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In vivo endomicroscopy improves detection of Barrett's esophagus–related neoplasia: a multicenter international randomized controlled trial

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Abstract

BACKGROUND—Confocal laser endomicroscopy (CLE) enables in vivo microscopic imaging of the gastrointestinal tract mucosa. However, there are limited data on endoscope-based eCLE for imaging Barrett's esophagus (BE).

OBJECTIVE—To compare high definition white light endoscopy alone (HDWLE) with random biopsy (RB) and HDWLE+eCLE and targeted biopsy (TB) for diagnosis of BE neoplasia.

DESIGN—Multicenter randomized controlled trial.

SETTING—Academic medical centers.

PATIENTS—Adult BE patients undergoing routine surveillance or referred for early neoplasia.

INTERVENTIONS—Patients were randomized to HDWLE+RB (Group 1) or HDWLE+eCLE +TB (Group 2). Real time diagnoses and management plans were recorded after HDWLE in both

groups, and after eCLE in Group 2. Blinded expert pathologic diagnosis was the reference standard.

MAIN OUTCOME MEASUREMENTS—Diagnostic yield, performance characteristics, clinical impact.

RESULTS—192 BE patients were studied. HDWLE+eCLE+TB led to a lower number of mucosal biopsies, higher diagnostic yield for neoplasia (34% vs. 7%, $p<.0001$) compared to HDWLE+RB but with comparable accuracy. HDWLE+eCLE+TB tripled the diagnostic yield for neoplasia (22% vs. 6%, $p=.002$) and would have obviated the need for any biopsy in 65% of patients. The addition of eCLE to HDWLE increased the sensitivity for neoplasia detection to 96% from 40% ($p<.0001$) without significant reduction in specificity. *In vivo* CLE changed the treatment plan in 36% of patients.

LIMITATIONS—Tertiary referral centers and expert endoscopists limiting generalizability.

CONCLUSIONS—Real time eCLE and targeted biopsy after HDWLE can improve the diagnostic yield and accuracy for neoplasia and significantly impact *in vivo* decision-making by altering the diagnosis and guiding therapy.

INTRODUCTION

Barrett's esophagus (BE) is a precancerous change in the epithelial lining of the esophagus that is associated with the development of esophageal adenocarcinoma (ECA). The current best, albeit imperfect, marker of neoplastic progression is dysplasia detected in mucosal biopsies obtained at the time of endoscopy. High grade dysplasia (HGD) is associated with a high rate of progression to invasive ECA^{2,3}. Detection and pathologic confirmation of early BE neoplasia (HGD or ECA) is important because endoscopic therapy or surgery lead to successful prevention or cure of cancer.²⁻⁸ Unfortunately, neoplasia in BE may not be clearly evident even with careful endoscopic inspection. Hence, the current standard of care includes routine endoscopic surveillance with high quality white light endoscopy (WLE) a standardized biopsy protocol involving systematic 4-quadrant biopsies every 1–2 cm of BE and targeted biopsies of any mucosal irregularities². A large number of biopsies may be required resulting in high cost and increased time, typically resulting in a low (1–10%) diagnostic yield for neoplasia^{9,10} and low adherence to practice guidelines¹¹.

Confocal laser endomicroscopy (CLE) is a relatively new endoscopic imaging technique that combines endoscopy and microscopic imaging of the gastrointestinal mucosa¹². Kiesslich *et al* first described the use of a fluorescein-aided endoscope-based CLE (eCLE) system for the diagnosis of BE and associated neoplasia using the Mainz confocal Barrett's classification system¹³. In a single center randomized crossover study, Dunbar *et al* demonstrated a greater diagnostic yield for the detection of BE neoplasia with *in vivo* eCLE¹⁴. Here, we report the results of our study that compared the diagnostic yield and accuracy of high definition white light endoscopy (HDWLE) and random biopsy with that of HDWLE plus real-time eCLE imaging and targeted tissue sampling of BE and determined the impact of *in vivo* eCLE on real time clinical decision-making in BE patients.

METHODS

Study Design and Patients

We performed a prospective, single blinded, multicenter randomized controlled trial comparing high definition white light endoscopy (HDWLE group) with HDWLE followed by endomicroscopy (HDWLE+eCLE group). We enrolled consecutive adult patients undergoing outpatient endoscopy for either routine surveillance of BE (surveillance group) or suspected or biopsy-proven unlocalized BE-associated HGD and/or early intramucosal ECA (neoplasia group) referred for confirmation of diagnosis and/or endoscopic therapy (Study Schema Figure 1). After informed consent was obtained, eligible patients were randomly assigned at each site to HDWLE alone or HDWLE+ECLE (1:1 allocation) using a centralized computer-generated permuted block randomization stratified by study site and by procedure indication (surveillance or suspected neoplasia) based on review of the endoscopic and pathology records. We excluded patients with BE < 1cm and > 10 cm, known ECA, advanced BE lesions 2 cm or more in size, Paris classification of 0-Ip (polypoid), 0-Is (protruding sessile), 0-IIa (flat elevated), or 0-IIb (flat), 4) any Paris 0-IIc (superficial shallow depressed) or 0-III (excavated) lesion, esophageal strictures or altered anatomy preventing passage of the endomicroscope, allergy to fluorescein or history of any severe anaphylactic reaction, and active gastrointestinal bleeding, coagulopathy or chronic anticoagulation, pregnancy, and contraindications to endoscopy due to medical instability. All patients gave written informed consent. This study was approved by each participating institution's institutional review board (IRB) or ethics committee. This trial was registered on Clinicaltrials.gov (registration number NCT00487695).

All 5 study sites were located at academic medical centers. At each site, one endoscopist performed all study procedures. All participating endoscopists had performed more than 50 eCLE procedures, were trained in eCLE image interpretation using the Mainz Confocal Barrett's Classification¹³, and had passed an image interpretation test of 30 BE eCLE images with a score of at least 80% of images interpreted correctly.

Study procedures

The study procedures are summarized in Figure 1. All study patients had careful examination of the upper GI tract with a HDWLE upper endoscope (EG2990i, EPKi HD processor, Pentax Medical Corporation, Montvale, New Jersey). The esophageal landmarks, length and detailed description of the BE, presence of esophagitis¹⁵, and the presence, location, and type (Paris classification¹⁶) of any lesions were recorded. The results of prior endoscopy and pathology were available to the study endoscopists, who were also served as the patient's consultant gastroenterologist. Annotation of HDWLE and eCLE images and completion of detailed esophageal maps and pathology forms were performed immediately after the procedure. Biopsies from lesions and random biopsies were placed in separate containers.

HDWLE Group

After examination with HDWLE, endoscopic diagnoses were recorded in real time based on the appearance of the BE mucosa. The management plan for lesions was made at the

discretion of the endoscopist and recorded, including the option to take a biopsy, perform an endoscopic mucosal resection (EMR), tattoo the lesion, or perform no intervention. Then, biopsies from suspicious lesions were obtained, or EMR performed. Four-quadrant mucosal biopsies were obtained every 2 cm from the entire length of the BE for surveillance patients, or every 1 cm in patients with BE and suspected neoplasia ("RB protocol").

HDWLE+eCLE Group

HDWLE was performed as detailed above. Then, immediately after HDWLE, fluorescein-aided eCLE^{13,14} imaging was performed with the endomicroscope (3870CIK Pentax, Tokyo, Japan) on visible mucosal lesions as well as on 4 quadrants every 2 cm from the entire BE length in surveillance patients and every 1 cm in patients referred for suspected neoplasia (supplementary video demonstrates eCLE). The eCLE diagnoses using the Mainz confocal Barrett's classification¹³ for all lesions and flat BE were documented for each imaging site in real time. The management plan based on the CLE findings was recorded for each imaging site.

Targeted mucosal biopsies (TB) were obtained or EMR performed only if there was eCLE evidence of neoplasia ("CLE-TB" samples). The targeted mucosal biopsy sampling of flat BE sites with neoplasia was made possible by careful release of endoscopic suction and placement of the biopsy forceps at the site of the endomicroscope lens located within 5 mm to the left of the suction polyp made by the endomicroscope.

In order to calculate performance characteristics for eCLE, 30% of eCLE-imaging sites of flat BE mucosa were biopsied (first and every third optical site thereafter) regardless of the eCLE diagnosis ("forced" biopsies). To allow subsequent matching of eCLE diagnoses with pathologic diagnoses, the mucosal samples that were designated as "forced" or "CLE-TB" biopsies were inked and placed in numbered cassettes in separate bottles. Biopsies from different quadrants at the same BE level were separated within the pathology cassette. After eCLE imaging and eCLE-guided TB, the RB protocol and EMR were performed. All lesions had to be either biopsied or be treated with EMR, according to the discretion of the endoscopists. These samples were obtained before the 4-quadrant random biopsy protocol.

Pathology

Formalin-fixed mucosal biopsy specimens were processed routinely at each study site and were blindly interpreted by 2 expert gastrointestinal pathologists (E.M, AM), who graded the severity of neoplasia in each specimen¹⁷ without knowledge of the prior pathology results, endoscopic findings, or group assignment. When there was a discordant reading, a third expert GI pathologist (J.G.) was consulted and a consensus reached. A final pathologic diagnosis was recorded for each individual biopsy and for each patient based on the worst grade of neoplasia identified.

Statistical Analysis

The final pathologic diagnosis served as the reference gold standard for calculation of study endpoints. The study pathologists were not aware of the endoscopy results. The primary outcome of this study was the diagnostic yield, calculated as the number of mucosal biopsy

samples with HGD or cancer by final pathologic diagnosis divided by the total number of mucosal biopsies obtained. The secondary endpoints included the total and median number of mucosal biopsies obtained by indication and the performance characteristics of HDWLE versus HDWLE+eCLE using a *per biopsy* and *per patient* analysis. The clinical impact of *in vivo* eCLE was also assessed by determining the proportion of patients with a correct change in endoscopic diagnosis and associated change in management plan before and after eCLE.

The study was designed to enroll 188 patients who were randomly assigned 1:1 to HDWLE alone or HDWLE+eCLE, for a statistical power of 80% with a two-sided significance level of 0.05 to detect a doubling of the primary study endpoint (diagnostic yield) from 18% for HDWLE to 36% for HDWLE+eCLE based on data from a prior study¹⁴. Randomization was stratified by study site and procedure indication (surveillance vs. suspected neoplasia). To allow for possible patient dropout, we planned to enroll approximately 100 patients per group.

For the HDWLE group, the denominator for the primary endpoint was the total number of biopsies obtained from lesions and from flat BE (random, 4-quadrant). For the HDWLE + eCLE group, the total number of mucosal biopsies used as the denominator for the primary endpoint was equal to the total number of CLE-targeted mucosal biopsies obtained. The “forced” biopsies diagnosed as non-neoplastic by eCLE with an associated plan “not to biopsy” and random 4-quadrant biopsies obtained after eCLE imaging and intervention as part of standard care were not included in the denominator of the primary endpoint.

To allow comparison of accuracy of HDWLE and HDWLE+CLE for detection of neoplasia using a *per biopsy* analysis, we calculated that a minimum sample size of 200 paired biopsy-image samples would be needed. Hence, the study protocol required a 30% “forced” sampling of flat BE sites in the HDLWE+eCLE group. *Per biopsy* and *per patient* analyses were performed separately for the primary and secondary outcomes. All statistical tests were two-sided and considered statistically significant at $p < 0.05$. The analyses were carried out using SAS (version 9.2, SAS Institute, Cary, NC) and Stata SE statistical software (version 8, StataCorp, College Station, TX).

RESULTS

A total of 212 patients were consented and enrolled from February 2010 to December 2011. The trial was ended due to completion of accrual. Twenty patients were excluded (11 from HDWLE only and 9 from HDWLE+eCLE group) due to absence of BE in biopsies or BE length outside specified range 1–10 cm ($n=9$), patient drop-out ($n=8$), esophageal stricture ($n=1$), aborted procedure due to incomplete sedation ($n=1$), and mass with advanced cancer at study visit ($n=1$)(Figure 1). Forty-six (24%) of the 192 patients had suspected or confirmed neoplasia at study entry. Table 1 summarizes the enrolled patient characteristics by study group. There were no significant differences in the age, gender, race, BE length, neoplasia prevalence, esophagitis, or presence of an esophageal lesion. The final diagnoses were determined by histopathologic interpretation of 1,371 esophageal tissues obtained by biopsy or EMR.

eCLE was performed successfully and safely in all patients. There were no adverse reactions to fluorescein administration. One patient in the HDWLE+eCLE group developed gastrointestinal bleeding immediately after EMR performed on a lesion with HGD. The patient was observed in the hospital for one day and did not require a blood transfusion.

Diagnostic Yield

Using a *per biopsy* analysis, the addition of eCLE to HDWLE endoscopy decreased the number of mucosal biopsies obtained during endoscopy and led to a 4.8-fold reduction in the total number of biopsies obtained (Figure 2A) and an overall decrease in the median number of biopsies obtained *per patient* (2 for HDWLE+eCLE versus 4 for HDWLE alone, $p < .0001$, Wilcoxon rank sum test) (Figure 2B) by allowing TB of abnormal BE mucosa. The reduction in median biopsy number was from 6 to 3 in the neoplasia group ($p = 0.0001$) and 3 to 1 in the surveillance group ($p < 0.0001$) comparing HDWLE and HDWLE+eCLE, respectively. With comparable sampling of neoplastic BE (41 in HDWLE+eCLE versus 40 in HDWLE-alone) and fewer biopsies of non-neoplastic BE, there was a higher diagnostic yield for neoplasia obtained using HDWLE+eCLE+TB approach (yield 40/119 or 34%) compared to HDWLE+RB (yield 41/580 or 7%, $p < 0.0001$) (Figure 3A).. The difference in diagnostic yield was seen mainly in the neoplasia group (45% with eCLE versus 9% for HDWLE alone, $p = 0.004$). The diagnostic yield was higher in the surveillance group (12% versus 5%) but this did not reach statistical significance.

Using a *per patient* analysis, the addition of eCLE to HDWLE led to a 2.7-fold higher diagnostic yield for neoplasia (6/98 or 22% vs. 21/94 or 6%, $p = .002$) (Figure 3B). This difference between HDWLE+eCLE+TB and HDWLE+RB was found primarily in patients with neoplasia (12/24 or 75% vs. 5/23 or 22%, $p = .0004$) (Figure 3B). There were 7 patients in the HDWLE+eCLE group with a normal HDWLE exam who had neoplasia detected only by eCLE in endoscopically normal-appearing BE. Images from one of these patients are shown in Figure 4.

Performance Characteristics

The prevalence of suspicious esophageal mucosal lesions visible by HDWLE imaging was low and comparable by group (Table 1). When analyzed on a *per biopsy* basis, the sensitivity of the HDWLE+eCLE+TB for the *in vivo* detection of BE neoplasia was higher than HDWLE+RB (86% versus 10%, respectively, $p < 0.0001$) with comparable overall accuracy (92% versus 93%, $p = 0.45$), despite lower specificity (Table 2). The decreased specificity of HDWLE+eCLE compared to HDWLE-alone was due to eCLE overdiagnosis of nondysplastic BE and gastric epithelium in 6 patients as focal HGD in BE. On the other hand, the NPV significantly increased when eCLE was added to HDWLE, increasing from 94% to 98% ($p = 0.006$). Using a *per patient* analysis, the addition of eCLE imaging to HDWLE increased the sensitivity of neoplasia detection from 40% to 95% ($p < 0.0001$) and increased the NPV from 90% to 98% ($p = 0.005$), with comparable accuracy (Table 2). All patients with any neoplasia were detected by HDWLE+eCLE, regardless of the presence or absence of any suspicious lesion. The exception was one patient with focal HGD in a single random biopsy obtained from endoscopically-normal BE after completion of eCLE imaging.

When the eCLE experience of the endoscopist was considered, the sensitivity, specificity, NPV, and accuracy for BE neoplasia detection using a *per biopsy* analysis were higher in endoscopists who had performed >75 esophageal eCLE procedures prior to the trial (Table 3).

Clinical Impact

We assessed the clinical impact of adding eCLE to HDWLE by determining the number of cases where eCLE changed management (i.e. changed the impression of a lesion from neoplastic to non-neoplastic, or changed the impression of flat BE mucosa from non-neoplastic to neoplastic). Of the 94 patients in the HDWLE+eCLE group, 32 (34%) had a correct change in dysplasia grade after eCLE when compared to initial HDWLE endoscopic findings. Of these 32 patients, 6 had lesions and 26 did not. In the latter group of patients with no lesions, 5 (19.2%) had endoscopically-inapparent HGD in flat BE mucosa only detected by eCLE. In the remaining 21 of the 26 (81%) patients with flat BE and no neoplasia, eCLE changed the plan from “biopsy” to “no biopsy”. In the 6 BE patients with indeterminate lesions by HDWLE, eCLE suggested neoplasia in two, leading to a change of the plan from “biopsy” to “perform EMR”. The remaining four patients had no eCLE findings supporting dysplasia and no EMR or biopsy was proposed. Standard-of-care biopsy of these lesions showed no neoplasia.

The other 62 of 94 (65%) patients in the HDWLE+eCLE group had concordant HDWLE and eCLE diagnoses, and thus, had no change in management plan.

DISCUSSION

HDWLE, careful inspection of BE and visible lesions, and systematic 4-quadrant random biopsies throughout the segment of BE are currently recommended for detection of neoplasia^{3,18,19}. Our trial demonstrates that HDWLE+eCLE+TB significantly enhances the diagnostic yield and accuracy of endoscopic surveillance. Indeed, this approach led to an 80% reduction in mucosal biopsies obtained by enabling more selective tissue sampling. Moreover, over two thirds of patients who underwent HDWLE+eCLE would have needed no mucosal biopsies, as eCLE did not show any neoplasia. With comparable detection of neoplastic areas, HDWLE+eCLE+TB provided a 5-fold higher diagnostic yield for detection of neoplasia compared to HDWLE+RB. Our study results are comparable to those of randomized crossover trial comparing standard WLE and standard WLE+eCLE¹⁴, which showed improved diagnostic yield and avoidance of unnecessary biopsies in the eCLE group.

Stratification of diagnostic yield by patient type showed that there was comparable detection of neoplasia by HDWLE and HDWLE+eCLE in the surveillance group due to the very low prevalence of HGD and ECA. Hence, eCLE might be best used selectively in patients with increased risk for neoplasia (patients with indeterminate lesions, those with unlocalized dysplasia in prior random biopsies). Alternatively, the absence of neoplasia by both HDWLE and eCLE in a low risk population could potentially eliminate the need for any mucosal biopsy. These approaches to BE neoplasia detection need prospective study and validation.

Overall, the addition of eCLE to HDWLE improved the *per patient* sensitivity and NPV to 95% and 98%, respectively, at the expense of a slight decrease in specificity and PPV to 92% and 77%. None of the patients with an upgraded diagnosis of HGD by eCLE had inappropriate therapy. One patient with focal HGD would have been missed by eCLE with a targeted biopsy approach if no random biopsies were obtained following our study protocol. However, the natural history of BE HGD can vary, with inconsistent reproducibility of focal HGD in numerous subsequent surveillance biopsies²⁰. Importantly, no cancers were missed with an HDWLE+eCLE+TB. Our study results are consistent with those of other studies of eCLE reporting high accuracy for diagnosis of BE neoplasia^{13,21}. A non-randomized single center study of eCLE reported a high accuracy rate of 98% for BE neoplasia²¹.

Guidelines developed by the American Society of Gastrointestinal Endoscopy (ASGE) suggest that a threshold of *per patient* sensitivity of 90% or greater, specificity of 80% or better, and NPV of 98% or greater, are needed to ensure optimal detection of neoplasia as well as eliminate the need for RB during endoscopic surveillance of BE²². Our study results show that HDWLE+eCLE surpasses the PIVI threshold, with a *per patient* sensitivity of 95%, specificity of 92% and NPV of 98%, supporting the use of HDWLE+eCLE+TB as an approach to the BE imaging.

Our study also showed that the *per patient* sensitivity, specificity, and accuracy for eCLE for BE neoplasia diagnosis was comparably good, regardless of endoscopist experience with CLE. However, using a *per biopsy* analysis, more experienced endomicroscopists had better sensitivity, specificity, and accuracy. CLE is not a procedure that is routinely taught in GI fellowship training programs and postgraduate training in CLE is also limited. CLE has not yet become widely adopted in the United States due to a number of factors, including equipment cost and availability, access to training, and lack of a CPT billing code. The accumulating scientific evidence supporting the use of CLE since it was first described in 2004²³, increased clinical experience in CLE, and the recent generation of optical endomicroscopy 2013 CPT codes may change physician interest and utilization of CLE in the United States. However, the performance characteristics, diagnostic yield, clinical utility, and cost-effectiveness of CLE for BE neoplasia in community practice are unknown and worthy of further study.

There are retrospective reports^{24,25,26} of *in vivo* CLE imaging for guiding therapy in BE. We demonstrate for the first time in a prospective study the significant impact of real time CLE on clinical decision-making. *In vivo* eCLE detection of neoplasia was associated with a change in the diagnosis and management of 34% of all BE patients studied. Traditionally, mucosal biopsies of BE are obtained during endoscopy and histopathology results are available in 3–7 days. This may result in the need for a second procedure visit for endoscopic therapy if dysplasia is found on biopsy. In our study, the addition of eCLE to HDWLE led to localization of microscopic neoplastic changes in BE, enabling initiation of appropriate endoscopic therapy prior to the availability of histopathology results in patients with indeterminate lesions and flat neoplasia. Conversely, the high NPV of eCLE coupled with a negative prior HDWLE examination allowed accurate differentiation of patients without neoplasia and a correct intra-procedural decision not to take mucosal biopsies.

There are technical differences between pCLE and eCLE, with each system providing different advantages. The eCLE system has a higher image resolution and larger field of view (more glands visible in each image). In addition, the application of suction through the endoscope may provide stability that may facilitate image acquisition with less motion artifact. The potential advantages of pCLE include the technical ease of placing a through-the-scope CLE probe on an area of interest and the possibility of adding a digital contrast enhancement technique, such as narrow band imaging (NBI). A recent study compared the diagnostic performance of pCLE in 101 patients²⁷. All patients had HDWLE with NBI with or without pCLE. The addition of pCLE to HDWLE+NBI increased sensitivity (68.3% and 34.2%, respectively) but lowered specificity (87.8% versus 92.7%, respectively). Without NBI, pCLE may have suboptimal sensitivity (12%) and PPV (18%) when used as a technique to potentially replace RB²⁸. A recent non-randomized study reported a lower sensitivity of eCLE (75.7% without considering prior NBI) compared to HDWLE+NBI (89%)²⁹. However, this 46 patient pilot study underpowered and the sensitivity of eCLE was low compared to other studies^{13,30}. No back-to-back comparison of eCLE and pCLE or HDWLE + NBI versus HDWLE + eCLE for accuracy or diagnostic yield for detection of BE neoplasia has yet been made. In the setting of other advanced imaging methods, CLE has not been shown to be superior.

Our study has several strengths, including the relatively large sample size, independent group design, stratified randomization, standardization of CLE criteria for BE neoplasia diagnosis, and assessment of endoscopist experience level. We also used blinded expert central GI pathology interpretation of all tissue samples, performed detailed mapping and co-localization of BE imaging sites, and tracked the sequential prediction of *in vivo* diagnosis and recording of treatment plan at each step.

Our study has several limitations. First, we included only academic centers with highly experienced endoscopists. Our results may not be generalizable to community-based physicians, less experienced practitioners and non-academic practices. Second, we used the eCLE system and our findings may not be generalizable to pCLE systems. Third, the endoscopists were not blinded to prior endoscopy and pathology results because these were necessary for standard medical care and *in vivo* decision-making regarding biopsy or EMR. However, pathologists provided blinded final diagnoses. Fourth, the study's lack of digital contrast enhancement techniques might also be considered a limitation. A single center crossover trial³¹ and a systematic review of 5 studies³¹ support the use of NBI for detection of neoplastic BE. A recent study involving expert endoscopists reported improved diagnostic yield for neoplasia with NBI-TB (30%) than WLE+RB (21%)³². While this trial demonstrated fewer biopsies were needed with NBI to detect neoplasia using a *per biopsy* analysis, there was no significant improvement using a *per patient* analysis³². We chose not use additional digital contrast enhancement techniques, such as NBI (Olympus) or IScan (Pentax), in order to determine the specific contribution of eCLE to WLE and minimize confounding. NBI-capable endoscopes are also not uniformly used by all gastroenterologists and no red-flag imaging modality has been shown to be more accurate than HDWLE. U.S. professional societies have not formally recommended routine use of NBI for BE surveillance^{33,18}. Finally, we studied population a partially enriched with BE neoplasia.

However, our study used centralized stratified randomization to balance allocation of BE patients with neoplasia and stratified our primary and secondary endpoints by type of patient.

In summary, the addition of *in vivo* imaging with eCLE to HDWLE is associated with improved targeting of neoplasia, decrease in unnecessary mucosal biopsies, and significant change in diagnosis and management plan in BE patients. The approach of real time CLE diagnosis and imaging-guided therapy represents a potential paradigm shift in BE surveillance. Research studies are needed to address training in CLE, comparative effectiveness studies of advanced endoscopic imaging techniques, the role of imaging-guided therapy, and advances in CLE devices and contrast agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Hur C, Miller M, Kong CY, et al. Trends in esophageal adenocarcinoma incidence and mortality. *Cancer*. 2013; 119:1149–58. [PubMed: 23303625]
2. American Gastroenterological A. Spechler SJ, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American Gastroenterological Association medical position statement on the management of Barrett's esophagus. *Gastroenterology*. 2011; 140:1084–91. [PubMed: 21376940]
3. Bennett C, Vakil N, Bergman J, et al. Consensus statements for management of Barrett's dysplasia and early-stage esophageal adenocarcinoma, based on a Delphi process. *Gastroenterology*. 2012; 143:336–46. [PubMed: 22537613]
4. Shaheen NJ, Sharma P, Overholt BF, et al. Radiofrequency ablation in Barrett's esophagus with dysplasia. *N Engl J Med*. 2009; 360:2277–88. [PubMed: 19474425]
5. Shaheen NJ, Greenwald BD, Peery AF, et al. Safety and efficacy of endoscopic spray cryotherapy for Barrett's esophagus with high-grade dysplasia. *Gastrointest Endosc*. 2010; 71:680–5. [PubMed: 20363409]
6. Ell C, May A, Pech O, et al. Curative endoscopic resection of early esophageal adenocarcinomas (Barrett's cancer). *Gastrointest Endosc*. 2007; 65:3–10. [PubMed: 17185072]
7. Prasad GA, Wang KK, Buttar NS, et al. Long-term survival following endoscopic and surgical treatment of high-grade dysplasia in Barrett's esophagus. *Gastroenterology*. 2007; 132:1226–33. [PubMed: 17408660]
8. Prasad GA, Wu TT, Wigle DA, et al. Endoscopic and surgical treatment of mucosal (T1a) esophageal adenocarcinoma in Barrett's esophagus. *Gastroenterology*. 2009; 137:815–23. [PubMed: 19524578]
9. Borovicka J, Fischer J, Neuweiler J, et al. Autofluorescence endoscopy in surveillance of Barrett's esophagus: a multicenter randomized trial on diagnostic efficacy. *Endoscopy*. 2006; 38:867–72. [PubMed: 16981102]
10. Ngamruengphong S, Sharma VK, Das A. Diagnostic yield of methylene blue chromoendoscopy for detecting specialized intestinal metaplasia and dysplasia in Barrett's esophagus: a meta-analysis. *Gastrointest Endosc*. 2009; 69:1021–8. [PubMed: 19215918]

11. Abrams JA, Kapel RC, Lindberg GM, et al. Adherence to biopsy guidelines for Barrett's esophagus surveillance in the community setting in the United States. *Clin Gastroenterol Hepatol*. 2009; 7:736–42. quiz 10. [PubMed: 19268726]
12. Polglase AL, McLaren WJ, Skinner SA, Kiesslich R, Neurath MF, Delaney PM. A fluorescence confocal endomicroscope for in vivo microscopy of the upper- and the lower-GI tract. *Gastrointest Endosc*. 2005; 62:686–95. [PubMed: 16246680]
13. Kiesslich R, Gossner L, Goetz M, et al. In vivo histology of Barrett's esophagus and associated neoplasia by confocal laser endomicroscopy. *Clin Gastroenterol Hepatol*. 2006; 4:979–87. [PubMed: 16843068]
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. [PubMed: 19559419]
15. Lundell LR, Dent J, Bennett JR, et al. Endoscopic assessment of oesophagitis: clinical and functional correlates and further validation of the Los Angeles classification. *Gut*. 1999; 45:172–80. [PubMed: 10403727]
16. Endoscopic Classification Review G. Update on the paris classification of superficial neoplastic lesions in the digestive tract. *Endoscopy*. 2005; 37:570–8. [PubMed: 15933932]
17. Dixon MF. Gastrointestinal epithelial neoplasia: Vienna revisited. *Gut*. 2002; 51:130–1. [PubMed: 12077106]
18. Committee ASoP. Evans JA, Early DS, et al. The role of endoscopy in Barrett's esophagus and other premalignant conditions of the esophagus. *Gastrointestinal endoscopy*. 2012; 76:1087–94. [PubMed: 23164510]
19. Spechler SJ, Sharma P, Souza RF, Inadomi JM, Shaheen NJ, Association AG. American Gastroenterological Association technical review on the management of Barrett's esophagus. *Gastroenterology*. 2011; 140:e18–52. quiz e13. [PubMed: 21376939]
20. Appelman HD, Umar A, Orlando RC, et al. Barrett's esophagus: natural history. *Ann N Y Acad Sci*. 2011; 1232:292–308. [PubMed: 21950819]
21. Trovato C, Sonzogni A, Ravizza D, et al. Confocal laser endomicroscopy for in vivo diagnosis of Barrett's oesophagus and associated neoplasia: A pilot study conducted in a single Italian centre. *Dig Liver Dis*. 2013
22. Sharma P, Savides TJ, Canto MI, et al. The American Society for Gastrointestinal Endoscopy PIVI (Preservation and Incorporation of Valuable Endoscopic Innovations) on imaging in Barrett's Esophagus. *Gastrointest Endosc*. 2012; 76:252–4. [PubMed: 22817781]
23. Kiesslich R, Burg J, Vieth M, et al. Confocal laser endoscopy for diagnosing intraepithelial neoplasias and colorectal cancer in vivo. *Gastroenterology*. 2004; 127:706–13. [PubMed: 15362025]
24. Johnson EA, De Lee R, Agni R, Pfau P, Reichelderfer M, Gopal DV. Probe-Based Confocal Laser Endomicroscopy to Guide Real-Time Endoscopic Therapy in Barrett's Esophagus with Dysplasia. *Case Rep Gastroenterol*. 2012; 6:285–92. [PubMed: 22754488]
25. Konda VJ, Chennat JS, Hart J, Waxman I. Confocal laser endomicroscopy: potential in the management of Barrett's esophagus. *Dis Esophagus*. 2010; 23:E21–31. [PubMed: 20626448]
26. Leung KK, Maru D, Abraham S, Hofstetter WL, Mehran R, Anandasabapathy S. Optical EMR: confocal endomicroscopy-targeted EMR of focal high-grade dysplasia in Barrett's esophagus. *Gastrointest Endosc*. 2009; 69:170–2. [PubMed: 18582880]
27. Sharma P, Meining AR, Coron E, et al. Real-time increased detection of neoplastic tissue in Barrett's esophagus with probe-based confocal laser endomicroscopy: final results of an international multicenter, prospective, randomized, controlled trial. *Gastrointest Endosc*. 2011; 74:465–72. [PubMed: 21741642]
28. Bajbouj M, Vieth M, Rosch T, et al. Probe-based confocal laser endomicroscopy compared with standard four-quadrant biopsy for evaluation of neoplasia in Barrett's esophagus. *Endoscopy*. 2010; 42:435–40. [PubMed: 20506064]
29. Jayasekera C, Taylor AC, Desmond PV, Macrae F, Williams R. Added value of narrow band imaging and confocal laser endomicroscopy in detecting Barrett's esophagus neoplasia. *Endoscopy*. 2012; 44:1089–95. [PubMed: 23188660]

30. Trovato C, Sonzogni A, Ravizza D, et al. Confocal laser endomicroscopy for in vivo diagnosis of Barrett's oesophagus and associated neoplasia: a pilot study conducted in a single Italian centre. *Dig Liver Dis.* 2013; 45:396–402. [PubMed: 23352281]
31. Wolfsen HC, Crook JE, Krishna M, et al. Prospective, controlled tandem endoscopy study of narrow band imaging for dysplasia detection in Barrett's Esophagus. *Gastroenterology.* 2008; 135:24–31. [PubMed: 18442484]
32. Sharma P, Hawes RH, Bansal A, et al. Standard endoscopy with random biopsies versus narrow band imaging targeted biopsies in Barrett's oesophagus: a prospective, international, randomised controlled trial. *Gut.* 2013; 62:15–21. [PubMed: 22315471]
33. Spechler SJ, Sharma P, Souza RF, Inadomi JM, Shaheen NJ, Association AG. American Gastroenterological Association medical position statement on the management of Barrett's esophagus. *Gastroenterology.* 2011; 140:1084–91. [PubMed: 21376940]

Take Home Message

The addition of confocal laser endomicroscopy to high definition white light endoscopy enables improved real-time endoscopic diagnosis of BE neoplasia, targeted biopsy of abnormal mucosa to reduce unnecessary mucosal biopsies, and potentially reduce costs. It may also positively influence patient care by changing the plan for immediate endoscopic management.

Figure 1.
Study Flow Diagram

Figure 2.

A: Comparison of the total number of mucosal biopsies obtained per patient from Barrett's esophagus patients (HDWLE alone versus HDWLE + CLE). HDWLE = high definition white light endoscopy, CLE= confocal laser endomicroscopy. P values shown for comparisons between groups were obtained using Student's t test.

B: Boxplots comparing the average number (with interquartile ranges) of mucosal biopsies obtained per patient from Barrett's esophagus patients (HDWLE alone versus HDWLE + CLE). P values obtained for all comparisons were significant ($p < 0.0001$ for all patients, $p = 0.0001$ for patients with neoplasia, $p < 0.0001$ for patients undergoing routine surveillance).

Figure 3.

A: Comparison of the diagnostic yield for neoplasia using a *per biopsy* analysis for HDWLE with random biopsy approach (n=580 specimens) versus HDWLE + CLE and targeted biopsy approach (110 specimens). HDWLE + RB = high definition white light endoscopy with random biopsy; HDWLE+CLE+TB= high definition white light endoscopy with confocal laser endomicroscopy and targeted biopsy. P values for comparisons between groups were obtained using Fisher's exact test.

B: Comparison of the diagnostic yield for neoplasia using a *per patient* analysis for HDWLE with random biopsy approach (n=580 specimens) versus HDWLE + CLE and targeted biopsy approach (110 specimens). HDWLE + RB = high definition white light endoscopy with random biopsy; HDWLE+CLE+TB= high definition white light endoscopy with confocal laser endomicroscopy and targeted biopsy. P values for comparisons between groups were obtained using Fisher's exact test.

Figure 4.

High resolution white light endoscopy (HDWLE) images of Barrett's esophagus with high grade dysplasia not detected by 2 prior endoscopies showing no suspicious neoplastic lesion (A and B). Multiple optical biopsy sites in BE imaged with confocal laser endomicroscopy (CLE) are shown with numbers (B). CLE images from sites 6 and 7 in image B after showing intestinal metaplastic glands with loss of normal mucosal pattern, darkening of epithelial cells, lack of goblet cells, glandular distortion, and a cribriform pattern, consistent with high grade dysplasia (C and D). Note the whitish squamous islands seen in the endoscopic and CLE images (arrows), with corresponding CLE image C suggesting partially buried dysplastic BE glands. *In vivo* diagnosis of neoplasia led to a decision to perform endoscopic mucosal resection (EMR) at the baseline procedure. Pathological examination of the EMR specimen confirmed the diagnosis of high grade dysplasia with partial pseudoregression.

Table 1

Patient and Specimen Characteristics (N=192)

	HD n=98	HD + CLE n=94
Routine surveillance, n(%)	75 (76.5%)	70 (74.5%)
Suspected neoplasia, n (%)	23 (23.5%)	24 (25.5%)
Median age, (range)	62 (26–79)	62 (32–82)
Male, n (%)	73 (74%)	70 (74%)
White, n (%)	97 (98%)	89 (95%)
Median BE length in cm (Prague M), range	2 (1–11)	3 (1–14)
BE lesion present, n (%)	21 (21.4%)	16 (17.1%)
Esophagitis present, n (%)	12 (12.2%)	13 (13.8%)
Baseline pathologic diagnosis (highest grade in any biopsy)		
BE neoplasia *, n (%)	25 (25.5%)	19 (20.2%)
BE no dysplasia/indefinite/low grade dysplasia, n(%)	73 (74.5%)	75 (79.8%)
Final pathologic diagnosis in patients (highest grade in any biopsy)		
BEneoplasia *, n (%)	14 (14.3%)	20 (21.2%)
BE no dysplasia/indefinite/low grade dysplasia, n (%)	83 (84.7%)	74 (79.8%)
Patients treated with endoscopic mucosal resection, n (%)	16 (16.3%)	20 (21.3%)
Final pathologic diagnosis in EMR specimens (n=28)	n=6	n=22
BE neoplasia *, n (%)	4(66.7%)	15(68.2%)
BE no dysplasia/indefinite/low grade dysplasia, n (%)	2(33.3%)	7(31.8%)

* neoplasia = high grade dysplasia or adenocarcinoma as highest grade in pathology sample

All differences between groups were not significant.

Table 2

Performance Characteristics for Diagnosis of Barrett's Esophagus-Related Neoplasia HDWLE versus HDWLE+CLE, by Group

	Per Biopsy Analysis n=978 biopsies		Per Patient Analysis n=192 patients	
	HDWLE n=580	HDWLE + CLE n=398	HDWLE n=98	HDWLE + CLE n=94
Sensitivity	10%	86%^a	40%	95%^a
Specificity	99%	93%^a	98%	92%
Positive Predictive Value	67%	65%	75%	77%
Negative Predictive Value	94%	98%^b	90%	98%^c
Accuracy	93%	92%	89%	93%

^a: p<0.0001,

^b: p=0.006.,

^c: p=0.005 for comparisons between groups using Fisher's exact test.

All other differences between groups were not statistically significant.

Table 3

Performance Characteristics of Endomicroscopy by Endomicroscopist Experience

	Per Biopsy Analysis n=978		Per Patient Analysis n=192	
	More Experienced Endomicroscopist [*]	Less Experienced Endomicroscopist ^{**}	More Experienced Endomicroscopist [*]	Less Experienced Endomicroscopist ^{**}
Sensitivity	97% ^a	67%	100%	100%
Specificity	92% ^b	88%	95%	71%
PPV	70%	56%	84%	71%
NPV	99% ^c	93%	100%	100%
Accuracy	96% ^c	84%	96%	83%

^{*} "More experienced" defined as > 75 prior esophageal CLE procedures performed

^{**} "Less experienced" defined as 75 or less prior esophageal CLE procedures performed

PPV = positive predictive value, NPV = negative predictive value

^a: p=0.0009,

^b: p=0.0004,

^c: p = 0.0001, for comparisons between groups using Fisher's exact test.