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Real-time diagnosis of Barrett's esophagus: a prospective, multicenter study comparing confocal laser endomicroscopy with conventional histology for the identification of intestinal metaplasia in new users

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Abstract

Introduction Endoscopic evaluation with high-definition white light endoscopy and random 4-quadrant biopsy (Seattle Protocol) is the current standard of care for the detection of Barrett's esophagus (BE). Recently, enhanced imaging technologies have become available to provide real-time diagnosis of intestinal metaplasia (IM) and dysplasia, reducing the need for tissue biopsy. Probe-based confocal laser endomicroscopy (pCLE) provides dynamic microscopic mucosal views, rapidly capturing digital images that become optical biopsies. This study examined the role of pCLE in BE screening and surveillance as compared to the Seattle Protocol.

Methods Patients undergoing BE screening or surveillance endoscopy were enrolled at eight US centers. Optical biopsy using pCLE was interpreted in real time. Endoscopists performing pCLE were new users with a median experience of 8.5 months and no formal training in surgical pathology. Seattle Protocol biopsies were then taken. Recorded pCLE images were reviewed by a blinded expert in optical biopsy interpretation.

Results Early pCLE users identified significantly more patients with IM than the Seattle Protocol overall (99/172 vs. 46/172, $p < 0.0001$). Early users of pCLE also identified significantly more patients with IM than the Seattle Protocol in the patients with visible columnar lined esophagus (75 vs. 31, $p < 0.0001$), but not in the 76 patients without columnar lined esophagus (24 vs. 15, $p = 0.067$). There was no statistically significant difference between early pCLE users and expert review.

Conclusion Optical biopsy using pCLE technology allows for the real-time evaluation of entire segments of columnar lined esophagus. Consequently, pCLE is considerably more sensitive in the detection of BE than the Seattle Protocol, which leaves a majority of epithelium unexamined. This effect is seen even in new users and increases with experience. Overall, pCLE provides a promising advance in Barrett's detection which will likely result in superior identification of individuals at risk for esophageal adenocarcinoma.

Keywords Confocal laser endomicroscopy · Barrett's esophagus · Endoscopy · Barrett's surveillance

The incidence of esophageal adenocarcinoma in the United States continues to rise, and with it comes an increased need for cost-effective methods for screening. Barrett's esophagus (BE) remains the most prominent risk factor for the development of adenocarcinoma. While the current standard for screening, particularly in high-risk patients, consists of

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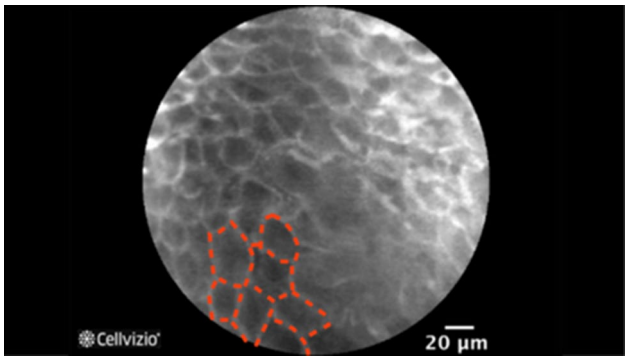

Fig. 1 pCLE of squamous epithelium

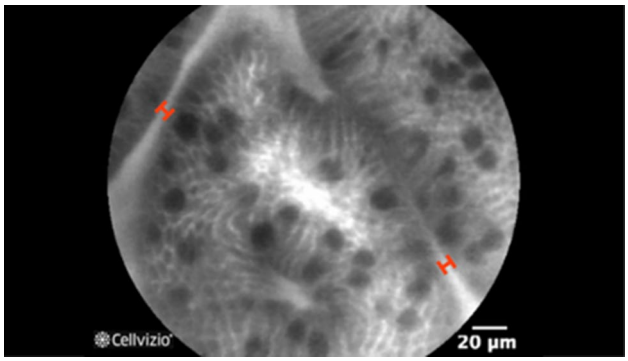

Fig. 2 pCLE of intestinal metaplasia with goblet cells

conventional white light endoscopy with random biopsy, several newer modalities have emerged over the past decade [1, 2]. One such adjunctive technology, probe-based confocal laser endomicroscopy (pCLE), utilizes principles of confocal microscopy to increase the resolution, imaging frame rates, contrast ratios, and depth of tissue penetration compared to white light endoscopy.

The probe-based confocal laser endomicroscopy (pCLE) system utilized consists of a laser-emitting probe that is passed through the working channel of a standard endoscope. Fluorescein contrast dye is injected intravenously into the patient just before the procedure, and it perfuses into the tissues within seconds. This contrast dye produces a fluorescent signal upon excitation from the fixed wavelength laser light emitted by the probe that is placed in contact with the mucosal surface. The resulting en-face images are provided at a depth of 100 µm, with a spatial resolution of 1 µm, and are displayed on a bedside tower display (Figs. 1, 2). Images are obtained and projected in real time to provide bedside video, which may also be recorded for later review. Several studies have addressed the application of pCLE in the context of BE surveillance, and some suggest that there is a role for this technology as an adjunctive enhancement that may even supplant the current practice standard [3–5]. Benefits that have been demonstrated include decreased time to diagnosis, targeted tissue biopsy sampling, and reduced number of procedures. In addition, pCLE may also have potential roles during therapeutic procedures that include localization of pathology, targeting of resections, guiding which therapy to use, and determining adequacy of treatment [3]. The goal of the present study is to examine the application of pCLE by early users in BE screening and surveillance as compared to the Seattle Protocol.

Materials and methods

Study design

Experienced endoscopists who were early users of pCLE (Cellvizio probe-based confocal laser endomicrosopy system, Mauna Kea Technologies, Paris, France) were recruited from eight centers across the United States. Early users were defined as < 2 years of experience with pCLE (median 8.5 months, interquartile range 0.25–10.1) and no formal pathology training. After obtaining IRB approval for each participating institution, all adult patients undergoing BE screening or surveillance endoscopy were eligible for enrollment unless they had a contraindication to fluorescein injection, a history of esophageal ablation for BE, were pregnant, or unable to provide consent. Data were collected prospectively. Demographic information recorded for each patient included age, gender, race, body mass index (BMI), history of BE or dysplasia, current or past use of proton pump inhibitors (including drug, dose, frequency, and duration), extra-esophageal manifestations of reflux, and history of anti-reflux procedure. Random, unique patient ID numbers were used to de-identify patients. No compensation was offered for participation in the study.

Diagnostic methods

The endoscopy technique was standardized across all centers and included routine white light and narrow band imaging evaluation, landmark identification, and recording of all visible columnar lined esophagus according to the Prague classification. Fluorescein was then administered intravenously for optical biopsy. Image capture was performed using full circumferential sweeps with the imaging probe from the squamocolumnar junction to the gastroesophageal junction. Optical biopsy using pCLE focused on columnar lined esophagus and was interpreted in real time and immediately after the procedure. Following the completion of pCLE image acquisition, the probe was removed and esophageal biopsies were taken per Seattle Protocol (four-quadrant biopsies starting at the squamocolumnar junction and proceeding in 1-cm

segments to the gastroesophageal junction). At the conclusion of the procedure, the endoscopist reviewed recorded pCLE videos and images. The identification of goblet cell intestinal metaplasia confirmed the presence of BE, while visualization of saw-toothed, irregular epithelial surfaces and pleomorphic cell sizes and shapes suggested dysplasia [3]. All videos and images were saved to an external hard drive for later review. The recorded pCLE images were reviewed by a single blinded expert with more than 7 years of experience in optical biopsy interpretation.

Statistical analysis

McNemar's test for paired data was utilized to compare proportions. The Kappa coefficient was used to evaluate inter-rater agreement. A p value ≤ 0.05 was treated as statistically significant. All analyses were performed using SAS software version 9.4 (© SAS Institute Inc., Cary, NC, USA).

Sample size estimation

Prior data were available from a similar protocol utilized by co-investigators in the present study, comparing 108 patients in paired analysis [6]. Using discordant pairs from the prior study, a sample size of 54 was identified using an alpha of 0.05 and power of 0.8. Three groups were identified in which to compare pCLE and traditional tissue biopsy: (1) patients with no history of Barrett's and abnormal squamocolumnar junction; (2) patients with no history of Barrett's and normal squamocolumnar junction; and (3) patients with a history of Barrett's undergoing surveillance. To ensure adequate representation from each of these groups, a total of 172 patients were enrolled. No less than 10 and no more than 30 patients were enrolled by each of the participating sites to maintain appropriate distribution of patients among the centers.

Results

A total of 172 patients were included in the study. No patients refused participation nor were any later excluded after enrollment. The mean age was 57.5 ± 14.7 with a mean BMI of 32.2 ± 7.9 kg/m^2. Sixty-one percent of participants were female. At least daily proton pump inhibitor (PPI) use was prevalent in 71.5% of patients, with a mean use of 6.2 ± 6.3 years. Twenty-seven patients had a previous diagnosis of BE. A total of 40 patients had prior foregut surgery, which included 30 fundoplications with hiatal hernia repair, two magnetic sphincter augmentations, two sleeve gastrectomies with hiatal hernia repair, two gastric bypasses, two lap bands, one lap band converted to sleeve gastrectomy without hiatal hernia repair, and one partial gastrectomy.

During endoscopy, 104 patients were found to have a hiatal hernia (median axial length 2 cm, range 1–15 cm), of which 16 were recurrent and 18 paraesophageal. A total of 11 patients had erosive esophagitis: 7 LA grade A, 3 LA grade B, and 1 LA grade D. Visually apparent columnar lined esophagus was found in 96 patients, with 8 having segments of 4 cm or greater. In these 96 patients, mean circumferential columnar lined esophagus length was 0.6 cm (median 0 cm, range 0–8 cm), and mean maximal length was 1.8 cm (median 1 cm, range 1–10 cm).

Endoscopists identified intestinal metaplasia (IM) in 99 patients (57.6%) using pCLE, and 12 of these did not have visible columnar lined esophagus. These early users also detected dysplasia in six patients (3.5%), and possible dysplasia in one patient. Tissue biopsy using the Seattle Protocol identified 46 (27%) patients with IM and two patients with dysplasia. This yielded a kappa coefficient of 0.25 (95%CI 0.13–0.36) comparing detection of IM. Both patients with dysplastic Barrett's esophagus on tissue biopsy were detected by early pCLE users.

Blinded expert review of discrepant IM detection yielded confirmation of IM detected with pCLE in 56 of 61 patients with negative tissue biopsies, disagreed with early users in two patients, and could not interpret three of the samples. In regard to the eight patients having IM on tissue biopsy that was not detected by early pCLE users, the blinded expert review identified IM in six of these eight patients. Figure 3 demonstrates the matched pair analyses of Seattle Protocol versus early users ($p < 0.0001$) and then early users versus expert review of the samples where novice users and Seattle Protocol did not agree ($p = 0.289$). Early pCLE users identified significantly more patients with IM than the Seattle Protocol overall (Fig. 4, 99/172 vs. 46/172, $p < 0.0001$). Early pCLE users also identified significantly more patients with IM than the Seattle Protocol in the patients with visible columnar lined esophagus (Fig. 5, 75 vs. 31, $p < 0.0001$), but not in the 76 patients without columnar lined esophagus (Fig. 6, 24 vs. 15, $p = 0.067$). There was no statistically significant difference between early pCLE users and expert review.

Discussion

The incidence of esophageal adenocarcinoma has risen over 800% over the past several decades and is still associated with a high mortality rate despite improvements in therapy such as endoscopic resection and ablation of early lesions, as well as more effective chemoradiotherapy for deeper tumors [7]. This discrepancy lies in the fact that most cancers are still detected at advanced stages. Improvements in risk stratification and surveillance of at-risk individuals are imperative. BE has been identified as the number one risk factor for

A

	Seattle Protocol IM detected	Seattle Protocol IM not detected
Early pCLE User IM detected	38	61
Early pCLE User IM not detected	8	65

B

	Expert Review IM detected	Expert Review IM not detected
Early pCLE User IM detected	56	2
Early pCLE User IM not detected	6	2

Fig. 3 **A** Matched pair analysis–Intestinal Metaplasia detected by Seattle Protocol versus early pCLE users. **B** Matched pair analysis—Discordant results of **A** intestinal metaplasia detected by early pCLE users versus expert review

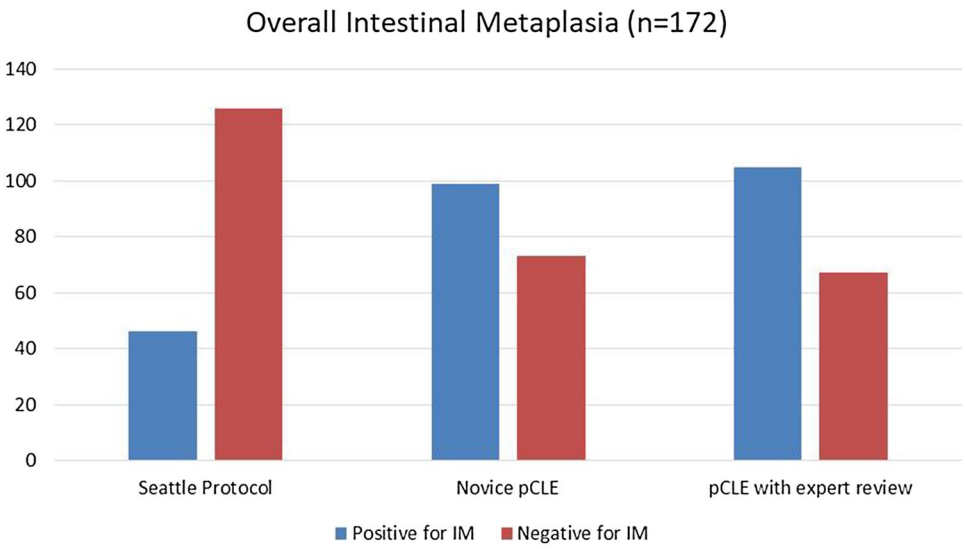

Fig. 4 Overall detection of IM between pCLE and Seattle Protocol

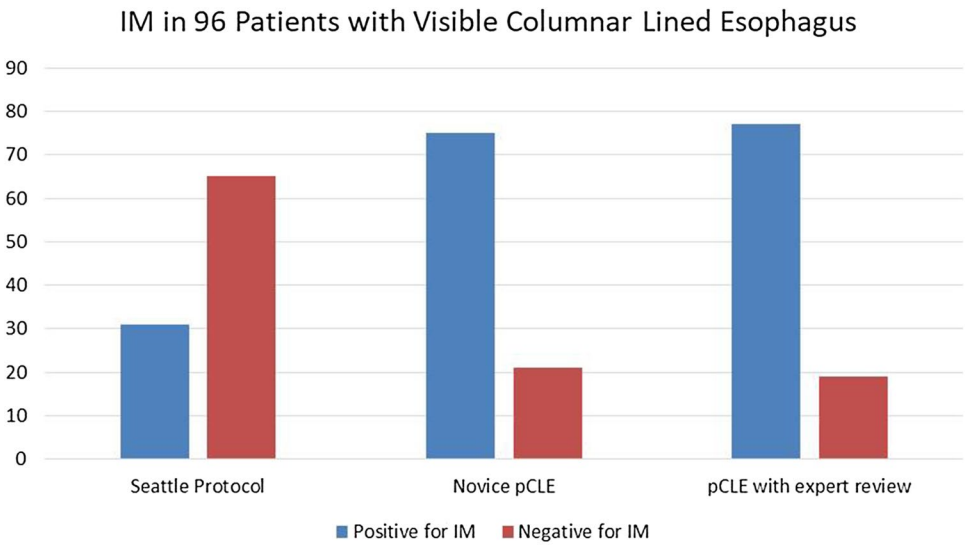

Fig. 5 Detection of IM with visible columnar lined esophagus

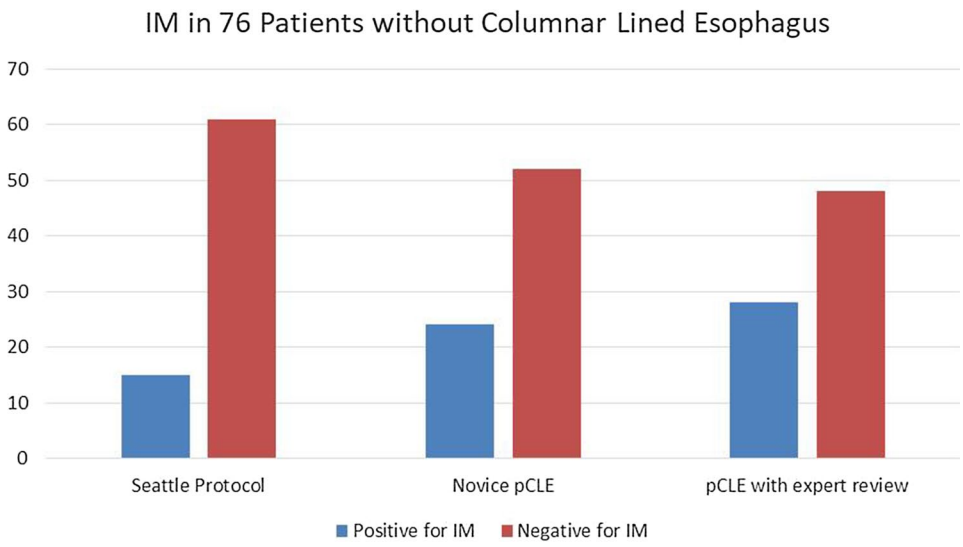

Fig. 6 Detection of IM without visible columnar lined esophagus

the development of esophageal adenocarcinoma. Compared to the general population, patients with BE have a 30- to 50-fold increased risk for developing adenocarcinoma of the esophagus [7]. Gastroesophageal reflux disease (GERD) has been identified as the major risk factor for development of BE. Unfortunately, there is no universally agreed upon protocol for screening patients with GERD to identify the at-risk population with BE. Even long-term surveillance strategies for patients diagnosed with BE are a source of disagreement among experts. One of the major criticisms is that random biopsies are expensive, imprecise, and time consuming. Current guidelines for BE diagnosis and surveillance, according to the Seattle Protocol, include four-quadrant random biopsies at 1- to 2-cm intervals [8–10]. This random biopsy protocol is inherently prone to sampling error, as very little of the esophageal surface area is actually sampled, depending on the length of the at-risk mucosa [11, 12]. Worse yet, despite the practice guidelines, there is inconsistent compliance with many practitioners taking far less than the recommended number of biopsies [13].

Despite well-documented shortcomings, the Seattle Protocol is the accepted "gold standard" for evaluation of esophageal mucosal metaplasia [14, 15]. However, the true sensitivity and specificity of the Seattle Protocol remains unknown as it can only be calculated if the entire Barrett's segment is subsequently removed and examined pathologically. Clearly, a study aimed at defining the true accuracy of the Seattle Protocol in defining non-dysplastic Barrett's esophagus would require an unreasonably invasive resection of benign epithelium. However, despite this limitation, there are ample data to suggest that the Seattle Protocol is insufficient to sample segments of Barrett's esophagus for dysplasia [11, 12]. In a particularly sobering study, Visrodia et al. showed that up to 25% of new cancers are missed at index endoscopy with "routine" biopsies [16]. Clearly, we need technology to improve the sampling rate in this disease and based on our data, pCLE appears to do just that.

Probe-based confocal laser endomicroscopy (pCLE) generates optical biopsies, providing physicians with instantaneous microscopic images of tissue. The present study demonstrates that Barrett's mucosa can be evaluated in real time with pCLE technology. This may have the potential to obviate the need for collecting traditional tissue biopsies, also eliminating the inherent delays in processing, reading, and reporting the standard pathology results. There has been a demonstrated 80% reduction in mucosal biopsies required during BE evaluation, due to more selective tissue sampling with the use of pCLE [17]. Although not the focus of the present study, the authors believe that pCLE may provide a quicker and broader sample of the at-risk mucosa than the traditional Seattle Protocol. The user can choose to scan the entire mucosa and review the recorded images after the procedure, as performed in the study protocol, or theoretically use the probe to "biopsy" in accordance with the Seattle Protocol itself, omitting the physical tissue biopsy. It is easy to conceive that even doubling or tripling the sampling rate with this technique would be quick, feasible, and accurate, as it allows a greater esophageal surface area to be examined as compared to the random biopsy method. As a result of the improved identification of specialized intestinal metaplasia as compared to the conventional Seattle Protocol, pCLE has the potential to result in a new standard of care for the diagnosis of BE.

A valid concern for clinicians looking to adopt new technology is the learning curve they will encounter before becoming clinically proficient with a new technique. This study shows that early users are able to accurately interpret optical images even at the early stages of using pCLE. Endoscopists performing pCLE were early users with a median experience of 8.5 months and no formal training in

surgical pathology. Early users identified 61 patients with BE that were not identified by the Seattle Protocol. A total of 55 were confirmed to have metaplasia by expert evaluation. This demonstrates that the limited sampling of the Seattle Protocol may grossly under-diagnoses intestinal metaplasia. The kappa coefficient of 25 demonstrates this significant difference. Conversely, there were eight patients who were identified to have BE on histology that were not identified by the endoscopist at initial pCLE review. Six of these cases, however, were identified as having BE during the independent review, suggesting that as endoscopists gain more experience with the technology, they will become more accurate.

Throughout the study, the authors identified and employed several strategies that aided in the ease of the procedure and interpretation of the images. Of the utmost importance is the quality of video image acquisition and review. Routine desufflation of the stomach prior to image acquisition helps bringing the mucosa into contact with the probe tip; an insufflated stomach tended to be more sensitive to patient respiratory movements. The authors also attached a silicone cap to the gastroscope tip to hold a section of mucosa steady for probe contact. With this area of mucosa pinned to the movements of the scope and probe tip, motion blur and inadvertent traversal across the mucosa were greatly reduced. Lastly, systematic post-procedure video review was critical. This was performed immediately following the procedure in a frame-by-frame manner. The potential for bias in this protocol was recognized and concerns were mitigated within the study design. First, the endoscopist did not know the results of the standard biopsies at the time they interpreted the pCLE. However, if intestinalization was identified by pCLE in real time, one could argue that the subsequent biopsies may be more "targeted" than pure Seattle Protocol. Since evidence suggests that within a segment of columnar lined esophagus the length of the intestinalized portion varies but, if present, is always found at the squamocolumnar junction, we controlled for potential bias by mandating that at minimum the squamocolumnar junction was always biopsied with both the optical and tissue techniques [18]. Additionally, the pCLE expert reviewer was blinded to the endoscopic appearance of the mucosa, the standard biopsy results, and the interpretation by the early user.

In this study, the use of pCLE identified twice as many cases of Barrett's esophagus than the Seattle Protocol. Furthermore, these cases of Barrett's esophagus were confirmed by an experienced independent reviewer, showing the ability of early users to quickly acquire the necessary skills to interpret these optical biopsies. Another interesting finding was that a much higher percentage of patients with visible columnar lined esophagus were found to have BE with pCLE versus conventional biopsy (80 vs. 32%). Endoscopists frequently encounter the situation where visual columnar lined esophagus is noted during endoscopy, yet conventional biopsies do not reveal Barrett's metaplasia. This study has shown that many of these patients in fact do have Barrett's esophagus as diagnosed with pCLE, despite negative histology. It is interesting to note in this study that there was 100% agreement between the endoscopists and the independent reviewer in regard to the interpretation of pCLE images in patients with visual columnar lined esophagus, despite the independent reviewer being blinded to the endoscopic findings. It is therefore more likely that the technique of physically taking tissue biopsies is inherently less accurate than carefully scanning of the squamocolumnar junction with the optical pCLE probe. In either situation, the present study confirms that Seattle Protocol biopsies do not appear to be as sensitive as pCLE at detecting BE in patients with visible columnar lined esophagus. Overall, pCLE provides a promising advance in Barrett's detection which will likely result in superior identification of individuals at risk for esophageal adenocarcinoma.

Compliance with ethical standards

Disclosures Drs. Cory Richardson, Paul Colavita, Christy Dunst, John Bagnato, Peter Billing, Kurt Birkenhagen, Francis Buckley, William Buitrago, Joseph Burnette, Phil Leggett, Howard McCollister, Kurt Stewart, Thomas Wang, Alvin Zfass, and Paul Severson have no conflicts of interest or financial ties to disclose.

References

1. Sharma P, Brill J, Canto M, DeMarco D, Fennerty B, Gupta N, Loren L, Lieberman D, Lightdale C, Montgomery E, Odze R, Tokar J, Kochman M (2015) White Paper AGA: advanced imaging in Barrett's esophagus. Clin Gastroenterol Hepatol 13:2209–2218
2. Gupta A, Attar BM, Koduru P, Murali AR, Go BT, Agarwal R (2014) Utility of confocal laser endomicroscopy in identifying high grade dysplasia and adenocarcinoma in Barrett's esophagus: a systematic review and meta-analysis. Eur J Gastroenterol Hepatol 4:369–377
3. Konda VJ, Chennat JS, Hart J, Waxman I (2010) Confocal laser endomicroscopy: potential in the management of Barrett's esophagus. Dis Esophagus 5:E21–E31
4. Bajbouj M, Vieth M, Rosch T, Miehlke S, Becker V, Anders M, Pohl H, Madisch A, Schuster T, Schmid RM, Meining A (2010) Probe-based confocal laser endomicroscopy with standard four-quadrant biopsy for evaluation of neoplasia in Barrett's esophagus. Endoscopy 42(6):435–440
5. Johnson E, De Lee R, Agni R, Pfau P, Reichelderfer M, Gopal DV (2012) Probe-based confocal laser endomicroscopy to guide real-time endoscopic therapy in Barrett's esophagus with dysplasia. Case Rep 6(2):285–292
6. Burnette J, Zfass A, Roch A, Bagnato J (2015) Utility of Probe-based Confocal Laser Endomicroscopy in screening work-up for Barrett's esophagus and detection of intestinal metaplasia [Abstract]. Presented at the Society of American Gastrointestinal and Endoscopic Surgeons Annual Meeting, Nashville
7. Pera M, Manterola C, Vidal O et al (2005) Epidemiology of esophageal adenocarcinoma. J Surg Oncol 92:151–159

8. Bennett C, Vakil N, Bergman J et al (2012) Consensus statements for management of Barrett's dysplasia and early-stage esophageal adenocarcinoma, based on a Delphi process. Gastroenterology 143:336–346
9. Committee ASoP. Evans JA, Early DS et al (2012) The role of endoscopy in Barrett's esophagus and other premalignant conditions of the esophagus. Gastrointest Endosc 76:1087–1094
10. Spechler SJ, Sharma P, Souza RF, Inadomi JM, Shaheen NJ (2011) American Gastroenterological Association technical review on the management of Barrett's esophagus. Gastroenterology 140:e18–e52
11. Kariv R, Plesec TP, Goldblum JR et al (2009) The Seattle protocol does not more reliably predict the detection of cancer at the time of esophagectomy than a less intensive surveillance protocol. Clin Gastroenterol Hepatol 7:653–658
12. Harrison R, Perry I, Haddadin W et al (2007) Detection of intestinal metaplasia in Barrett's esophagus: an observational comparator study suggests the need for a minimum of eight biopsies. Am J Gastroenterol 102:1154–1161
13. Abrams JA, Kapel RC, Lindberg GM et al (2009) Adherence to biopsy guidelines for Barrett's esophagus surveillance in the community setting in the United States. Clin Gastroenterol Hepatol 7:736–742
14. Sharma P, Allen J et al (2018) Improving quality of care in patients with Barrett's esophagus by measuring and improving neoplasia detection rates. Gastrointest Endosc 87(5):1195–1197
15. Shaheen NJ, Falk GW, Lyer PG et al (2016) ACG clinical guideline: diagnosis and management of Barrett's esophagus. Am J Gastroenterol 111:30–50
16. Visrodia K, Singh S, Krishnamoorthi R, Ahlquist DA, Wang KK, Iyer PG, Katzka DA (2016) Systematic review with meta-analysis: prevalent vs. incident oesophageal adenocarcinoma and high-grade dysplasia in Barrett's oesophagus. Aliment Pharmacol Ther 44(8):775–784
17. Canto MI, Anandasabapathy S, Brugge W, Falk GW, Dunbar KB, Zhang Z, Woods K, Almario JA, Schell U, Goldblum J, Maitra A, Montgomery E, Kiesslich R (2014) In vivo endomicroscopy improves detection of Barrett's esophagus-related neoplasia: a multicenter international randomized controlled trial. Gastrointest Endosc 2:211–221
18. Chandrasoma P, DeMeester T (2016) A New Pathologic Assessment of Gastroesophageal Reflux Disease: The Squamo-Oxyntic Gap. Adv Exp Med Biol 908:41–78