Confocal endomicroscopy (CEM) improves efficiency of Barrett surveillance

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Abbreviations: BE, Barrett's esophagus; CLE, columnar lined epithelium; SIM, specialized intestinal metaplasia; CEM, confocal endomicroscopy; EGD, esophagogastroduodenoscopy; GEJ, gastro-esophageal junction; SCG, squamo-columnar junction; HGD, high grade dysplasia; LGD, low grade dysplasia; SE, standard of error

Background: Endoscopists with extensive experience with confocal endomicroscopy (CEM) have demonstrated that this technology is useful for Barrett's esophagus (BE) surveillance. However, data on endoscopists with minimal experience with this technique are limited.

Hypothesis: For BE surveillance, an endoscopist with minimal experience in CEM-guided biopsy would achieve a similar diagnostic yield with fewer biopsies when compared to the random 4-quadrant biopsy protocol.

Objective: To compare the diagnostic yields of CEM-guided biopsy technique with the random 4-quadrant biopsy protocol.

Design: Randomized controlled trial.

Setting: Tertiary care center.

Patients: Patients with BE.

Methods: Out of 18 patients who underwent routine BE surveillance, 11 and 7 were randomly assigned to group A (CEM-guided) and to group B (random 4-quadrant biopsy), respectively. The pathologists were blinded to all clinical information.

Results: Mean length of endoscopic Barrett was similar in both groups, (5.1 vs. 6.3 cm, $p=0.51$). The diagnostic yields for detecting SIM (63.6% vs. 59.5%, $p=0.5$), low grade dysplasia (11.6% vs. 11.2%, $p=NS$), high grade dysplasia (10.1% vs. 11.5%, $p=0.88$). Although the total number of individual mucosal biopsy performed were 52% lower in the CEM group (129 vs. 269), the overall diagnostic yield (85.3% vs. 82.2%, $p=0.53$) was similar in both groups.

Limitations: Small sample size.

Conclusions: For BE surveillance, limited data suggested that endoscopists with minimal experience in CEM can effectively use this technology for "smart" biopsy to decrease the need for intense tissue sampling but without lowering the diagnostic yield in detecting dysplasia.

Introduction

Barrett's esophagus (BE) is a common complication of chronic gastro-esophageal reflux disease. An estimated 700,000 patients in the US alone have BE and, compared to general population, they are at 30-125 times higher risk of developing esophageal cancer.¹ BE is characterized by endoscopically distinguishable columnar lined epithelium (CLE) along with the histological identification of characteristic specialized intestinal metaplasia (SIM). Histologically, SIM is characterized by intestinal type crypts and goblet cells distended with mucus and interspersed

between columnar type of epithelial cells. Dysplasia and esophageal adenocarcinoma are known to develop only from areas of SIM and it is SIM that defines BE and confers the increased risk for adenocarcinoma. The distribution of SIM in the CLE is often focal, is not identifiable by conventional endoscopy, and requires a time-consuming and costly process of intense tissue sampling of the CLE during endoscopy.^{1,2} The number of biopsies necessary to detect intestinal metaplasia has not been defined although in clinical practice random 4-quadrant random biopsy is taken every 1-2 cm of the segment with CLE during surveillance (standard protocol).³ In published controlled trials, the rate of detection of SIM with the random 4-quadrant biopsy protocol has varied from 57% to 68%.⁴⁻⁹ Numerous endoscopic techniques including chromoendoscopy, magnification endoscopy, high-resolution endoscopy, narrow band and autofluorescence imaging have been shown mixed results in improving the detection of dysplasia compared with the standard protocol.¹⁰

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The technique of fluorescence-aided confocal endomicroscopy (CEM) has been recently introduced which allows real-time in vivo microscopy of the mucosal layer of the gastrointestinal tract and may increase detection of early neoplasia by enabling “smart”, guided biopsies.^{11,12} An initial report demonstrated that CEM, unlike any other endoscopic technique, can provide a real-time diagnosis of SIM in areas of CLE by identifying the pathognomic goblet cells and potentially can be very helpful in targeted identification of Barrett’s associated lesions with high accuracy.¹³ In addition, a recent study indicated that CEM-guided biopsy can increase the diagnostic yield of Barrett-associated dysplasia with decreasing the number of biopsies compared to standard random 4-quadrant biopsy.¹⁴ However, these studies were performed by endoscopists with extensive experience in CEM. It is unknown if endoscopists with minimal experience (only performing 10 procedures prior to the study) in this technology are able to achieve a similar result.

The aim of the study was to compare the diagnostic yields of CEM-guided biopsy technique with the random 4-quadrant biopsy protocol in patients undergoing BE surveillance.

Methods

Initial esophagogastroduodenoscopy (EGD)

After obtaining informed consent patients, all eligible patients underwent an EGD under conscious sedation. This procedure was performed with a commercially available confocal endomicroscope. All procedures were performed by a primary investigator (AD) who was experienced in conventional endoscopy with more than 15 years of post-fellowship training and has performed approximately 15000 diagnostic and therapeutic endoscopies. Patients with endoscopic esophagitis (Los Angeles classification A to D), mucosal irregularity or nodules suspicious for dysplastic or cancerous lesion, and those with obvious mass lesions were excluded.

After initial endoscopic evaluation of the esophagus and proximal stomach, positions of the diaphragmatic pinch, the muscular gastro-esophageal junction (GEJ), the squamo-columnar junction (SCJ), and the length of the CLE were carefully noted. All measured in centimeters from the incisor teeth. The study was approved by The Mayo Clinic Institutional Review Board.

Randomization

Patients were randomly assigned to group A and B in a one-to-one ratio. A randomized treatment allocation schedule was created by using a computerized random number generator. To prevent selection bias, the allocation schedule was concealed by storing it on a biostatistic randomization website. Each patient was randomly provided a treatment assignment number by this website. So the patient and clinical staff would not know the treatment assignment until after the patient has been consented.

Group A: CEM-guided biopsy protocol

The confocal endomicroscope (Pentax EC-3870CIK, Pentax of America, Montvale, NJ) is a standard video endoscope with

an integrated confocal laser microscope at the distal tip, which delivers an argon ion laser at an excitation wavelength 488 nm. The confocal image data are collected at scanning rates 0.8 to 1.6 frames/s (512×1024 to 1024×1024 pixels) with an optical slice thickness of 7 µm, lateral resolution of 0.7 µm and a field of view of 475×475 µm; the range of the Z-axis is 0-250 µm below the surface layer.

A single dose fluorescein (5-10 ml of a 10%), a contrast agent, was injected intravenously for mucosal staining for approximately 30-60 min. After fluorescein administration, CEM was performed in a circular manner at four quadrants every 2 cm (every 1 cm in patients with history of high grade dysplasia [HGD]) starting at the level of the muscular GEJ and proceeding proximally to the SCJ. During CEM, the distal tip of the endoscope was placed in gentle contact with the mucosa and the position of the focal plane of imaging was adjusted electronically. In every quadrant, targeted regions with villous, dark, regular, cylindrical epithelial cells interspersed with characteristic goblets cells were biopsied by using standard biopsy forceps and were sent for histopathology in a separate appropriately labeled biopsy container (**Fig. 1**). Targeted biopsy was possible with this endoscope because of the proximity of the accessory channel and the endomicroscopic window at the distal tip of the endoscope. After all study biopsies had been obtained, random biopsies were acquired from every quadrant which had not been sampled earlier at 1-2 cm interval for compliance with standard practice, but these biopsies were not be part of the primary analysis of the study (**Fig. 1**).

Group B: Random biopsy protocol

Random 4-quadrant biopsy protocol was carried out using standard biopsy forceps every 2 cm (every 1 cm in patients with history of HGD) starting at the level of the muscular GEJ and proceeding proximally to the SCJ.

Histo-pathological assessment

Every biopsy specimen was placed in 10% formalin in a separate labeled biopsy container and processed in a standard fashion (paraffin embedding, staining with hematoxylin & eosin and alcian blue at pH 2.5). All biopsies were assessed by an experienced gastrointestinal pathologist with an interest in BE, and all diagnoses of dysplasia were confirmed by an additional pathologist. The pathologists were blinded to the group allocation of the patients and details of the endoscopic evaluation. Each pathologist reported separately on each biopsy sample specifically mentioning the presence or absence of SIM in addition to the revised Vienna classification format for BE and associated neoplasia.¹⁵ **Figure 2** showed the randomization process.

Statistical analysis

The demographic variables (age, gender), length of BE, proportion of patients with dysplasia were compared in both groups. Unpaired t-test and Chi-squared test (or Fisher’s exact test when appropriate) were used to compare the groups for continuous and categorical data, respectively. The p value was considered significant if less than 0.05. SAS 9.0 (SAS Institute Inc., Cary, NC) was employed for all data analysis.

Results

A total of 18 (mean age: 72.6, standard error: 2.2 years, 16 men) patients were randomized in to Group A (n=11) and Group B

(n=7)(Fig. 2). A few confocal images of normal esophageal epithelium, BE, and HGD were shown in figures 3 and 4. The results were summarized in table 1.

Although the total number of individual mucosal biopsy

Figure 1. CEM was performed at every 1-2 cm of BE in all 4 quadrants. Presence of BE by CEM in any or more quadrant (for example, in quadrants 2, 3 in B) was sampled by targeted biopsy and these biopsies were used for comparative analysis. The quadrants negative for BE by CEM were sampled by random biopsy (for example, quadrants 1 and 4 in C) at the completion of all CEM-guided biopsy but were excluded from primary analysis.

Figure 2. Randomization process.

Figure 3. A) Normal columnar epithelium without goblet cells; B) BE: goblet cells (black arrows) and narrow lumen (white arrow).

Figure 4. A)HGD: loss of basal border (circle); B)Another example of HGD: disorganized architecture (circle) next to a nondysplastic Barrett's gland (arrows).

performed were 52% lower in CEM group (129 vs. 269), the individual diagnostic yields for detecting SIM, LGD, HGD, and the overall diagnostic yield were similar in both groups.

Discussion

Our results suggested that an endoscopist with minimal experience in CEM can effectively use this technology to reduce the number to biopsies without decreasing the overall diagnostic yield when compared to the random biopsy protocol in BE surveillance. Several reports indicated high accuracy and inter-observer agreement among inexperienced endoscopists with CEM for the detection of BE and Barrett-associated neoplasm.^{17,18} These studies suggested that, although extensive training and

experience will be required to tap the full potential of CEM, even with minimal experience, endoscopists can effectively use this technology to differentiate normal from neoplastic lesions.

The first prospective, randomized controlled trial showed that CEM was able to eliminate approximately 60% of biopsies in patients undergoing BE surveillance because no dysplasia was observed during CEM.¹⁸ Similar to our study, although Group A had 52% lower in number of biopsies compared to Group B, the overall diagnostic yield was similar between them. Also, Dunbar et al.¹⁸ reported, comparing to the random 4-quadrant biopsy protocol, the diagnostic yield of CEM-group was almost doubled for neoplasia (33% vs. 17%). However, our study did not demonstrate this difference likely because of a small number of patients. The above favorable results of using CEM in detecting

Table 1. Results of the study

Variable	Group A (n=11)	Group B (n=7)	p
Mean length of BE	5.1 (SE:1.3) cm	6.3 (SE: 0.9) cm	0.51
Number of biopsy	129	269	<0.001
Number of biopsy per patient	11.7	38.4	<0.001
SIM (%)	82(63.6)	160(59.5)	0.50
LGD (%)	15(11.6)	30(11.2)	NS
HGD (%)	13 (10.1)	31(11.5)	0.88
Normal gastric mucosa (%) (false positive)	19(14.7)	48(17.8)	0.53
Overall diagnostic yield (%)	110/129 (85.3)	221/266 (82.2)	0.53

BE/Barrett-associated dysplasia were supported by Kiesslich et al.¹³ which showed that CEM-guided biopsy can identify these lesions with high sensitivity (92.9-98.4%) and specificity 94.1-98.4%). However, these studies were performed by endoscopists with extensive experience in CEM. So the results may not be representative of endoscopists with lesser experience. Our results were likely more “realistic” because the endoscopist (AD) had only minimal training in CEM prior to the study. It should be noted that our primary endpoint of this study was not to determine the performance characteristics of CEM but rather to examine the diagnostic yields of BE and BE-associated dysplasia between the CEM-guided biopsy technique and the standard 4-quadrant protocol.

Recent evidence suggests that narrow band and autofluorescence imaging can be used effectively in conjunction with CEM as “red-flag” techniques in BE surveillance because these techniques are able to evaluate the entire area of BE and their main limitation is low specificity.¹⁷ As a result, these optical methods can screen the mucosa for suspicious sites. Then the sites can be further examined by CEM for “smart,” targeted biopsies.

There are several limitations of the study should be mentioned. First, the sample size was small and uneven (11:7 instead of 1:1 ratio) due to relocation of the primary investigator (AD). Second, only one endoscopist performed all the procedures. Hence, generalization of our results is limited. Third, a crossover design was deemed to be not feasible in our clinical practice as most patients in a BE surveillance program were not required to have repeat endoscopies at 4-6 weeks intervals. However, to minimize bias, the same endoscopist performed the procedures in all patients included in this study using the same endoscope, and also, the pathologist was blinded. Fourth, we did not incorporate methylene blue based chromoendoscopy or even narrow band imaging in this study because these are currently not considered part of standard clinical practice in most centers. Fifth, differentiation between low grade dysplasia (LGD) and HGD was not possible in this study because nuclei were not readily visible by fluorescein. Other contrast agent such as acriflavin that stains nuclei selectively can overcome this problem, but acriflavin is currently not available in the USA for use in human beings.

In conclusion, limited data suggested that endoscopists with minimal experience in CEM can effectively use this technology for “smart,” targeted biopsy to decrease the need for intense tissue sampling but without lowering the diagnostic yield in detecting dysplasia. Further controlled studies with larger sample size are needed.

References

1. Spechler SJ. Clinical practice. Barrett's Esophagus. *N Engl J Med* 2002; 346:836-42.
2. Shaheen NJ. Advances in Barrett's esophagus and esophageal adenocarcinoma. *Gastroenterology* 2005; 128:1554-66.
3. Sampliner RE; Practice Parameters Committee of the American College of Gastroenterology. Updated guidelines for the diagnosis, surveillance, and therapy of Barrett's esophagus. *Am J Gastroenterol* 2002; 97:1888-95.
4. Wo JM, Ray MB, Mayfield-Stokes S, Al-Sabbagh G, Gebrael F, Slone SP, et al. Comparison of methylene blue-directed biopsies and conventional biopsies in the detection of intestinal metaplasia and dysplasia in Barrett's esophagus: a preliminary study. *Gastrointest Endosc* 2001; 54:294-301.
5. Ragunath K, Krasner N, Raman VS, Haqqani MT, Cheung WY. A randomized, prospective cross-over trial comparing methylene blue-directed biopsy and conventional random biopsy for detecting intestinal metaplasia and dysplasia in Barrett's esophagus. *Endoscopy* 2003; 35:998-1003.
6. Kara MA, Peters FP, Rosmolen WD, Krishnadath KK, ten Kate FJ, Fockens P, et al. High-resolution endoscopy plus chromoendoscopy or narrow-band imaging in Barrett's esophagus: a prospective randomized crossover study. *Endoscopy* 2005; 37:929-36.
7. Hoffman A, Kiesslich R, Bender A, Neurath MF, Nafe B, Herrmann G, et al. Acetic acid-guided biopsies after magnifying endoscopy compared with random biopsies in the detection of Barrett's esophagus. *Gastrointest Endosc* 2006; 64:1-8.
8. Lim CH, Rotimi O, Dexter SP, Axon AT. Randomized crossover study that used methylene blue or random 4-quadrant biopsy for the diagnosis of dysplasia in Barrett's esophagus. *Gastrointest Endosc* 2006; 64:195-9.
9. Gossner L, Pech O, May A, Vieth M, Stolte M, Ell C. Comparison of methylene blue-directed biopsies and four-quadrant biopsies in the detection of high-grade intraepithelial neoplasia and early cancer in Barrett's oesophagus. *Dig Liver Dis* 2006; 38:724-9.
10. Curvers WL, van den Broek FJ, Reitsma JB, Dekker E, Bergman JJ. Systematic review of narrow-band imaging for the detection and differentiation of abnormalities in the esophagus and stomach (with video). *Gastrointest Endosc* 2009; 69:307-17.
11. Hoffman A, Goetz M, Vieth M, Galle PR, Neurath MF, Kiesslich R. Confocal laser endomicroscopy: technical status and current indications. *Endoscopy* 2006; 38:1275-83.
12. Kiesslich R, Goetz M, Lammersdorf K, Schneider C, Burg J, Stolte M, et al. Chromoscopy-guided endomicroscopy increases the diagnostic yield of intraepithelial neoplasia in ulcerative colitis. *Gastroenterology* 2007; 132:874-82.
13. Kiesslich R, Gossner L, Goetz M, Dahmann A, Vieth M, Stolte M, et al. In vivo histology of Barrett's esophagus and associated neoplasia by confocal laser endomicroscopy. *Clin Gastroenterol Hepatol* 2006; 4:979-87.
14. Dunbar KB, Okolo P 3rd, Montgomery E, Canto MI. Confocal laser endomicroscopy in Barrett's esophagus and endoscopically inapparent Barrett's neoplasia: a prospective, randomized, double-blind, controlled, crossover trial. *Gastrointest Endosc* 2009; 70:645-54.
15. Geboes K, Van Eyken P. The diagnosis of dysplasia and malignancy in Barrett's esophagus. *Histoathology* 2000; 37:99-107.
16. Nguyen VX, Nguyen CC, Leighton JA, Pasha SF, De Petris G, Gurudu S, et al. Accuracy and inter-observer agreement in identifying neoplastic gastrointestinal mucosal pathology by probe-based confocal laser endomicroscopy (pCLE). *Gastrointest Endosc* 2009; 69: AB364.
17. Wallace MB, Sharma P, Lightdale C, Wolfsen H, Coron E, Buchner A, et al. Preliminary accuracy and interobserver agreement for the detection of intraepithelial neoplasia in Barrett's esophagus with probe-based confocal laser endomicroscopy. *Gastrointest Endosc* 2010; 72:19-24.
18. Dunbar KB, Okolo P 3rd, Montgomery E, Canto MI. Confocal laser endomicroscopy in Barrett's esophagus and endoscopically inapparent Barrett's neoplasia: a prospective, randomized, double-blind, controlled, crossover trial. *Gastrointest Endosc* 2009; 70:645-54.