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Confocal Laser Endomicroscopy in the Field of Esophageal Diseases

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Abstract

Confocal laser endomicroscopy (CLE) is a new endoscopic imaging technology that allows real-time, high-resolution observation of tomographic images of mucosal cells and subcellular levels *in vivo*, detecting microscopic structural changes in mucosal morphology, and its *in vivo* immediate pathological diagnostic capability can avoid delays in mucosal pathological diagnosis and reduce the pain caused by repeated biopsies. CLE is known as "optical biopsy" and compared with other endoscopic techniques, it has obvious advantages. CLE systems include probe-based confocal laser endomicroscopy (pCLE) and endoscope-based confocal laser endomicroscopy (eCLE). Since 2006, CLE has been widely used for the evaluation of various lesions in the digestive system, including esophageal, gastric, and colonic neoplasia, pancreatic cysts and solid lesions, and inflammatory bowel disease. The advent of CLE has made *in vivo* microscopic imaging possible, which has changed the endoscopic screening and diagnosis of multiple gastrointestinal (GI) lesions. However, the value of its use in GI diseases is still controversial. In this review, we focus on the application of CLE in the field of esophageal diseases.

Keywords

Confocal Laser Endomicroscopy (CLE), Barrett's Esophagus (BE), High-Grade Dysplasia (HGD), Esophagus Cancer (EC), Gastroesophageal Reflux Disease (GERD)

1. Introduction

This review is about the application of confocal laser endomicroscopy (CLE) in the field of esophageal diseases, which involves the diagnosis, treatment, monitoring, and follow-up of Barrett's esophagus (BE), Esophagus Cancer (EC), and

gastroesophageal reflux disease (GERD) in a more objective and specific way.

The birth of CLE technology and its application in the clinic is a milestone that makes it possible to image the lumen of the gastrointestinal tract *in vivo* microscopically. The most significant advantage of CLE is that endoscopy can obtain histological images of the mucosal surface and subsurface structures *in vivo* without biopsy and histopathological examination, analyze the state of nuclei, crypt, and capillaries, and make an immediate high-resolution histological diagnosis of the mucosa, which is known as "optical biopsy". CLE has been widely used for the evaluation of various lesions in the gastrointestinal tract, including esophageal, gastric, and colonic neoplasia, pancreatic cysts and solid lesions, and inflammatory bowel disease. Because of the insidious clinical symptoms and complex diagnostic procedures in esophageal disease, we focused our review on this area.

In this review, we found that most studies demonstrate significant advantages of CLE in diagnosing esophageal disease, improving the sensitivity, specificity, and accuracy of the diagnosis, with possible gainful benefits in combination with other endoscopes. CLE can guide the endoscopic treatment of BE and endoscopic targeted biopsies with similar accuracy to conventional biopsies but can significantly reduce the number of biopsy samples required. However, high cost, difficulty in image interpretation, and narrow field of view are still significant limitations of CLE, so more research and newer techniques are needed to serve the current challenges.

2. How CLE Works

Confocal laser endomicroscopy (CLE) is a combination of confocal laser microscope and traditional electron endoscope. Confocal laser microscope technology is now more mature, when scanning its illumination point and detection imaging point are always located in the same plane so that the imaging image is not affected by scattered light from other sections of the observed object and can get higher resolution imaging. CLE uses a combination of imaging fiber bundle and objective lens to place the light source into the body, and its single fiber acts as both an illumination point source and a detection pinhole. The laser beam from the laser is first directed through the beam splitter to the tissue under observation at the confocal point with precision. The fluorescent material in the tissue is irradiated and emits fluorescence, which can be projected in all directions. Only the fluorescence that is focused on the detection pinhole through the objective lens and beam splitter can form a point image, which is then received by the detector through the pinhole for imaging. Only the fluorescence emitted by the tissue in the focal plane of the objective can pass through the detection pinhole and be detected by the detector. In contrast, the light signal outside the focal plane (above and below) is shielded, so it cannot be imaged.

The pinhole has a crucial role in imaging, and the size of the pinhole diameter directly affects the contrast and resolution of the image. When the laser beam

scans the observed tissue point by point, the photomultiplier tube behind the pinhole also obtains the confocal image of the corresponding light point by point, converts it into a digital signal for transmission to the computer, and finally assembles the confocal image of the whole focal plane on the screen, so that the focal plane is located on different levels of the observed tissue in turn, so that the image of the corresponding optical cross-section of the tissue can be obtained layer by layer.

From the principle of action of CLE, we can find that the generation of images requires the fluorescent contrast agent. At present, fluorescent contrast agents available in human tissues include sodium fluorescein, acriflavine hydrochloride, tetracycline and cresol violet. Contrast agents can be applied systemic (sodium fluorescein or tetracycline) or mucosal topical (acridine hydrochloride or cresol violet). Sodium fluorescein has been used safely for decades in ophthalmic examinations. It is now the most commonly used fluorescent contrast agent in CLE examinations because it is inexpensive, safe, and non-mutagenic. Fluorescein sodium can be visualized within 15 seconds after intravenous injection to visualize individual cells under CLE for up to 30 minutes: fluorescein sodium first binds to serum proteins, while unbound staining molecules stain the interstitial vessels and gradually penetrate into the whole mucosa and bind to the extracellular matrix and basement membrane, thus showing the crypt structure of the mucosa, epithelial cells, the connective tissue of the lamina propria, blood vessels, and red blood cells. And the connective tissue of the lamina propria of the mucosa and the microvasculature can produce a clear contrast. Because sodium fluorescein could not penetrate the lipid-like membrane of the cells and bind to the acidic material of the nucleus, the structure of the nucleus could not be shown, and the appearance was "dark". All patients showed temporary yellowing of skin and urine after fluorescein sodium injection, which returned to normal after 24 hours of drinking more water. In addition, fluorescent contrast agents commonly used in research are acridine yellow hydrochloride, which can bind to nucleic acids in the cytoplasm and nucleus and can be absorbed within seconds after local application. Still, it is limited to imaging the surface layer of the gastrointestinal (GI) mucosa, which can identify intraepithelial neoplasia and carcinoma of the GI tract. Still, it is rarely used in humans because of the risk of inducing cell mutation and causing cancer. The imaging effect of other contrast agents such as mepurophenol, tetracycline and porphyrin is not very ideal, so it is still necessary to study new contrast agents, especially for specific molecules.

3. The Application in the Field of Esophageal Diseases

3.1. Barrett's Esophagus and Associated Neoplastic Lesions

Barrett's esophagus (BE) is a pathology in which the normal compound squamous epithelium of the lower esophagus above 1 cm from the junction of the esophagus and gastric mucosa (dentate line) is replaced by a single layer of colum-

nar epithelium with chemosis [1], which can develop in the following order: chemosis, low-grade dysplasia (LGD), high-grade dysplasia (HGD), and esophageal adenocarcinoma (EAC) [2]. The incidence of EAC is rapidly increasing in western countries, with an overall 5-year survival rate of approximately 20%. Still, the early intervention of dysplasia in BE, including endoscopic ablation and endoscopic mucosal resection (EMR), can increase the survival rate to 90% [3] [4]. BE is the only known precancerous lesion of EAC [5], and 80% of EAC associated with BE are closely related [6]. Considering the increased risk of EAC in patients with BE, its endoscopic screening and surveillance every 3 - 5 years to detect early and reduce mortality from EAC. However, many dysplastic lesions have a flat structure and patchy distribution in BE, which are difficult to detect even with high-resolution white light endoscopy (HR-WLE) [7] [8]. HR-WLE combined with Seattle four-quadrant biopsy is currently recommended as the gold standard for diagnosing and monitoring BE [1] [9]. However, the histological biopsy method is relatively complex and difficult to standardize for inexperienced endoscopists, with the poor interobserver agreement [10] [11] [12], while increasing the financial burden and bleeding risk for patients. In addition, the uneven distribution of dysplasia in the BE segment can cause sampling errors [13]. Thus, previous studies have shown that HR-WLE combined with Seattle four-quadrant biopsy may miss dysplasia in approximately 50% of patients with Barrett's inconspicuous neoplastic lesions [14] without being clinically effective in preventing Barrett-associated cancers [15]. Therefore, advanced endoscopic imaging techniques are needed to diagnose dysplasia in patients with Barrett.

Advanced imaging techniques include chromoendoscopy, narrow-band imaging (NBI)-stained endoscopy, optical coherence tomography (OCT), autofluorescence endoscopy, and CLE [16] [17] [18] [19]. In particular, CLE allows *in vivo* histologic diagnosis of esophageal mucosal disease without waiting for specimen processing and histopathologic interpretation. Its particular focus is on detecting occult tumors. Although the use of CLE in BE has not yet completely replaced endoscopic biopsy, a study by Dunbar *et al.* [20] showed that CLE increased the detection of dysplasia compared with conventional endoscopy. In contrast, the number of random biopsies was reduced.

1) The application of CLE in the diagnosis and prediction of BE and BE-associated tumors

The first application of CLE in BE was reported by Kiesslich [19] in 2006. In that study, the Barrett classification of the Mainz confocal criteria was developed based on targeted biopsies of BE patients with or without neoplastic lesions, which for the first time differentiated BE, Barrett's tumors by identifying the cellular and vascular features of each entity. It had a diagnostic sensitivity of 93% and specificity of 98% for Barrett's tumors and 98% sensitivity and 94% specificity for non-dysplasia in BE. There was a high inter- and intra-observer agreement with kappa values of 0.84 and 0.89, respectively.

In a prospective, double-blind, multicenter study, Wallace *et al.* [21] men-

tioned that the sensitivity and specificity of using probe-based confocal laser endomicroscopy (pCLE) *in vivo* to diagnose BE-associated tumors were 88% and 96%, respectively. DiPietro *et al.* [22] found that the sensitivity and specificity of pCLE for detecting High-grade neoplasia/intramucosal cancer (IMC) in the BE population were 100.0% and 53.6%, respectively. The sensitivity and specificity of NBI + magnifying endoscopy were 57.1% and 74.1%, respectively. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of CLE for the diagnosis of low-grade intraepithelial neoplasia of the esophagus were 75.0%, 88.6%, 70.6%, 90.7%, and 85.0%, respectively, in a study by Gao Lijun *et al.* [23] The sensitivity, specificity, PPV, NPV, and accuracy for diagnosing high-grade intraepithelial neoplasia of the esophagus were 85.7%, 92.3%, 85.7%, 92.3%, and 90.0%, respectively.

Sharma *et al.* [24] conducted the first multicenter international randomized prospective study in 2011 in which 101 patients with BE were included who underwent high-definition white light endoscopy (HD-WLE), NBI, and pCLE intending to compare the performance of pCLE combined with HD-WLE with HD-WLE alone for the detection of BE-associated neoplastic lesions. The study was performed with histology as the gold standard. The results showed that the specificity and sensitivity of HD-WLE alone for detecting HGD were 34% and 93%, respectively, while the specificity and sensitivity of pCLE combined with HD-WLE were 68% and 88%, respectively. Sharma concluded that pCLE combined with HD-WLE significantly improved the detection of BE-associated tumors and may be useful in managing and following up with patients with BE, except that the results were not statistically different.

A 2012 study by Peiqi Long [25] noted the high sensitivity and specificity of CLE for the diagnosis of BE. I-scan combined with CLE can show the main morphological features of Barrett's epithelium and allow tissue staging of BE, which is not achieved by conventional endoscopy, but further studies with large samples are needed to confirm this due to the small sample size of the study.

A meta-analysis comparing NBI with CLE showed that the pooled sensitivity of both for detecting HGD/EAC was similar. Still, CLE significantly increased the detection rate of HGD/EAC per lesion [26].

Studies by Wallace [21] and Leggett [27] *et al.* found sensitivities of 88% and 76% and specificities of 96% and 79% for diagnosing BE-associated tumors using pCLE *in vivo* and *in vitro*, respectively. And in Wallace's study, accuracy was the same between experienced and inexperienced observers, indicating a shorter learning curve.

Certain studies have concluded that CLE is superior to preoperative and conventional biopsies for diagnosing BE and associated neoplastic lesions.

In a retrospective study by Caillol *et al.* [28], 31 patients were included from 2013 to 2015 with 35 endoscopic examinations. The histological findings of endoscopic resection were normal/inflammatory in 3 cases, non-dysplastic BE with intestinal degeneration in 8 cases, LGD in 10 cases, and HGD/EAC in 14 cases.

71% (25/35) of cases were correctly diagnosed by pCLE, and 43% (15/35) by pre-excisional biopsy. pCLE detected HGD/EAC with a sensitivity, specificity, and accuracy were 92.9%, 71.4%, and 80%, respectively, and 78.6%, 61.9%, and 68.6% for histological biopsy. However, the difference in support of pCLE was not statistically significant ($P = 0.2$).

In a 2018 study by Richardson *et al.* [29], twice as many BE cases were identified using pCLE than Seattle four-quadrant biopsy. Tissue biopsies using the Seattle protocol identified intestinal metaplasia (IM) in 46/172 patients, whereas pCLE identified IM in 99/172 patients ($P < 0.0001$). Another interesting finding was the much higher percentage of BE patients with columnar epithelial esophagus visible under pCLE compared to conventional biopsy (80% vs. 32%). This study suggests that pCLE is more sensitive than the Seattle protocol in detecting BE, with a proportion of patients with negative histology actually diagnosed as IM-positive under pCLE. Overall, pCLE offers a promising advance in the detection of Barrett. This may be because multiple random biopsies may miss some esophageal tumors, whereas CLE allows the observation of the entire BE segment in real time.

Tofteland's [30] study showed that GI pathologists had high accuracy and substantial interobserver agreement for diagnosing BE dysplasia with pCLE compared to pathologists interpreting the results of BE histology specimens. Pathologists appeared to have similar accuracy and interobserver agreement as endoscopists. These results provided further support of endoscopists accurately interpreting the *in vivo* optical histology provided by pCLE.

The diagnostic performance of CLE+ targeted biopsy versus standard endoscopic biopsy for Barrett's tumors has been compared in several studies.

For example, the diagnostic efficiency of endoscope-based confocal laser endomicroscopy (eCLE)+targeted biopsy was compared with standard endoscopic biopsy in a prospective randomized, double-blind crossover trial [20]. The results showed that eCLE with targeted biopsy improved the diagnosis of severe dysplasia in BE (33.7% vs. 17.2%) and reduced the average number of biopsy specimens required compared to standard randomized biopsy. Notably, this study was not designed to assess diagnostic accuracy because mucosal biopsies during eCLE were performed only on lesions suspected of BE with HGD.

Subsequently, in a prospective randomized controlled trial by Canto *et al.* [31] in 2014, the 192 BE patients were randomly assigned to the HD-WLE + random biopsy group or the HD-WLE + eCLE + targeted biopsy group. The results showed that: HD-WLE combined with eCLE increased the sensitivity of tumor detection from 40% to 96% ($P < 0.0001$) and significantly reduced the number of biopsies required (eCLE-targeted biopsies reduced the number of biopsies by 4.8 times compared to random biopsies). In addition, CLE changed the treatment regimen in 36% of patients. This study demonstrated the superiority of the targeted biopsy approach over standard randomized biopsy.

Because metabolically active dysplastic cells are more likely to be admixed with

2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose(2-NBDG), areas of dysplasia are more visible on microendoscopy. The intensity of 2-NBDG fluorescence correlates with the degree of dysplasia, as described in early clinical [32] [33] [34].

In a 2012 study by Gorospe *et al.* [35], a new simplified set of fluorescence intensity criteria for CLE was developed based on the local fluorescent marker of 2-NBDG, which remained highly accurate in the detection of dysplasia even when used by endoscopists with no experience in CLE and with the high interobserver agreement but not yet validated *in vivo*.

The purpose of using the marker technology is to reduce the number of sites evaluated by pCLE. The choice of autofluorescence imaging (AFI) marker technology is based on previous feasibility evidence that positive AFI signals are associated with molecular aberrations [36].

Di Pietro *et al.* [22] demonstrated that the multimodal method of pCLE combined with AFI achieved a sensitivity of 96.4% and specificity of 74.1% in diagnosing BE-associated neoplastic lesions. The additional use of pCLE in the AFI target area reduced the AFI diagnosis of HGD/IMC and dysplasia of any grade from false positive rates from 82.7% and 69.5% to 69.7% and 48.7%, respectively.

A study by Vithayathil *et al.* [14] found that in BE patients with inconspicuous dysplasia, AFI-guided pCLE had similar sensitivity and accuracy for diagnosing dysplasia compared with standard HR-WLE + Seattle protocol biopsies. Still, the number of biopsies under AFI-targeted pCLE was much lower than under conventional protocols. However, AFI has yet to be widely available and is unlikely to be the ideal marker technique for future applications.

CLE criteria for diagnosing Barrett-associated neoplastic lesions have been established [37] [38].

The Miami criteria for pCLE were proposed based on five structural features of Barrett's tumors (epithelial irregularity, variable epithelial width, glandular fusion, dark areas, and irregular blood vessels) to differentiate between normal squamous epithelium, BE with or without dysplasia, and IMC. The sensitivity and specificity for detecting dysplasia using the criterion were 88% and 96%, respectively, with a substantial interobserver agreement (kappa 0.72) [21].

Subsequently, in 2011, Gaddam *et al.* [37] developed and validated a new diagnostic criterion (including six indicators: absence of cup cells; enlarged and irregular cells; variable gland size and spacing; variable gland shape; altered glandular epithelial thickness; and irregular or jagged glandular epithelial surface) for the prediction of BE-associated HGD based on high-quality video of pCLE in 50 BE patients. The results showed that the overall accuracy, sensitivity, specificity, PPV, and NPV for the diagnosis of BE-associated HGD were 81.51%, 76%, 85%, 76.05%, and 84.97%, respectively, and the agreement between endoscopists and novice endoscopists was also high, indicating a short learning curve for the diagnostic criterion.

In recent years Pietro *et al.* [38] proposed a diagnostic system for identifying

BE with LGD, which had diagnostic sensitivity, specificity, accuracy, interobserver agreement, and area under the receiver operating characteristic curve of 81.9%, 74.6%, 78.3%, 0.654, and 0.888, respectively.

However, conflicting findings make the interpretation of CLE performance challenging.

In 2008 Pohl *et al.* [39] established CLE diagnostic criteria for normal esophagus and BE-associated tumors based on 201 biopsies from 15 BE patients. The study confirmed that CLE had a high NPV for diagnosing endoscopic invisible tumors in BE; however, the sensitivity needed improvement. Subsequently, in 2009, Bajbouj *et al.* [40] compared the correlation between pCLE diagnostic criteria for Barrett's tumors and histopathological findings of biopsies from Seattle four-quadrant biopsy and showed that pCLE had a specificity and NPV of more than 90% in excluding Barrett's tumors, but the PPV and sensitivity remained low. Similarly, a 2017 study by Shah *et al.* [41] showed that pCLE had high specificity (98%) but also low sensitivity (67%) for detecting Barrett-associated dysplasia and cancer, and the lack of incremental benefit of pCLE compared to HD-WLE and NBI may limit its usefulness in BE clinical applications.

Both Pohl and Bajbouj showed that CLE was no less competent than histological biopsy in excluding Barrett's tumors; however, CLE may not replace standard biopsy techniques, as their studies failed to confirm the value of CLE in the diagnosis of BE-associated tumors, possibly because the pCLE diagnostic criteria developed were too strict or the patients included were not representative (the incidence of BE-associated tumors was low).

A cross-sectional study published in 2012 by Jayasekera *et al.* [42] showed that the most accurate method for detecting HGD in BE was HD-WLE combined with NBI rather than eCLE but made the argument for targeted biopsy without random biopsy in detecting HGD and IMC. A prospective randomized trial by Wallace *et al.* [43] showed no significant benefit to adding CLE to HD-WLE, and the reason for the failure was unclear.

In summary, although CLE has significant imaging advantages, previous studies have provided mixed reviews of its value in diagnosing BE and BE-associated tumors. Large studies are needed to further validate it.

2) CLE in BE and BE-associated tumors surveillance follow-up

In a study by Bertani *et al.* [44], patients were divided into two groups: 50 underwent HD-WLE only, and 50 underwent pCLE in addition to HD-WLE. Results showed that pCLE detected dysplasia events more frequently than HD-WLE in a BE surveillance program and may improve the efficiency of BE patient follow-up. However, the study did not conduct a prospective trial to further determine whether the higher dysplasia detection rate provided by pCLE could improve the efficacy of the BE surveillance program.

Moreover, according to the latest European Society for Gastrointestinal Endoscopy (ESGE) guidelines, HD-WLE is still strongly recommended for BE surveillance. Routine use of advanced endoscopic imaging, including CLE, has yet

to be recommended [45].

3) CLE in guiding endoscopic treatment of BE and BE-associated tumors

CLE plays various roles during endoscopic treatment by providing a real-time dysplasia evaluation. First, CLE can localize and predict pathology. Second, CLE can help the endoscopist identify targets for biopsy and resection in monitoring and treating BE [19] [20] [39] [46]. In addition, CLE can guide which treatment to use, determine the adequacy of treatment, and gauge the need for further treatment.

In a case reported by Leung *et al.* [46], CLE-guided targeted EMR in a patient with focal HGD of BE. Unlike ablative modalities, EMR has the advantage of pathologic confirmation, and in addition, targeted EMR reduces the risk of stenosis formation and perforation due to large circumferential EMR.

In the Konda VJ study [47], Case 1 was a 66-year-old white male diagnosed with BE for two years who was referred for a nodule showing HGD. The initial pre-endoscopic plan was to perform focal EMR of any visible lesions followed by radiofrequency ablation. Two lesions were identified based on white light endoscopy (WLE) and NBI examination. pCLE was then performed on both lesions, and the results were consistent with WLE and NBI. Subsequent pCLE examination of the remainder of the BE revealed multifocal neoplastic lesions, although WLE or NBI did not detect any irregularities in these areas. The endoscopist therefore chose to perform a complete EMR of the 4 cm segment using a band ligation technique. Nodal pathology revealed IMC, and the remaining resected specimen showed diffuse HGD. Other cases also involved post-treatment evaluation of the margins of the lesion after repeat EMR using pCLE and additional EMR by pCLE in areas that were not immediately apparent under HD-WLE.

In a retrospective caseseries, Johnson *et al.* [48] endoscopic treated four patients with pathologically confirmed BE with high-grade intraepithelial neoplasia and subsequently examined the area around the tumor resection site using pCLE to clarify the presence of IM, dysplasia, and residual cancer cells. The results showed that one patient still had a neoplastic lesion at the surgical margins and underwent a second EMR immediately; one patient presented with discontinuous BE under pCLE and was treated with radiofrequency ablation after confirmation by targeted biopsy. This study demonstrated that in BE patients with neoplastic lesions, pCLE could be a good guide for targeted biopsies and for assessing the accuracy of endoscopic treatments such as EMR or radiofrequency ablation, allowing real-time monitoring of dysplasia and marking the lesion area for targeted therapy of dysplasia.

In a multicenter randomized controlled trial by Wallace MB [43], BE patients underwent HD-WLE or HD-WLE + pCLE monitoring after ablation, with biopsy plus ablation in patients with suspected dysplasia and otherwise biopsy only. The primary outcome indicator of the study was the proportion of best-treated patients (defined as free of dysplasia). Due to the lack of statistical difference in the proportion of best-treated patients between the two groups, this study was

terminated early, concluding that there was no evidence adding pCLE to HD-WLE in patients after ablation improved outcomes.

In 2014, a prospective pilot study by Dolak *et al.* [49] included 38 patients with BE-associated tumors. The patients were first examined with HD-WLE combined with NBI, then another endoscopist completed eCLE, and each side marked the observed tumor borders. Finally, EMR or endoscopic submucosal dissection (ESD) was performed on the suspected tissue. The results showed that compared with HD-WLE combined with NBI examination, eCLE still identified additional tumor tissue in 7 of 38 patients (18%) (two combined lesions, two medial and lateral tumor extensions of Barrett's epithelium, and three extensions of previously undetected subsquamous tumors). eCLE, according to Dolak, by assessing the lateralization of BE-associated tumors and extensions of subsquamous tumors, appeared to be a means to optimize the endoscopic treatment of BE-associated tumor lesions. However, this was only a pilot study and needed further validation in a randomized controlled clinical trial.

Unusually, a study by Michael B *et al.* [43] evaluated the impact of using pCLE to guide ablative treatments in BE. The primary aim was to assess whether adding pCLE to confirm findings with HD-WLE would result in a higher proportion of optimally treated patients. This study did not provide evidence that the combination of HD-WLE and pCLE was superior to HD-WLE alone to assess the completeness of ablation of BE. There was a slightly higher proportion of patients with residual dysplasia in the HD-WLE group and a slightly higher proportion with metaplasia in the HD-WLE + pCLE group; however, with the small numbers, these differences were not of statistical significance, and the clinical significance is unknown.

4) CLE in the monitoring of BE and BE-associated tumors after endoscopic treatment

In 2020, a study by Jana Krajciova *et al.* [50] evaluated the ability of pCLE to detect persistent or recurrent tumor formation or intestinal IM after BORN endoscopic treatment and showed no significant differences in sensitivity, specificity, PPV, and NPV between pCLE and biopsy for the diagnosis of recurrent/persistent IM; the diagnostic accuracy was 100% for pCLE (95% CI, 93.6% - 100%) and 94.6% (95% CI, 85.1% - 98.9%) for biopsy, P = 0.25; pCLE detected significantly more cupped cells in patients with IM than biopsy, P = 0.01.

3.2. Esophagus Cancer

Esophagus cancer (EC) mainly consists of two pathological types: esophageal adenocarcinoma and esophageal squamous cell carcinoma (ESCC), and is one of the most common malignant tumors of the digestive system, ranking 9th in incidence and 5th in mortality worldwide [51]. EC is usually asymptomatic or mildly symptomatic in the early stages, and most patients are already in the middle to late stages when they are seen. Its prognosis is poor, and the 5-year survival rate is generally low [52]. Therefore, early endoscopic evaluation of EC is particularly

important for timely intervention and determination of prognosis.

The study by Pech *et al.* [53] concluded that CLE could provide virtual histology of early ESCC with high accuracy and facilitate rapid diagnosis in the context of conventional endoscopy.

The study by Liu, H *et al.* [54] aimed to compare the endoscopic features of cells and intrapapillary capillary loops (IPCLs) in the normal esophagus and superficial esophageal squamous cell carcinoma (SESC). The results showed that compared to controls, CLE observed a significantly higher proportion of irregularly arranged squamous epithelial cells (79.4% vs. 10.0%, $P < 0.001$), increased IPCLs diameter (26.0 μm vs. 19.2 μm, $P < 0.001$) and increased irregularly shaped IPCLs (82.4% vs. 36.7%, $P = 0.0002$) in the SESC group. In this study, approximately 35.5% of CLE images were of good quality, and there was fair interobserver agreement in predicting cancerous mucosa. The authors concluded that CLE could be used to distinguish between cancerous and normal epithelium, which made it potentially valuable for the early detection of EC.

A prospective study by Jing Guo *et al.* [55] evaluated the role of pCLE in identifying and differentiating esophageal neoplastic from non-neoplastic lesions, the first pCLE-based surface maturation scoring (SMS) system to identify squamous cell carcinoma (SCC) and esophageal squamous intraepithelial neoplasia (ESIN). They performed biopsies of only microscopic abnormalities, suggesting that targeted biopsies of pCLE could effectively reduce the number of tissue biopsy specimens required without reducing tumor detection. This has important implications for areas with a high prevalence of SCC and for areas where endoscopic treatment is rapidly evolving. In conclusion, this prospective trial demonstrated the potential of pCLE with targeted biopsy for diagnosing and identifying esophageal squamous neoplasia (ESN) in either a real-time or offline setting. The microscopic diagnostic system of SMS has been shown to be applicable to pCLE (CLE can accurately predict ESIN with the SMS system and has been validated by eCLE; however, the SMS system has not been previously applied to pCLE).

The studies described above demonstrate that using CLE can reliably distinguish between the normal esophagus and ESCC, emphasizing the potential of CLE for early detection of ESCC and allowing for timely treatment. However, training is required in operational techniques for locating CLE probes in the area of interest, and working with a pathologist is required to gain a thorough knowledge of mucosal histopathology. In addition, obtaining good images of the esophagus is a potential challenge for CLE.

3.3. Gastroesophageal Reflux Disease

The clinical manifestations of gastroesophageal reflux disease (GERD) are diverse, with reflux and heartburn being the typical symptoms occurring in about 50% of cases [56] [57]. Throat discomfort, foreign body sensation, hoarseness, cough, or asthma is the main symptoms in some patients. To date, there is no standard tool for diagnosing GERD. Typically, GERD is a non-erosive reflux disease (NERD) without endoscopic abnormalities. CLE has been shown to be

an effective tool for identifying and diagnosing GERD.

A study by Chu Chuanlian [58] in 2012 pointed out that CLE could clearly observe the morphology of capillary climbing and the changes of tube diameter and cell gap in the esophageal mucosal cells, subcellular and interstitial intraepithelial papillae, providing a new valuable examination method for the diagnosis of NERD, enriching the pathophysiological mechanism of NERD and providing a new theoretical basis for the treatment of NERD.

Disruption of epithelial barrier function (EBF) is a critical mechanism in GERD. A study by Pritesh *et al.* [59] compared whether pCLE and mucosal integrity testing (MIT) could assess EBF. pCLE was found unable to differentiate GERD from non-GERD, whereas MIT could, thus being more promising.

RapatPittayanon's study [60] compared the diagnostic value of flexible spectral imaging color enhancement (FICE) and pCLE for minimal change esophageal reflux disease (MERD). The results showed that the accuracy of FICE and pCLE were 79% and 87%, the sensitivity was 94% and 97%, the specificity was 50% and 66%, the PPV was 79% and 85%, and the NPV was 82% and 92%, respectively. The authors concluded that both FICE and pCLE had good operational properties and contribute to diagnosing MERD. However, pCLE was more consistent in the diagnosis of MERD across observers.

A study by Eunju Jeong [61] assessed cellular and vascular changes in GERD patients by CLE *in vivo* and *in vitro*. The results showed: Patients with esophageal reflux disease (ERD) and NERD exhibited wider cellular gaps on CLE images than control patients. The diameter, number, and cross-sectional area of IPCLs were significantly larger in the ERD group than in the NERD group. The irregular shape of IPCLs was observed in both ERD and NERD patients. The authors concluded that irregularly shaped IPCLs were significantly associated with a positive diagnosis of GERD and that CLE had high sensitivity and accuracy in diagnosing NERD.

4. Future Directions

The emergence of CLE as a new imaging technology marks a qualitative change in endoscopy from superficial to deep, from macroscopic to microscopic, and from morphology to histology. The popular application of this new technology plays an essential role in diagnosing and treating GI diseases, and this review focuses on the application of CLE in esophageal diseases. In summary, CLE has the potential to significantly improve the management of BE patients by better detecting and diagnosing BE-associated tumors and guiding endoscopic targeted biopsies and treatment. CLE also involves the application of ESCC and GERD.

CLE facilitates the *in vivo* diagnosis of BE and associated dysplasia, and its accuracy in detecting HGD is comparable to conventional biopsy. However, there is still room for improvement in detecting other grades of dysplasia. At the current state of the technology, a virtual histologic biopsy of CLE cannot replace a conventional biopsy. Still, CLE can enhance the monitoring of BE by providing real-time evaluation and identification of suspicious dysplastic lesions.

The main limitation of CLE is its narrow observation field, which is prone to sampling errors depending on the placement of the CLE probe in the esophageal mucosa. Therefore, it will be vital to combine CLE with appropriate red-flag techniques in the future. In addition, the available CLE contrast agents are non-specific for dysplasia because they rely solely on vascular and cellular staining patterns.

A full wide-field examination of the esophagus can now be performed with volumetric laser endomicroscopy (VLE), a novel imaging technology similar to OCT [62]. VLE combined with CLE provides the dual benefits of wide-field imaging *in vivo* environment and high magnification mucosal dysplasia observation [63].

The development of new fluorescent contrast agents and molecular probes, which can be stained specifically under microendoscopy, will enable easier detection of lesions, allow understanding of the pathophysiological causes of diseases, and study the interconnection between cells and tissues in terms of physiological functions. Meanwhile, the application of molecular markers for early diagnosis and targeted treatment of GI tumors is also a direction for further research on CLE.

Although CLE has drawbacks, it can reduce tissue damage and improve diagnostic accuracy to a certain extent. CLE, combined with other imaging methods, can improve the detection rate of esophageal or gastric cancer and precancerous lesions and avoid unnecessary biopsies. Further technological innovation and standardization of CLE will make it more adaptable to routine clinical applications.

Newer, higher-resolution microendoscopic systems are under development. Multiphoton microscopy is now available for *vitro* specimens, providing high-resolution images without fluorescent dyes, including nuclear visualization [64]. Spectral-encoded confocal microscopy is another emerging technology that allows imaging of larger areas of the GI mucosa, providing both high cellular resolution and detailed images of mucosal structures [65]. These and other new endoscopic technology will undoubtedly advance the field and allow for improved imaging of the GI mucosa.

Advances in artificial intelligence provide a means to address the inherent subjectivity and cumbersomeness in human image interpretation and complement CLE diagnostics well in medical diagnostics. Computer-aided diagnosis has the potential to help operators interpret images in real-time during CLE procedures and represents an interesting area for future research.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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List of Abbreviations

CLE: Confocal Laser Endomicroscopy
GI: Gastrointestinal
BE: Barrett's Esophagus
LGD: Low-Grade Dysplasia
HGD: High-Grade Dysplasia
EAC: Esophageal Adenocarcinoma
EMR: Endoscopic Mucosal Resection
HR-WLE: High-Resolution White Light Endoscopy
NBI: Narrow-Band Imaging
pCLE: Probe-Based Confocal Laser Endomicroscopy
IMC: Intramucosal Cancer
PPV: Positive Predictive Value
NPV: Negative Predictive Value
HD-WLE: High-Definition White Light Endoscopy
IM: Intestinal Metaplasia
eCLE: Endoscope-Based Confocal Laser Endomicroscopy
2-NBDG: 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose
AFI: Autofluorescence Imaging
WLE: white Light Endoscopy
EC: Esophagus Cancer
ESCC: Esophageal Squamous Cell Carcinoma
IPCLs: Intrapapillary Capillary Loops
SMS: Surface Maturation Scoring
GERD: Gastroesophageal Reflux Disease
NERD: Non-Erosive Reflux Disease
FICE: Flexible Spectral Imaging Color Enhancement
MERD: Minimal Change Esophageal Reflux Disease
ERD: Esophageal Reflux Disease