz classification and the Miami classification. The Mainz classification distinguishes neoplastic from hyperplastic polyps of the colon based on a dark, irregularly thickened epithelial layer characteristic of epithelial dysplasia.[5] The Miami classification was similarly established for pCLE to distinguish normal and pathological GI conditions.[3] During the review of articles and analysis of results, the types of system used should be considered. Because eCLE has been discontinued, pCLE is likely to dominate the market. Both systems have inherent limitations compared with WLE, including a very small field of view, high cost of the technology, long learning curve, and extra time needed to view the images during endoscopy.

CLE FOR DIAGNOSIS OF *H. PYLORI IN VIVO*

H. pylori is an accepted major etiological factor in gastric

diseases, such as chronic gastritis, peptic ulcer, and gastric carcinoma.[6,7] Kiesslich et al.[8] first reported in 2004 that *H. pylori* was detected *in vivo* by eCLE in a single patient. The study suggested that CLE permits immediate imaging of live bacteria in the stomach, and thus can be used for diagnosis of *H. pylori* infection during ongoing gastroscopy. eCLE was performed using topical acriflavine. Chinese researchers reproduced this using eCLE in 2010.[9] In this study, the authors concluded that eCLE findings, including white spots, neutrophils, and microabscesses, showed positive association with *H. pylori* diagnosis. To our knowledge, these studies were the first to show that CLE can detect bacteria; however, this indication for CLE is very limited because of the fact that eCLE is no longer available and because acriflavine accumulates in the nuclei, leading to a concern of potential mutagenic risk. Wang et al.[10] analyzed eCLE features of gastritis caused by *H. pylori* infection. They concluded that eCLE can accurately show the histological severity of gastritis associated with *H. pylori* infection. To our knowledge, this was the first study to show gastropathy associated with *H. pylori* infection using eCLE. After several initial studies, no further study was reported on this subject. Moreover, no studies compared other methods for detecting *H. pylori*. The possibility of detecting either localization or dispersion of *H. pylori* residue in the stomach and the role of CLE in detecting *H. pylori* will be clinically limited due to CLE's narrow range of examination.

eCLE FOR DIAGNOSIS OF CANCER AND PREMALIGNANT CONDITIONS

To the best of our knowledge, the first trial of scanning for gastric cancer was reported by Japanese researchers in 2006. A prototype of an eCLE system with acriflavine was used and the possibility of diagnosis of malignancy in more than half the cases was reported.[11] The next study on human gastric cancer, also performed in Japan, was conducted using eCLE and 500 mg fluorescein sodium IV as a contrast agent.[12] eCLE had an accuracy of 94% to 96% for the diagnosis of malignancy among 27 patients with EGC, when compared directly with histological biopsies. IV fluorescein had pros and cons compared to acriflavine. IV fluorescein allows visualization of ductal structures and deeper levels; however, acriflavine can stain the surface layer of mucosa intensely, allowing identification of the cell structure and nuclei, but the deeper layer is not revealed. In this study, the accuracy of diagnosis of gastric cancer by eCLE in two different pathologists was 94.2%, and 96.2%, respectively. The accuracy decreased substantially when poor images and inaccessible lesions were included. The characterization of cancer in upper GI mucosa using eCLE with fluorescein sodium was subsequently studied in China.[13]

In this study, eight gastric cancer patients, six with superficial esophageal carcinoma, and 10 normal individuals were evaluated; however, eCLE could provide clear images of gland architecture and microvascular structures, and made it possible to differentiate early carcinoma and normal mucosa.[13]

In 2008, Zhang et al.[14] tried to establish classification of the gastric pit patterns using eCLE and the relationship between the patterns and relevant histopathologic findings.[15] They reported seven types of gastric pit patterns. Type A (round pits) represented normal mucosa with fundic glands; type B (noncontinuous, short, rod-like) corporal mucosa with histologic gastritis; type C (continuous, short, rod-like) normal mucosa with pyloric glands; type D (elongated and tortuous, branch-like) antral mucosa with histologic gastritis; type E (the number of pits decreasing and pits prominently dilating) chronic atrophic gastritis; and type F (villus-like appearance, interstitium in the center and goblet cells appearing) intestinal metaplasia. Type G was subdivided into G1 (normal pits disappearing, with the appearance of diffusely atypical cells), which represented signet ring cell carcinoma and poorly differentiated adenocarcinoma, and G2 (normal pits disappearing with the appearance of atypical glands), which represented differentiated tubular adenoma. To the best of our knowledge, this study is the first to systematically classify pit patterns and correlate them with disease spectrums in the stomach. The sensitivity and specificity of the type G pattern for predicting gastric cancer were 90.0% and 99.4%. Although this study introduced the systematic classification of eCLE findings corresponding to histologic findings, interobserver and intraobserver variability was not evaluated.

In 2010, Li et al.[16] tried to determine the feasibility of eCLE using fluorescein sodium for identification and grading of gastric intraepithelial neoplasia (GIN). Thirty-three patients with histologically confirmed normal mucosa (n=4) and non-neoplastic (n=12) and neoplastic (n=17; including 12 low-grade IN and 5 high-grade intraepithelial neoplasia [HGIN]) lesions were evaluated to establish the diagnostic criteria for GIN (Table 1). These criteria were evaluated in 75 consecutive patients. The sensitivity, specificity, and positive and negative likelihood ratios of eCLE diagnosis for 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 in a comparison between the endoscopist and GI pathologist. However, not all parameters that represent the accuracy of eCLE for GIN were as positive as eCLE for gastric intestinal metaplasia (GIM). Detailed examination of the cell nuclei is mandatory to determine GIN histologically, but a fundamental limitation of CLE with fluorescein is the inability to define the nuclear structure. This can hinder precise CLE diagnosis of GIN, resulting in a reduced diagnostic accuracy compared to GIM.

In the largest study published on the use of eCLE with fluorescein sodium for the characterization of gastric superficial cancerous lesions, 182 patients were enrolled in phase I to establish morphologic criteria for gastric superficial cancerous lesions, and 1,786 patients were enrolled in phase II for prospective validation.[17] CLE criteria for cancer/HGIN lesions were irregularity in glandular size and shape, disorganized or destroyed pits and glands, irregular cells with disordered appearance, severe stratification, loss of cell polarity, and irregular shape and caliber of vessels (Table 1). The study was then used to establish a simplified two-tiered CLE classification of

Table 1. Endomicroscopic Classification of Gastric Lesions[16,17]

	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)
Non-neoplastic lesion (not IM, not IN)	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
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: 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	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.

Table 2. Simple Tow-Tiered Confocal Laser Endomicroscopy Classification for Gastric Superficial Lesions[17]

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

HGIN, high grade intraepithelial neoplasia.

non-cancerous lesions and EGC/HGIN lesions (Table 2). Using these criteria, eCLE had higher sensitivity (88.9%), specificity (99.3%), and accuracy (98.8%) for the diagnosis of gastric superficial cancer/HGIN lesions than WLE (sensitivity, 72.2%; specificity, 95.1%; and accuracy, 94.1%).[17]

GIM is a risk factor that leads to the development of intestinal-type gastric cancer. A group of 28 Chinese patients with known intestinal metaplasia underwent eCLE, and CLE criteria for diagnosis of GIM were developed. In addition, 53 consecutive patients with known or suspected GIM were prospectively evaluated. The sensitivities of conventional endoscopy and CLE for GIM were 36.88% versus 98.13%, and the specificities were 91.59% versus 95.33%, respectively. The κ value for the correlation with histological findings was 0.25 for conventional endoscopy versus 0.94 for CLE.[15] Furthermore, trials were conducted to identify and grade intestinal metaplasia and atrophic gastritis that were confirmed as precancerous lesions of gastric cancer by eCLE. CLE showed the distinct features of intestinal metaplasia, and when used with targeted biopsies, it is superior to WLE with standard biopsies for the detection and surveillance of GIM.[18]

Recently, Liu et al.[19] reported a new application of eCLE for grading of atrophic gastritis. In this study, eCLE was compared to chromoendoscopy and narrow band imaging (NBI) for diagnosing atrophic gastritis. eCLE had a higher sensitivity, specificity, and accuracy compared with NBI and CE, which were equivalent for the diagnosis of atrophic gastritis. The sensitivity and specificity of eCLE for diagnosing non-metaplastic atrophy were 86.76% and 91.89%, respectively, and for metaplastic atrophy were 91.94% and 96.86%, respectively. Both interobserver and intraobserver agreement were high.

pCLE FOR DIAGNOSIS OF CANCER AND PREMALIGNANT CONDITIONS

Compared to eCLE, pCLE has rarely been studied for gastric lesions. Pittayanon et al.[20] assessed the diagnostic yield of magnifying endoscopy with flexible spectral imaging color enhancement (ME-FICE) and pCLE with fluorescein sodium for GIM. The overall criteria from ME-FICE plus pCLE provided the highest sensitivity, specificity, positive predictive value, negative predictive value, and accuracy at 96%, 90%,

86%, 97%, and 92%, respectively. This study showed that a combination of broad-field (red-flag) techniques with pCLE would be a promising tool for screening gastric lesions. An additional study that compared the diagnostic accuracy of autofluorescence imaging (AFI), magnifying NBI, and pCLE with WLE for the diagnosis of GIM, using histology as the gold standard.[21] pCLE was more accurate than AFI and WLE for the diagnosis of GIM.

CLE APPLICATION FOR ENDOSCOPIC RESECTION AND ENDOSCOPIC SUBMUCOSAL DISSECTION

Korean researchers compared the accuracy of pCLE and WLE for EGCs scheduled for endoscopic submucosal dissection (ESD).[22] For complete ESD, correct pre-ESD histologic diagnosis is mandatory. Furthermore, multiple pre-ESD biopsies can seriously hamper subsequent ESD because inflammatory change thereafter often results in fibrosis of the submucosal layer. In this study, combining pCLE with conventional WLE significantly improved the accuracy of pre-ESD histologic diagnosis. This study showed that pCLE has a strong beneficial role in the cases of EGC that are candidates for subsequent ESD. The same group also studied eCLE for this purpose and demonstrated similar results.[23] A Study on the use of pCLE to examine the delineation of margins of gastric epithelial tumors with ESD is being conducted by Korean researchers.

CONCLUSIONS

Although conventional histology is still the gold standard for diagnosis, endoscopists can take advantage of CLE. Based on the published papers, CLE shows the clinical benefits of targeted biopsy by aiding selection of appropriate biopsy sites for suspicious lesions, which possibly increases the rate of positive biopsy results and reduces the number of biopsies. However, currently available studies on CLE for gastric lesions are very limited because of a variety of factors. Most studies deal with detection of GIM and atrophy by eCLE. The clinical value of characterization of IM and atrophy by CLE is not high because of its limited area of examination, despite the ability of CLE to replace the laborious Sydney System 5-biop-

sy protocol. Further studies including coordination with red-flag technologies and molecular imaging are needed to extend CLE applications into clinical areas.

Conflicts of Interest

The author has no financial conflicts of interest.

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