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# Confocal Laser Endomicroscopy and Molecular Imaging in Barrett Esophagus and Stomach

Kwi-Sook Choi and Hwoon-Yong Jung

Department of Gastroenterology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Detection of premalignant lesions in the upper gastrointestinal tract may facilitate endoscopic treatment and improve survival. Despite technological advances in white light endoscopy, its ability to detect premalignant lesions remains limited. Early detection could be improved by using advanced endoscopic imaging techniques, such as magnification endoscopy, narrow band imaging, i-scanning, flexible spectral imaging color enhancement, autofluorescence imaging, and confocal laser endomicroscopy (CLE), as these techniques may increase the rate of detection of mucosal abnormalities and allow optical diagnosis. The present review focuses on advanced endoscopic imaging techniques based on the use of CLE for diagnosing premalignant lesions in Barrett esophagus and stomach.

**Key Words:** Barrett esophagus; Stomach neoplasms; Endoscopy; Confocal laser endomicroscopy; Molecular imaging

## INTRODUCTION

Upper gastrointestinal (GI) cancer is one of the most common malignancies worldwide. To improve the survival of patients with upper GI tract malignancies, early detection of premalignant lesions and cancers is important. Gastric cancer, which is usually detected at a late stage, is the second most common cause of cancer-related death worldwide. Atrophic gastritis, intestinal metaplasia, and epithelial dysplasia of the stomach are common in Asia and the risk of gastric cancer is high. Although the proportion of gastric cancers detected early has increased considerably with the national health screening program in Korea and Japan, conventional white light endoscopy (WLE) cannot accurately differentiate and diagnose preneoplastic gastric conditions. Barrett esophagus (BE) is a precursor to adenocarcinoma of the esophagus. Therefore, current guidelines recommend that individuals with BE undergo periodic endoscopic surveillance with multiple biopsies. However, this surveillance strategy is limited by random sampling error, inconsistent histopathological interpretation, and delay in diagnosis.

Endoscopic imaging has undergone several technical revolutions over the past two decades. New imaging techniques can be subdivided into wide-field imaging systems, which enable examination of the entire luminal surface area, and high-resolution imaging with smaller fields of view, which provide an optical biopsy of the tissue. Wide-field imaging techniques include chromoendoscopy, virtual chromoendoscopy (narrow band imaging [NBI], flexible spectral imaging color enhancement, and i-scan), autofluorescence imaging (AFI), and magnification endoscopy. High-resolution imaging techniques include confocal laser endomicroscopy (CLE) and high-resolution microendoscopy. These modalities can accurately estimate the extent of lesions, which is essential for endoscopic treatment, and they are therefore associated with an increased potential for curative treatment and improved patient outcomes.

CLE enables the endoscopist to obtain real-time *in vivo* histologic images or optical biopsies during endoscopy. Here, we focus on the use of advanced endoscopic imaging using CLE for the diagnosis of premalignant lesions in BE and the stomach.

## TECHNOLOGY

The term confocal refers to the alignment of both the illumination and collection systems in the same focal plane.[1] The laser light is reflected from the tissue and refocused onto the detection system by the same lens; therefore, only the return-

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**Correspondence:** Hwoon-Yong Jung
Department of Gastroenterology, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 138-736, Korea
**Tel:** +82-2-3010-3197, **Fax:** +82-2-476-0824, **E-mail:** hyjung@amc.seoul.kr

ing light that is refocused through the pinhole is detected, providing high-resolution images.[2] Natural tissue fluorescence is limited at the laser wavelength used for CLE; therefore, exogenous fluorescent agents are applied either topically or systemically. Intravenous fluorescein is the most widely used fluorescent agent to date; this nontoxic agent is approved by the Food and Drug Administration for retinal angiography. It highlights the lamina propria, intercellular spaces, and fills the capillaries, but does not stain the nuclei. Topical fluorescence agents are currently the only option for cell nuclei imaging, which is essential for diagnosis and grading of intraepithelial neoplasia.[3] Acriflavine hydrochloride 0.05% is a topical contrast agent that is applied with a spray catheter. It stains the cell nuclei and enables imaging of the surface but not the deeper mucosa.[4] Acriflavine accumulates in the nuclei and carries a potential mutagenic risk. Cresyl violet is another topical contrast agent that provides cytoplasmic enrichment and thereby enables negative visualization of nuclear morphology.[5]

Currently, two CLE-based systems are available: endoscope-integrated CLE (eCLE; Pentax, Tokyo, Japan) and probe-based CLE (pCLE; Cellovizio; Mauna Kea Technologies, Paris, France). Both systems provide 1,000-fold magnification and use a laser with a wavelength of 488 nm (blue light). In eCLE, a confocal probe is integrated in the tip of the endoscope, which reduces the flexibility of the tip; therefore, certain regions of the fundus and cardia are difficult to investigate owing to limitations in retroflexion. The field of view is 475×475 μm, with a lateral resolution of 0.7 μm and axial resolution of 1 μm. Images are acquired at a manually adjustable scan rate of 1.6 frames per second at a resolution of 1,024×512 pixels, or at 0.8 frames per second at a resolution of 1,024×1,024 pixels, with an adjustable scanning depth ranging from 0 to 250 μm with control to around 4-μm increments.[2] In contrast, pCLE involves mini probes that can be advanced through the accessory channel of standard endoscopes; it uses fixed laser power at a frame rate of 12 frames per second and a depth of imaging of 70 to 130 μm for the GI tract and 55 to 65 μm for the ultrahigh-definition (HD) probe.[2] The lateral resolution of pCLE is 1 μm, which is a 43% decrease in resolution compared to eCLE.[6]

The CLE procedure is as follows: a standard WLE examination is performed first, using either the eCLE endoscope or that used with the pCLE probe. After locating the areas of interest, the contrast agent is applied. The tip of the eCLE scope or pCLE probe is placed gently on the mucosa of interest, and images are acquired. A stable position is important for image acquisition, which can be achieved during eCLE by using suction and during pCLE by use of a translucent cap.[7] The working channel of eCLE is located 5 mm to the right of the confocal lens. Therefore, when suction is applied to the mucosa, the resulting intramucosal hemorrhage is located 5 mm to the right of the area evaluated by using eCLE.[2] In pCLE, mild pressure is applied to the tissue with the confocal probe and the resulting reddish mucosa can guide subsequent acquisition of biopsy samples for histopathologic diagnosis.[3] Handling the endoscope to achieve a stable position without motion artifacts and image interpretation requires training.

## CLINICAL APPLICATIONS IN BARRETT ESOPHAGUS

Several studies have investigated the role of CLE in the evaluation of suspicious lesions in BE. The first study on CLE in BE included 63 patients analyzed with eCLE and was published in 2006 by Kiesslich and colleagues.[8] These authors developed the confocal Barrett classification, which uses the cellular and vascular architecture to distinguish between gastric-type epithelium, Barrett epithelium, and neoplasia. Gastric epithelium is characterized by a regular, columnar-lined epithelium with round, glandular openings, typical cobble-stone appearance, and regular-shaped capillaries visible in the deeper mucosa. BE shows columnar-lined epithelium with dark mucin in goblet cells, a villiform pattern, and regular-shaped capillaries in the upper and deeper mucosa. Neoplastic BE is characterized by black cells with irregular borders and shapes, high dark contrast to the surrounding tissue, and irregular leaking capillaries in the upper and deeper mucosa. In an investigator-masked evaluation of eCLE, this classification system predicted histologic findings of BE and neoplastic BE with a sensitivity of 98.1% and 92.9% and a specificity of 94.1% and 98.4%, respectively (accuracy, 96.8% and 97.4%). Interobserver and intraobserver agreement were high, with a mean $\kappa$ value of 0.843 and 0.892, respectively.

The first study of pCLE in BE was published in 2008 by Pohl and colleagues.[9] In this two-phase, prospective, two-center trial, they established criteria for the diagnosis of BE neoplasia based on 95 biopsies obtained from 15 patients and tested these criteria on 201 biopsies from the remaining patients without visible focal changes. They identified five neoplastic criteria suggestive of BE neoplasia, which included irregular epithelial lining, variable width of the epithelial lining, glandular fusion, presence of dark areas (decreased fluorescein uptake), and an irregular vascular pattern. The pCLE diagnosis of neoplasia was based on the presence of at least two of these neoplastic criteria. The sensitivity and specificity for two independent investigators were 75% and 88.8%, and 75% and 91%, respectively, translating at best into a positive predictive value of 44.4% and a negative predictive value of 98.8% with good interobserver agreement ($\kappa$=0.6).

A prospective, double-blind, randomized, crossover study of eCLE in BE involving 39 patients showed that eCLE with

targeted biopsy almost doubled the diagnostic yield for neoplasia (34% vs. 17%) and was equivalent to the standard endoscopy with a four-quadrant biopsy protocol for the final diagnosis of neoplasia.[10] In this study, two-thirds of the patients in the surveillance group did not require mucosal biopsy and two cases of high-grade dysplasia were identified through eCLE with target biopsy alone. However, two cases of high-grade dysplasia were detected only by performing standard endoscopy with random biopsy. This study showed the limitations of CLE, which are associated with its small field of view and make this technique prone to sampling error.

In a multicenter study of pCLE that included 670 pairs of biopsies from 68 BE patients, the specificity and negative predictive value of pCLE for excluding neoplasia were 0.97 and 0.93 for the blinded evaluation, and 0.95 and 0.92 for the on site assessment, respectively.[11] However, the positive predictive values and sensitivity were poor for both settings (blinded, 46%/28% and on site, 18%/12%, respectively), and the specificity decreased significantly from 95% to 59% on a per patient basis when investigators were blinded to the endoscopic findings. These results suggest that correct image interpretation depends on the simultaneous elucidation of endoscopic and confocal images.

In another prospective, double-blind, multicenter pCLE study, a training set of 20 images with known histology was first reviewed to standardize image interpretation, followed by the blinded review of 20 unknown images.[12] The sensitivity for the diagnosis of neoplasia for the 11 endoscopists (only four had prior pCLE experience) was 88%, and the specificity was 96% with substantial agreement on the pCLE diagnosis ($\kappa$=0.72). These results suggest that pCLE has high accuracy and reliability for the diagnosis of neoplasia in BE and a short learning curve for the interpretation of images.

The pCLE criteria for the diagnosis of BE neoplasia were further refined in the Miami Classification and the KC Confocal Criteria. The Miami Classification system is based on a consensus that pCLE users reached during a meeting held in Miami in 2009.[13] Following this classification, high-grade dysplasia in BE is characterized by villiform structures, dark irregularly thickened epithelial borders, and dilated irregular vessels, whereas adenocarcinoma in BE is characterized by disorganized or complete loss of villiform structures and crypts, dark columnar cells, and dilated irregular vessels.

The KC Confocal Criteria are diagnostic pCLE criteria for dysplasia in BE generated by the Kansas City group.[14] These criteria are based on 50 pCLE videos and included saw-toothed epithelial surfaces, noneasily identifiable goblet cells, nonequidistant glands, unequal size and shape of glands, enlarged cells, and irregular and nonequidistant cells. The presence of two or more criteria provided the best accuracy for differentiation between dysplasia and nondysplasia. The use of these criteria yielded an overall accuracy of 81.5% for diagnosing dysplasia, and overall agreement of the criteria was substantial ($\kappa$=0.61), with no differences between experts and nonexperts. After a structured teaching session, no differences in accuracy or agreement were detected between experienced and nonexperienced observers, suggesting a short learning curve. Using these novel pCLE criteria, the accuracy and interobserver agreement among GI pathologists were also evaluated.[15] The sensitivity and specificity for detecting dysplasia were 85% and 70%, respectively, with substantial interobserver agreement ($\kappa$=0.65) among three GI pathologists.

A large, prospective, international, multicenter trial involving 101 BE patients compared the sensitivity and specificity of pCLE in addition to HD-WLE with HD-WLE alone for the detection of high-grade dysplasia and early carcinoma.[16] The sensitivity and specificity for HD-WLE were 34.2% and 92.7%, respectively, compared with 68.3% and 87.8%, respectively, for HD-WLE or pCLE, which was statistically significant. The sensitivity and specificity for HD-WLE or NBI were 45.0% and 88.2%, respectively, compared with 75.8% and 84.2%, respectively, for HD-WLE, NBI, or pCLE, which was also statistically significant.

A cross-sectional study assessed the benefit of the addition of eCLE to HD-WLE and NBI for detecting BE neoplasia.[17] For the detection of high-grade dysplasia and intramucosal cancer, the respective sensitivity, specificity, and accuracy were as follows: HD-WLE, 79.1%, 83.1%, and 82.8%; NBI, 89.0%, 80.1%, and 81.4%; and eCLE, 75.7%, 80.0%, and 79.9%. The diagnostic yield of the targeted biopsy protocol was superior to that of the Seattle protocol across all modalities. However, the addition of eCLE had no impact on patient outcomes and required considerable time and cost. Therefore, the authors concluded that a targeted biopsy protocol guided by HD-WLE and NBI was the most efficacious approach. The interim results of another large, international, multicenter trial of eCLE in BE showed that high-resolution endoscopy with eCLE followed by targeted biopsy improved the detection of BE neoplasia with significantly fewer biopsies compared to high-resolution endoscopy with random biopsy.[18] The final results of this study may provide additional information about the added benefit of eCLE.

A multicenter, randomized, controlled trial investigated whether the use of pCLE in addition to HD-WLE could aid in the determination of residual BE after mucosal ablation or resection of BE neoplasia.[19] However, this study was closed after the interim analysis because of low conditional power resulting from the lack of difference between groups and higher than expected residual BE in both arms.

## CLINICAL APPLICATIONS IN THE STOMACH

CLE allows direct *in vivo* identification of *Helicobacter pylori* infection[20] and good visualization of gastric pit patterns, making it a useful tool for *in vivo* diagnosis of premalignant lesions and gastric cancer.[21]

The gastric pits, which are openings of the gastric glands, are the basic units of the microstructure of the surface of the gastric mucosa. Seven different pit patterns detected by performing eCLE have been described that help distinguish between normal mucosa, gastritis, intestinal metaplasia, atrophy, and gastric cancer.[21] Normal mucosa with fundic glands is characterized by round pits with a round opening, and corporal mucosa with histologic gastritis is defined by noncontinuous, short, rod-like pits with a short, thread-like opening. Normal mucosa with pyloric glands is characterized by continuous, short, rod-like pits with a slit-like opening, and antral mucosa with histologic gastritis shows elongated and tortuous, branch-like pits. Epithelial cells in intestinal metaplasia are more slender and brighter than normal gastric epithelial cells and show a villous-like appearance, central interstitium, and black goblet cells. Atrophic gastritis is characterized by a decreased number of pits and a prominently dilated pit lumen. In gastric cancer, normal pit patterns disappear with the appearance of atypical cells. In a blinded, prospective study of gastric pit patterns that included 132 patients, Zhang and colleagues[21] reported that the diagnostic accuracy of eCLE for the detection of atrophy and gastric cancer was 97.5% and 97.1%, respectively.

In an early case report describing the *in vivo* detection of *H. pylori* by eCLE, *H. pylori* appeared as tiny rods in close association with the gastric epithelium owing to its active uptake of acriflavine.[20] In a prospective study involving 103 patients, eCLE for *H. pylori* infection showed any of the three following features: white spots resembling *H. pylori* organisms, neutrophils, and microabscesses.[22] The accuracy, sensitivity, and specificity of eCLE for the diagnosis of *H. pylori* infection were 92.8%, 89.2%, and 95.7%, respectively, with good interobserver agreement ($\kappa$=0.78). Another study of eCLE for *H. pylori* gastritis that included 118 patients showed that eCLE could accurately determine the histologic severity of *H. pylori* infection-associated gastritis.[23]

A prospective study of eCLE involving 267 sites from 53 patients showed good results for the diagnosis and classification of intestinal metaplasia.[24] The sensitivity of conventional endoscopy and eCLE for detecting gastric intestinal metaplasia was 36.88% and 98.13%, and the specificity was 91.59% and 95.33%, respectively. The $\kappa$-value for the correlation with histological findings was 0.25 for conventional endoscopy and 0.94 for eCLE. In a study comparing experienced and inexperienced confocal endoscopists, interpretation of *in vivo* eCLE images by the experienced group was associated with higher sensitivity (95.2% vs. 61.9%) and higher specificity (93.3% vs. 62.2%) for the diagnosis of gastric intestinal metaplasia than that by the inexperienced group.[25] The agreement between the interpretation by the experienced group and histology for gastric intestinal metaplasia was also higher than that for the inexperienced group ($\kappa$=0.864 vs. $\kappa$=0.217). However, the sensitivity and specificity of the interpretation of *ex vivo* CLE images for the diagnosis of gastric adenocarcinoma were similar between groups. A recent study compared the diagnostic performance of AFI, magnifying NBI, pCLE, and WLE for the diagnosis of gastric intestinal metaplasia by using histology as the gold standard.[26] For diagnosing gastric intestinal metaplasia in 125 sites of 20 patients, real-time pCLE had better sensitivity (90.9% vs. 37.9%) and accuracy (88.0% vs. 64.8%) than WLE. The sensitivity (90.9% vs. 68.2%), specificity (84.7% vs. 69.5%), and accuracy (88% vs. 68.8%) of real-time pCLE were better than those of AFI. The sensitivity, specificity, and accuracy of real-time pCLE and magnifying NBI for diagnosing intestinal metaplasia were similar.

The accuracy of CLE for the diagnosis of gastric cancer and premalignant lesions has been shown in several studies. As assessed by two pathologists in 27 patients, eCLE has a diagnostic accuracy of approximately 95% for gastric cancer if the image quality is good (16 of 27 cases were excluded because of inaccessibility or poor images).[27] In a study that used eCLE to differentiate gastric hyperplastic polyps from adenomas, the overall accuracy of eCLE for *in vivo* diagnosis was 90% and its overall accuracy for differentiating hyperplastic polyps from adenomas was 97%, with good interobserver agreement ($\kappa$=0.83).[28] In hyperplastic polyps, columnar epithelium cells were arranged in a regular pattern encircling the openings of dilated, elongated, or branch-like pits. Adenomas were characterized by irregularly shaped black cells encircled with white interstices after intravenous fluorescein and high gray-scale cells with irregular size and enlarged nuclei after topical acriflavine administration. Architectural changes in adenomas include irregular ridges or villi with a cerebriform shape, focal asymmetric ridge distortion, and distorted or ridge-like glandular openings.

In the largest published study on the use of CLE for the detection 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.[29] Two-tiered CLE imaging criteria for gastric superficial lesions were developed in phase I. CLE criteria for cancer/high-grade intraepithelial neoplastic 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. Using these criteria, eCLE had higher sensitivity (88.9%), specificity (99.3%), and accuracy (98.8%) for the diagnosis of gastric superficial cancer/high-grade intraepithelial neoplastic lesions than WLE (sensitivity, 72.2%; specificity, 95.1%; and accuracy, 94.1%).

A prospective, double-blind, feasibility study on the use of eCLE with fluorescein as the fluorescent agent showed low diagnostic accuracy (79.8%) for gastric intraepithelial neoplasia and low sensitivity (66.7%) in distinguishing between low- and high-grade intraepithelial neoplasia.[30] This result can be explained based on the inability of CLE using fluorescein to accurately assess the nuclear-cytoplasmic ratio, nuclear pleomorphism, and hyperchromatism, which are important pathologic features for grading the degree of neoplasia. In the present study, chromoendoscopy was only applied in selected cases (10 lesions in the validation study) with a small number of enrolled patients (75 patients in the validation study). Therefore, these preliminary findings should be validated in a larger cohort with a systematic red-flag technique and biopsy protocol at different centers.

A study assessing the value of eCLE for phenotypic diagnosis of gastric cancer showed that eCLE could discriminate between undifferentiated and differentiated lesions, and aids in detecting differentiated gastric cancers with an intestinal phenotype.[31] Gastric cancers with an intestinal phenotype have a better prognosis and show a brush border (a narrow black line around crypt cells), goblet cells (round black spots in crypt cells), or both on eCLE. The sensitivity, specificity, and accuracy for identifying an intestinal phenotype by eCLE were approximately 88%, 80%, and 85%, respectively.

Endoscopic mucosal resection (EMR) including endoscopic submucosal dissection (ESD) has become a standard treatment for gastric premalignant lesions and selected early gastric cancers, especially in Asia. In most cases, multiple biopsies before resection are important to establish the diagnosis. However, discrepancies between the histology of biopsy specimens and that of resected lesions can result in underestimation of the grade of neoplasia. In addition, multiple biopsies may limit subsequent dissection because of fibrosis from scarring. CLE enables the endoscopist to obtain an *in vivo* optical biopsy, which improves pre-EMR diagnosis. It offers the potential to decrease the number of biopsy specimens, and can also direct biopsies to target highly suspicious areas. CLE may even help differentiate lesions that are not amenable to EMR and determine the margins of a lesion before endoscopic resection. Two studies compared the accuracy of conventional endoscopic biopsy and CLE before EMR.[32,33] In one prospective, comparative study of eCLE involving 35 lesions from 31 patients, eCLE had significantly higher diagnostic accuracy than conventional endoscopic biopsies for differentiating between gastric adenomas and adenocarcinomas (94.2% vs. 85.7%).[32] eCLE showed better accuracy for discriminating differentiated from undifferentiated adenocarcinoma than conventional endoscopic biopsy (95.4% vs. 84.2%; $p$=0.146), although this difference did not reach statistical significance, most likely because of the small number of undifferentiated carcinomas in this study ($n$=5). Approximately 10% of patients (3/31) would have undergone surgery instead of ESD if the CLE classification showing undifferentiated cancer had been used instead of biopsy. Another prospective study of pCLE involving 54 lesions showed that the overall accuracy of pCLE for the diagnosis of adenocarcinoma was higher than that of conventional endoscopic biopsies (90.7% vs. 85.2%), although the difference was not statistically significant ($p$=0.065).[33] The combined accuracy of conventional endoscopic biopsy and pCLE was significantly higher than that of biopsies alone (98.1% vs. 85.2%), and the combined accuracy for cancer differentiation was 93.5%.

Extended or repeat endoscopic treatment is possible in many patients with incomplete resection on the lateral margin. However, the presence of residual neoplastic mucosa or an incomplete resection interface is difficult to detect with conventional endoscopy. In a study assessing the use of eCLE for *in vivo* prediction of EMR completeness, eCLE was performed 2 weeks after EMR in 24 patients.[34] The accuracy of eCLE for predicting incomplete resection of original lesions was 91.7%, with sensitivity and specificity of 100.0% and 89.5%, respectively. The residual lesions were treated with additional EMR guided by eCLE.

## MOLECULAR IMAGING

Molecular imaging involves fluorescent labeling of individual cells for the detection of molecular signatures *in vivo*.[2] Its objective is the identification and characterization of lesions based on their molecular fingerprint rather than morphology and to ultimately increase the efficiency of endoscopic screening and surveillance.[35] Molecular imaging enables visualization of disease-specific morphologic or functional tissue alterations, and can therefore provide information for individualized, molecular-targeted therapy. It comprises wide-field techniques for facilitating detection of lesions and microscopic high-resolution techniques such as CLE for *in vivo* characterization. The high-resolution techniques enable *in vivo* histology and intravital immunostaining, which makes CLE an ideal imaging device for on-site characterization of a lesion and visualization of the intravital interaction of targeted drugs within a tumor.[36] Exogenous fluorescent agents usually target a disease-specific biomarker and serve as molecular beacons. Such probes include labeled peptides and antibodies, nanoparticles, and probes with tumor-specific activation properties. The ben-

efit of targeted agents is the potential to achieve a high signal to background ratio through selective binding to a molecular target.

Several studies have used fluorescence-labeled antibodies against epitopes that are frequently overexpressed in GI cancers, such as vascular epithelial growth factor or epidermal growth factor receptor (EGFR). An anti-EGFR antibody has been developed that binds to colonic neoplasia; however, no targeting antibodies have been developed for BE neoplasia.[37] Peptides are promising for use as novel molecular probes that identify disease-specific cell surface targets and can be fluorescence-labeled for detection. A small affinity peptide specific for dysplasia in BE (peptide sequence, SNFYMPL) has been developed and tested in EMR specimens.[38] The fluorescence intensity was measured for each specimen and was highest in neoplastic BE tissue and lowest in squamous and gastric mucosa. These results show the potential future use of fluorescence-labeled peptides to target dysplasia on screening endoscopy and guide tissue biopsy in patients at increased risk for developing adenocarcinoma, which could increase the yield of detection of premalignant mucosa. Future efforts will focus on the *in vivo* validation of peptide binding to dysplastic esophageal mucosa. A molecular imaging study showed that alterations in cell surface glycans are associated with the progression from BE to adenocarcinoma and lead to specific changes in lectin binding patterns.[39] Fluorescent lectins applied topically bound preferentially to dysplastic tissue in BE in biopsy specimens and in esophagus specimens removed during esophagectomy. Lectins are relatively inexpensive and nontoxic imaging probes that can be used in conjunction with conventional fluorescence endoscopes to screen for the presence of dysplasia in the context of BE to help guide patient management. A recent study reported the *in vivo* molecular imaging of BE with CLE using a fluorescence-labeled peptide specific for esophageal neoplasia (ASY*-fluorescein isothiocyanate [FITC]).[40] A fluorescence-labeled peptide can be topically and safely administered during endoscopy to comprehensively cover the mucosal surface of the distal esophagus. Peptide binding occurs in less than five minutes, which facilitates their practical use in a busy clinical unit. Specific binding of the peptide to Barrett neoplasia has been shown in real time using pCLE *in vivo*.

In a proof of principle study in pigs, FITC-labeled antibodies targeting EGFR and survivin were topically applied to healthy porcine esophageal and gastric mucosa or administered via submucosal injection, and pCLE was performed to study expression patterns *in vivo*.[41] In the esophagus, both EGFR and survivin localized predominantly to the keratinocyte progenitor cells. In the stomach, EGFR localized to progenitor zone cells and some epithelial cells. Localization of survivin was similar, but involved more surface epithelial cells. This study demonstrated the feasibility of using CLE and topical administration of FITC-labeled antibodies for *in vivo* localization of EGFR and survivin in the esophageal and gastric mucosa. An *in vivo* molecular imaging study of gastric cancer used a human-murine xenograft model for EGFR-specific staining on human gastric cancer cells.[42] The results showed that *in vivo* microscopic and macroscopic molecular imaging of gastric cancer is feasible in a human-murine xenograft model with both diagnostic and therapeutic antibodies (cetuximab) targeting EGFR1. In another study, the monoclonal antibody MG7, which is a specific molecular marker of gastric cancer, was labeled with fluorescent agents to enable *in vivo* real-time imaging by CLE.[43] MG7 was capable of labeling human xenografts in a mouse model and stained 96% (22/23) of human gastric cancer specimens *ex vivo*, whereas the healthy mucosa was stained in only 22% (5/23) of specimens.

## CONCLUSIONS

Recent advances in endoscopic technologies have enabled the visualization of finer mucosal patterns and microvascular structures and provided a tool for optical diagnosis. CLE allows real-time histologic diagnosis of the mucosal layer of the GI tract at cellular and subcellular resolution with a high diagnostic yield for neoplasia. This technique has a strong impact on diagnostic and therapeutic approaches to premalignant lesions and cancer of the upper GI tract. However, CLE has inherent limitations compared to wide-field imaging techniques, including a small field of view, the cost of the technology, learning curve, and extratime needed to view the images during endoscopy. Moreover, the clinical use of CLE is limited by a lack of widespread availability. Additionally, a consensus is needed on the diagnostic criteria of CLE, particularly for gastric premalignant and malignant lesions. Large, prospective, multicenter studies are required to properly assess the diagnostic potential and advantages of CLE in gastric lesions. Further technological improvements in the near future will enable CLE to be incorporated with specific wide-field imaging techniques to allow more rapid detection at an early stage and precise risk stratification, which may facilitate treatment decisions.

Molecular imaging may be useful for the detection of suspicious lesions and could predict response to treatment. Although molecular imaging has not yet entered clinical practice, it holds the promise of rapid, early diagnosis of GI cancer and prediction of response to targeted therapy.

### Conflicts of Interest

The authors have no financial conflicts of interest.

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