Accuracy and interrater reliability for the diagnosis of Barrett's neoplasia among users of a novel, portable high-resolution microendoscope

P. M. Vila, **M. J. Kingsley**, **A. D. Polydorides**, **M.-A. Protano**, **M. C. Pierce**, **J. Sauk**, **M. K. Kim**, **K. Patel**, **J. H. Godbold**, **J. D. Waye**, **R. Richards-Kortum**, and **S. Anandasabapathy**

Summary

The high-resolution microendoscope (HRME) is a novel imaging modality that may be useful in the surveillance of Barrett's esophagus in low-resource or community-based settings. In order to assess accuracy and interrater reliability of microendoscopists in identifying Barrett's-associated neoplasia using HRME images, we recruited 20 gastroenterologists with no microendoscopic experience and three expert microendoscopists in a large academic hospital in New York City to interpret HRME images. They prospectively reviewed 40 HRME images from 28 consecutive patients undergoing surveillance for metaplasia and low-grade dysplasia and/or evaluation for high-grade dysplasia or cancer. Images were reviewed in a blinded fashion, after a 4-minute training with 11 representative images. All imaged sites were biopsied and interpreted by an expert pathologist. Sensitivity of all endoscopists for identification of high-grade dysplasia or cancer was 0.90 (95% confidence interval [CI]: 0.88–0.92) and specificity was 0.82 (95% CI: 0.79–0.85). Positive and negative predictive values were 0.72 (95% CI: 0.68–0.77) and 0.94 (95% CI: 0.92–0.96), respectively. No significant differences in accuracy were observed between experts and novices (0.90 vs. 0.84). The kappa statistic for all raters was 0.56 (95% CI: 0.54–0.58), and the difference between groups was not significant (0.64 vs. 0.55). These data suggest that gastroenterologists can diagnose Barrett's-related neoplasia on HRME images with high sensitivity and specificity, without the aid of prior microendoscopy experience.

Introduction

Barrett's esophagus (BE) is a precancerous condition arising from chronic acid-related injury to the distal esophagus. Patients with BE have a 30-fold increased risk of developing esophageal cancer, one of the fastest rising cancers in the United States today.[1] While esophagogastroduodenoscopy with four-quadrant biopsies is the gold standard for surveying patients with BE, the efficiency and accuracy of this approach are less than optimal. Random biopsy protocols, however, have been shown to miss >50% of all dysplastic lesions.[2–4] Moreover, the diagnostic yield of random biopsies is low, leading to a large number of unnecessary, non-neoplastic biopsies with added procedure time and cost.[5–7]

High-resolution optical imaging technologies such as confocal laser microendoscopy (CLE) have been used to provide in vivo histological data to aid in the diagnosis of gastrointestinal neoplasia[8] and with the diagnosis of BE.[9] CLE has been shown to increase the diagnostic yield of endoscopic surveillance in BE.[5] However, current usage is mostly limited to academic centers due to both the high cost of these platforms (>$125 000) and the steep learning curve required for image interpretation.[10] Our group has developed a low-cost (<$4000), portable, battery-operated, high-resolution microendoscope (HRME) that provides subcellular imaging of the epithelium when used in conjunction with a nuclear-specific topical contrast agent.[11] The device consists of a 1-mm diameter, flexible, fiber-optic probe that is passed through the accessory channel of an endoscope and can provide a

real-time view of the mucosa when placed in gentle contact with the mucosal surface.[12] When used with topical proflavine hemisulfate 0.01% (w/v) for fluorescent contrast, the device provides high-resolution images that can be used to delineate normal squamous epithelium from Barrett's metaplasia and further distinguish intraepithelial neoplasia (high-grade dysplasia [HGD] or cancer).[13] Because this device is portable and of significantly lower cost than other 'optical biopsy' technologies, it may be a feasible alternative to CLE in community-based settings or areas outside of tertiary care centers. However, the accuracy and interrater reliability of new users in interpreting these microendoscopic images has not been evaluated previously.

The goal of this pilot study was to assess the accuracy of the interpretation of HRME images by gastroenterologists to diagnose BE-associated neoplasia (HGD and cancer) and also to determine whether general gastroenterologists without prior experience in microendoscopy could be rapidly trained to interpret HRME images. To this end, we evaluated both the accuracy and the interrater reliability of HRME image interpretation using in vivo images obtained with HRME.

Methods

HRME

Technical details on the HRME design and assembly (Fig. 1) have been thoroughly described in Muldoon *et al*. [11] and Pierce *et al*.,[13] and the use of this device in endoscopy has been previously described by Muldoon *et al*.[12] Briefly, the system operates as a compact, battery-powered fluorescence microscope, coupled to a flexible, 1-mm diameter fiber-optic imaging probe. Light-emitting diode illumination (output spectrum centered at a wavelength of 455 nm) is delivered from the HRME unit, through the imaging probe, to the tissue surface. Light returning from the tissue is transmitted through the same probe back to the HRME unit and imaged onto a charge-coupled device camera through a 490-nm long pass filter. Images are displayed on a computer screen in real-time at 12 frames per second. The probe used in the current study provides a 750-μm diameter field of view with 4.5 μm spatial resolution and can be reused at least 50–70 times before replacement, after being disinfected with Cidex in between patient use (Fig. 2). A comparison between technical specifications in CLE and HRME is shown in Table 1.

HRME image acquisition

Consecutive patients undergoing BE surveillance for metaplasia, dysplasia, and/or evaluation for endoscopic therapy for HGD or intramucosal cancer were enrolled in an institutional review board-approved protocol. A single endoscopist performed standard upper endoscopic examination using a high-definition white-light endoscope. Suspicious and non-suspicious areas were then targeted for HRME imaging. In each patient, 2–4 mL of 0.01% proflavine hemisulfate was sprayed on the mucosa using a standard endoscopic spray catheter. Proflavine, which was used under an Investigational New Drug (IND) application from the Food and Drug Administration (IND 102 217), is a bright fluorescent contrast agent that selectively labels cell nuclei, with peak absorption and emission wavelengths of 445 nm and 515 nm, respectively. The HRME probe was inserted through the endoscope accessory channel and gently placed against the mucosa. Images were displayed as continuously streaming video throughout the procedure, with short video sequences captured with the probe placed at each site of interest. In order to ensure accurate histopathologic correlation, the level and quadrant of each site was recorded, and a dimple was placed at the time of imaging using the 1-mm HRME probe tip. A biopsy was obtained from this area within 10 seconds of imaging with the HRME. Each biopsy specimen from an imaging site

was labeled and packaged separately at this time. All biopsies were interpreted by an expert gastrointestinal pathologist who was blinded to the HRME interpretation.

Representative still images (.jpg format) were subsequently extracted from the video file (.avi format) for analysis. Image selection was performed by two of the authors (P. M. V. and R. R. K.), neither of whom are endoscopists and were not responsible for collecting or rating images. Images were selected if the following criteria were fulfilled: nuclei were visible in greater than 50% of the image, and >50% of the image was clearly visible (in focus, and not obscured by motion artifact). For each movie recorded one representative frame was chosen using one image per biopsy. Eleven high-quality images were selected for training, and 40 were selected for the test set. Twenty gastroenterologists with no prior experience in microendoscopy, along with three expert endoscopists with experience in over 50 HRME cases, completed the training and test set in a blinded fashion. The three expert endoscopists were included in the study to serve as a reference for comparison with gastroenterologists who had no prior microendoscopic experience. A 4-minute training session was provided in a controlled setting with a prepared script, using images from the training set as examples to illustrate HRME image features, including both glandular (size, shape, and density) and nuclear (size, variability, and crowding) criteria for each pathologic category (Fig. 3). This classification system was created based on previously developed criteria from prior work in scanning confocal microscopy[9, 14, 15] and subsequently adapted to HRME.[11] Image-classification criteria were explained in detail to all raters during the training session. Raters were then given 10 seconds per image to view and classify each image in the test set as non-neoplastic (benign squamous, benign cardia, intestinal metaplasia (IM), or low-grade dysplasia [LGD]) or neoplastic (HGD and cancer). Images were shown only once, and were not duplicated in the training and test sets. All raters evaluated all images in the test and were given 10 seconds to observe each image.

Statistical analysis

Interrater reliability between all endoscopists and within subgroups was assessed using the unweighted kappa statistic.[16] The calculations were performed using the MAGREE function macro in SAS (Version 9.2, SAS Institute, Inc., Cary, NC, USA), modeled after a previously reported method.[17] Measures of diagnostic accuracy were calculated for each individual rater and then averaged within each group (HRME expert, HRME novice, etc.). The kappa values were interpreted as a value of 1.0 (perfect agreement), 0.8–1.0 (high agreement), 0.6–0.8 (substantial agreement), 0.4–0.6 (moderate agreement), 0.2–0.4 (fair agreement), and 0.0–0.2 (slight agreement).[18] A two-sided *P*-value of <0.05 was considered to demonstrate a statistically significant difference, using the normal *z*-test.

Results

Images were obtained from a total of 28 patients. The mean age of the patients was 63.3 years. Sixty-eight percent of the imaged population were male and 32% were female. Eighty-two percent of patients identified themselves as White, 11% as Hispanic, 3.5% as Black, and 3.5% did not specify. Among the 28 patients, nine had no evidence of BE, seven had IM, four had LGD, and eight had evidence of neoplasia, as defined by either HGD or esophageal adenocarcinoma (EAC).

A total of 67 sites were imaged from the full set of 28 patients, with 35 sites showing a normal gastroesophageal junction (normal squamous or normal gastric mucosa), 14 sites showing IM, eight sites showing LGD, and 10 sites showing neoplasia (Table 2). Of the 18 sites that revealed LGD or neoplasia, four were from areas that were not initially deemed suspicious during white-light endoscopy. The diagnosis at all sites was obtained through standard histopathology as interpreted by a single expert pathologist (A. D. P). From the 67

sites, a total of 51 high-quality images were chosen for inclusion in the study based on previously mentioned exclusion criteria. Images were divided into a training and test set, each including images corresponding to all types of diagnoses encountered in the study (Table 2). There were no complications or adverse events related to imaging with the HRME or use of topical proflavine, and the entire imaging process added 6–8 minutes to the regular endoscopic evaluation.

The overall sensitivity of all 23 raters for the identification of neoplasia (HGD or EAC) was 90% (95% confidence interval [CI]: 88–92%), and the specificity was 82% (95% CI: 79–85), yielding a positive predictive value of 72% (95% CI: 68–77%) and a negative predictive value (NPV) of 94% (95% CI: 92–96%). No statistically significant differences in overall accuracy were observed between the expert and novice groups (90% vs. 84%), although one novice rater was an outlier (Figs 4, 5). The kappa statistic for interrater reliability was moderate at 0.56 (95% CI: 0.54–0.58). While higher agreement was seen among experts, the difference between expert and novices did not achieve statistical significance (0.64 vs. 0.55).

We also examined the accuracy of raters in identifying any Barrett's mucosa; that is, distinguishing Barrett's metaplasia with LGD and neoplasia (HGD and adenocarcinoma) from the normal gastroesophageal junction (gastric cardia and squamous-lined esophagus). The sensitivity in all raters was 93% (95% CI: 91–95), and the specificity was 74% (95% CI: 70–78). The specificity was 91% (95% CI: 82–100) in the expert group, which was significantly higher than the specificity of the novice group at 71% (95% CI: 67–76). The kappa statistic for interrater reliability among rater groups in classifying normal versus abnormal images was 0.83 in the expert group and 0.56 in the novice group, a statistically significant difference.

Discussion

This preliminary study demonstrates that general gastroenterologists with no prior microendoscopy experience can be rapidly trained to interpret HRME images obtained from a portable fiber-bundle device with a high degree of diagnostic accuracy (sensitivity = 90%, specificity = 82%) and moderate interrater reliability (kappa = 0.56). When experts and novices were compared, there was no statistically significant difference in the sensitivity (92% vs. 90%) or specificity (89% vs. 81%) of identifying Barrett's neoplasia, suggesting that new users can be rapidly trained to interpret HRME images. Among experts and novices the NPV for the detection of neoplasia (HGD and cancer) was 96% and 94%, respectively. This result suggests that community-based gastroenterologists with no advanced or microendoscopic experience may be trained to use this tool to select areas for biopsy more efficiently, potentially increasing diagnostic yield and improving surveillance efficacy. These preliminary accuracy rates are promising and approach those reported in similarly designed studies using confocal microendoscopic platforms (sensitivity 88%, specificity 96%).[19] A follow-up, prospective trial is ongoing to examine this accuracy with a larger number of patients in a real-time setting.

While we evaluated the ability of users to identify any BE as well as Barrett's-associated neoplasia, we chose not to evaluate the accuracy of the device for the identification of all dysplasia, but limited the analysis to HGD or cancer. Even among expert pathologists there is significant variability in the diagnosis of LGD,[20, 21] and similar studies using existing CLE platforms have also limited their evaluation of accuracy in the diagnosis of only HGD or cancer.[22] From a clinical standpoint this is the most important distinction to make because a diagnosis of either HGD or cancer necessitates intervention, whereas a diagnosis of BE or LGD would prompt continued surveillance.

The ideal role for microendoscopy is not for screening, but in a surveillance setting where it can be used to enhance diagnostic accuracy and reduce the number of biopsies obtained. With this in mind the high NPV seen in both new and expert users is favorable for ruling out cancers in the setting of surveillance. Because of the small field of view (720 μm diameter) of this and other 'optical biopsy' technologies,[19] the time needed to evaluate a long segment of Barrett's would be prohibitively long. Given this limitation, we feel that the best role for this type of microendoscopic imaging is in more accurately defining areas of abnormality seen on high-definition white-light endoscopy or other 'red flag' imaging modalities (narrow-band imaging, autofluorescence imaging, etc.), many of which have been shown to have high sensitivity but a high false positive rate.[23] Therefore, while we would not propose using a microendoscopic device such as the HRME alone as a primary screening modality, the accuracy rates seen in both expert and non-expert endoscopists in this study were promising and suggest that HRME may be used in conjunction with a widefield modality to more effectively evaluate suspicious areas seen on white-light endoscopy.

The accuracy of new users must also be interpreted in the context of the extremely short time frame given for the training. Based on the excellent accuracy of the three expert microendoscopists (sensitivity = 92%, specificity = 89%) as compared with the gastroenterologists who received a 4-minute training as their only experience with HRME (sensitivity = 90%, specificity = 81%), we expect further improvement in accuracy with increased duration of training and use, especially with improvements in specificity. The high accuracy for the detection of neoplasia among experts suggests another potential role for the device in high-risk surveillance settings, where it may enable real-time decision making, in conjunction with other imaging modalities. In this context an HRME diagnosis of neoplasia from a previously indeterminate lesion could trigger an immediate endoscopic mucosal resection, obviating the delay associated with histopathologic confirmation and a subsequent therapeutic procedure.

The high NPV achieved in this study suggests that first examining areas of concern with HRME could prevent many unnecessary, costly biopsies. Especially in light of the current recommendations for taking random, four-quadrant biopsies within the Barrett's segment, [24, 25] the potential exists for saving a significant number of unnecessary biopsies when this device is used, as biopsies would only be used to confirm abnormal pathology detected on HRME. This could potentially improve the overall cost-effectiveness of endoscopic surveillance, as the method of surveillance biopsies has been estimated to cost between $350 and $1200 for each procedure.[26] However, a formal cost-effectiveness analysis would be required in the future to prove that this applies in actual use.

One of the strengths of this study is that the raters were blinded to the clinical context of the HRME image because the gastroenterologists were given no additional clues that could help classify the lesions, such as the location or appearance of the lesion on white-light endoscopy (e.g. ulceration and nodularity). This allowed us to observe the diagnostic accuracy of the HRME image alone. An ongoing study is evaluating the real-time diagnostic accuracy of the device among a single expert (>50 HRME cases) in a prospective trial. This prospective trial should help determine whether the HRME can be used in conjunction with white-light endoscopy to more accurately guide biopsy sampling and facilitate real-time decision making.

A prior study with a similar study design showed that CLE with fluorescein, a systemic contrast agent, yielded 88% sensitivity and 96% specificity for the detection of neoplasia in the setting of BE, comparable with our findings using HRME.[19] While most studies examining the accuracy of CLE have used intravenous fluorescein as a contrast agent, some

have combined intravenous fluorescein and topical acriflavine in order to increase visualization of epithelial surfaces. For example, Pohl *et al.* reported a sensitivity and specificity of 75% and 89–91%, respectively, using probe-based CLE in the diagnosis of Barrett's-associated neoplasia.[22] Because we used cellular features such as nuclear spacing and nuclear size to classify HRME images with a nuclear stain (proflavine), this may be a large part of the reason why we were able to obtain such high accuracy and good interrater reliability with HRME.

In Europe, Asia, and Australia, acriflavine has been used for several years as a fluorescent contrast agent in investigational imaging studies in the esophagus,[27] similar to those reported with our HRME. (Acriflavine is a commercially available mixture of proflavine and its N-methyl quaternary salt, euflavine.) Proflavine and other acridine-derived dyes are postulated to bind DNA reversibly and non-covalently by intercalating between base pairs. As a result, these dyes are well suited for use as nuclear contrast agents. Acridine-related compounds have been shown to induce DNA strand breaks in bacteria and yeast. However, in cultured human cells clastogenic effects have been seen, but acridines have not been shown to induce a wide range of other mutagenic effects.[28] While no comprehensive long-term studies of proflavine have been published, proflavine-containing compounds have been used extensively without reported mutagenic effects in humans. Proflavine has been used for a number of years as a topical antiseptic agent and is widely used as component of Triple Dye, a solution applied to the umbilicus of newborns to prevent infection. The proflavine concentration used in this study is 17.5 times lower than that of proflavine in Triple Dye. [29]

Other compounds including methylene blue, crystal violet, and toluidine blue are routinely used to enhance visual contrast between normal and neoplastic tissues in the gastrointestinal tract and oral cavity, despite recognition that these agents interact with DNA and uncertainty existing over possible carcinogenicity.[30] In vitro assays have been reported in which photoactivated methylene blue was more than twice as mutagenic as proflavine under identical conditions.[31] The mutagenic effect of toluidine blue was also confirmed in vitro. [32]

A limitation of this study is that we did not perfectly simulate the conditions of a clinical endoscopy during the evaluation of the HRME. First, raters were shown static images of esophageal and gastric tissue as opposed to real-time, dynamic images. Using static images allowed the authors to better illustrate and explain the characteristics of HRME images representative of normal mucosa, BE, and neoplasia. In addition this provided the advantage of removing poor-quality images with motion artifact. Testing raters using static, high-quality images as a standard allowed better evaluation of their comprehension and application of diagnostic criteria obtained during the training. Evaluating images from the HRME and diagnosing changes in the esophageal mucosa in this way is slightly different from commenting on a moving image at the point of care. The test conditions were also very controlled, as the raters were not allowed to go back to prior images or spend more than 10 seconds evaluating an image, unlike in actual endoscopic practice. Despite this the novice raters managed to attain a very high level of accuracy and moderate interrater reliability in the face of the time constraint, which suggests that the accuracy could be even higher when raters are allowed to view the images on their own. Earlier studies examining the accuracy of confocal microendoscopic images used a similar method and reported similar accuracy rates.[9]

An additional limitation of our study was that the population of patients examined in our study reflects the population of a large academic center. While the proportion of sites sampled that revealed HGD or EAC were similar to recent studies using CLE,[33] the

prevalence in our population likely represents a higher prevalence than has been reported in larger reviews.[34] Given the higher proportion of patients with HGD or cancer in our population, we would expect a lower NPV as compared with the NPV that would be obtained in a population undergoing surveillance in the community. This suggests that the NPV seen in a community setting may be even higher than the 94% NPV we obtained in this study. Although a further limitation of this study is the relatively small number of patients who underwent imaging, continued evaluation of the HRME is ongoing as part of a prospective trial.

Finally, a limitation of the device itself is that it needs to be combined with a widefield optical imaging device, such as white-light endoscopy. While it was beyond the scope of this study to evaluate how this technology performs in conjunction with white-light endoscopy, this is currently being evaluated in a prospective trial. We believe that the purpose of this device will be to add specificity to the already high sensitivity in the detection of cancer with the use of white-light endoscopy and other widefield technology, such as narrow-band imaging and chromoendoscopy.

In summary our current results suggest that novice microendoscopists were able to achieve high accuracy and moderate interrater reliability for the discrimination of neoplasia in images obtained using a novel, low-cost microendoscope. While these results are encouraging, further research is ongoing to establish the accuracy and interrater reliability in a clinical setting in real time.

Acknowledgments

References

1. Centers for Disease Control and Prevention. United States Cancer Statistics: 1999–2005 Incidence, Wonder on-Line Database. Atlanta: United States Department of Health and Human Services, CDC, National Cancer Institute; 2008.

2. Thomson BNJ, Cade RJ. Oesophagectomy for early adenocarcinoma and dysplasia arising in Barrett's oesophagus. ANZ J Surg. 2003; 73:121–124. [PubMed: 12608973]

3. Dellon ES, Shaheen NJ. Does screening for Barrett's esophagus and adenocarcinoma of the esophagus prolong survival? J Clin Oncol. 2005; 23:4478–4482. [PubMed: 16002837]

4. van Sandick JW, van Lanschot JJB, Kuiken BW, Tytgat GNJ, Offerhaus GJA, Obertop H. Impact of endoscopic biopsy surveillance of Barrett's oesophagus on pathological stage and clinical outcome of Barrett's carcinoma. Gut. 1998; 43:216–222. [PubMed: 10189847]

5. Dunbar KB, Okolo P, 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; 70:645–654. [PubMed: 19559419]

6. Sharma P, Topalovski M, Mayo MS, Weston AP. Methylene blue chromoendoscopy for detection of short-segment Barrett's esophagus. Gastrointest Endosc. 2001; 54:289–293. [PubMed: 11522967]

7. Canto MI, Setrakian S, Willis JE, Chak A, Petras RE, Sivak MV. Methylene blue staining of dysplastic and nondysplastic Barrett's esophagus: an in vivo and ex vivo study. Endoscopy. 2001; 33:391–400. [PubMed: 11396755]

8. Kiesslich R, Burg J, Vieth M, et al. Confocal laser endoscopy for diagnosing intraepithelial neoplasias and colorectal cancer in vivo. Gastroenterology. 2004; 127(3):706–713. [PubMed: 15362025]

9. 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; 4:979–987. [PubMed: 16843068]

10. Dunbar KB, Montgomery EA, Canto MI. The learning curve of in vivo confocal laser endomicroscopy for prediction of Barrett's esophagus. Gastroenterology. 2008; 134:A62–A63.

11. Muldoon T, Anandasabapathy S, Maru D, Richards-Kortum R. High-resolution imaging in Barrett's esophagus: a novel, low-cost endoscopic microscope. Gastrointest Endosc. 2008; 68:737–744. [PubMed: 18926182]

12. Muldoon TJ, Pierce MC, Nida DL, Williams MD, Gillenwater A, Richards-Kortum R. Subcellular-resolution molecular imaging within living tissue by fiber microendoscopy. Opt Express. 2007; 15:16413–16423. [PubMed: 19550931]

13. Pierce M, Yu D, Richards-Kortum R. High-resolution fiber-optic microendoscopy for in situ cellular imaging. J Vis Exp. 2011; 47:e2306.

14. Inoue H, Cho JY, Satodate H, et al. Development of virtual histology and virtual biopsy using laserscanning confocal microscopy. Scand J Gastroenterol. 2003; 38:37–39.

15. Inoue H, Igari T, Nishikage T, Ami K, Yoshida T, Iwai T. A novel method of virtual histopathology using laser-scanning confocal microscopy in-vitro with untreated fresh specimens from the gastrointestinal mucosa. Endoscopy. 2000; 32(6):439–443. [PubMed: 10863908]

16. Fleiss, JL.; Levin, B.; Paik, MC. Statistical Methods for Rates and Proportions. 3rd edn. New York: John Wiley & Sons; 2003.

17. Posner KL, Sampson PD, Caplan RA, Ward RJ, Cheney FW. Measuring interrater reliability among multiple raters – an example of methods for nominal data. Stat Med. 1990; 9:1103–1115. [PubMed: 2244082]

18. Landis JR, Koch GG. Measurement of observer agreement for categorical data. Biometrics. 1977; 33:159–174. [PubMed: 843571]

19. Wallace MB, Sharma P, Lightdale C, 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. [PubMed: 20381042]

20. Maru DM. Barrett's esophagus: diagnostic challenges and recent developments. Ann Diagn Pathol. 2009; 13:212–221. [PubMed: 19433303]

21. Kerkhof M, Van Dekken H, Steyerberg EW, et al. Grading of dysplasia in Barrett's oesophagus: substantial interobserver variation between general and gastrointestinal pathologists. Histopathology. 2007; 50:920–927. [PubMed: 17543082]

22. Pohl H, Rösch T, Vieth M, et al. Miniprobe confocal laser microscopy for the detection of invisible neoplasia in patients with Barrett's oesophagus. Gut. 2008; 57:1648–1653. [PubMed: 18755886]

23. Curvers W, Baak L, Kiesslich R, et al. Chromoendoscopy and narrow-band imaging compared with high-resolution magnification endoscopy in Barrett's esophagus. Gastroenterology. 2008; 134:670–679. [PubMed: 18242603]

24. Barritt AS, Shaheen NJ. Should patients with Barrett's oesophagus be kept under surveillance? The case against. Best Pract Res Clin Gastroenterol. 2008; 22:741–750. [PubMed: 18656827]

25. Hirota WK, Zuckerman MJ, Adler DG, et al. ASGE guideline: the role of endoscopy in the surveillance of premalignant conditions of the upper GI tract. Gastrointest Endosc. 2006; 63:570–580. [PubMed: 16564854]

26. Inadomi JM, Sampliner R, Lagergren J, Lieberman D, Fendrick AM, Vakil N. Screening and surveillance for Barrett esophagus in high-risk groups: a cost-utility analysis. Ann Intern Med. 2003; 138:176–186. [PubMed: 12558356]

27. 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; 62:686–695. [PubMed: 16246680]

28. Ferguson LR, Denny WA. The genetic toxicology of acridines. Mutat Res. 1991; 258:123–160. [PubMed: 1881402]

29. Janssen PA, Selwood BL, Dobson SR, Peacock D, Thiessen PN. To dye or not to dye: a randomized, clinical trial of a triple dye/alcohol regime versus dry cord care. Pediatrics. 2003; 111:15–20. [PubMed: 12509548]

30. Olliver JR, Wild CP, Sahay P, Dexter S, Hardie LJ. Chromoendoscopy with methylene blue and associated DNA damage in Barrett's oesophagus. Lancet. 2003; 362:373–374. [PubMed: 12907012]

31. Brendel M. Different photodynamic action of proflavine and methylene blue on bacteriophage 2. Mutation induction in extracellularly treated serratiaphage kappa. Mol Gen Genet. 1973; 120:171–180. [PubMed: 4568530]

32. Dunipace AJ, Beaven R, Noblitt T, Li YM, Zunt S, Stookey G. Mutagenic potential of toluidine blue evaluated in the Ames test. Mutat Res. 1992; 279:255–259. [PubMed: 1377780]

33. Sharma P, Meining A, 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; 74:465–472. [PubMed: 21741642]

34. Yousef F, Cardwell C, Cantwell MM, Galway K, Johnston BT, Murray L. The incidence of esophageal cancer and high grade dysplasia in Barrett's esophagus: a systematic review and meta-analysis. Am J Epidemiol. 2008; 168:237–249. [PubMed: 18550563]
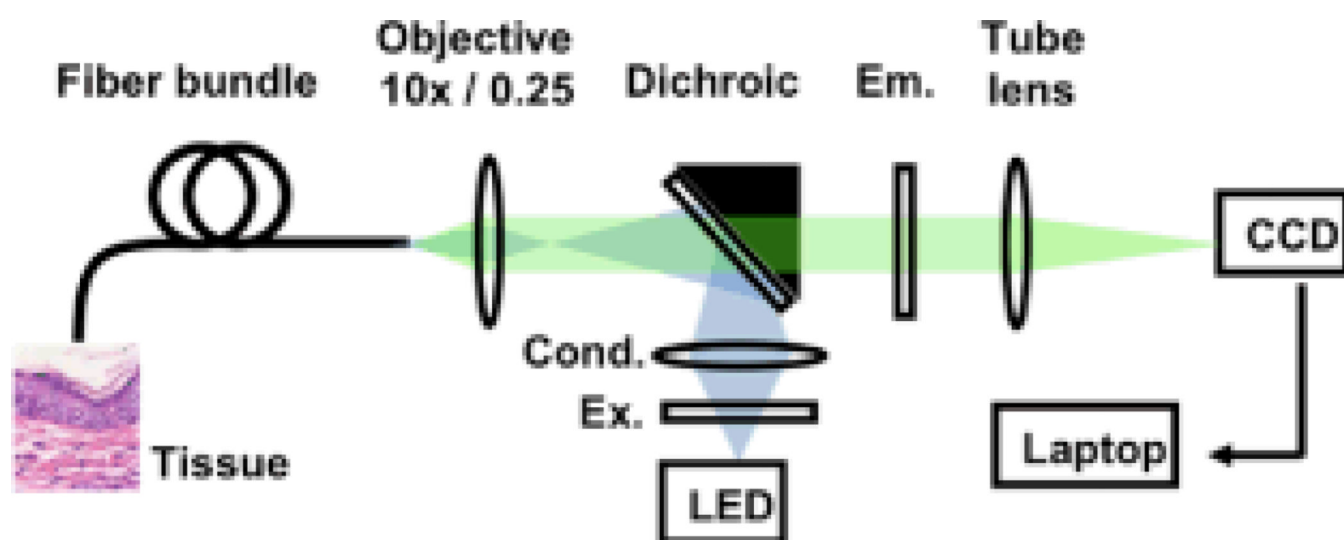
Figure 1.
Schematic diagram of the high-resolution microendoscope (HRME). CCD, charge-coupled device; Cond, condenser lens; Em, emission filter; Ex, excitation filter; LED, light-emitting diode.
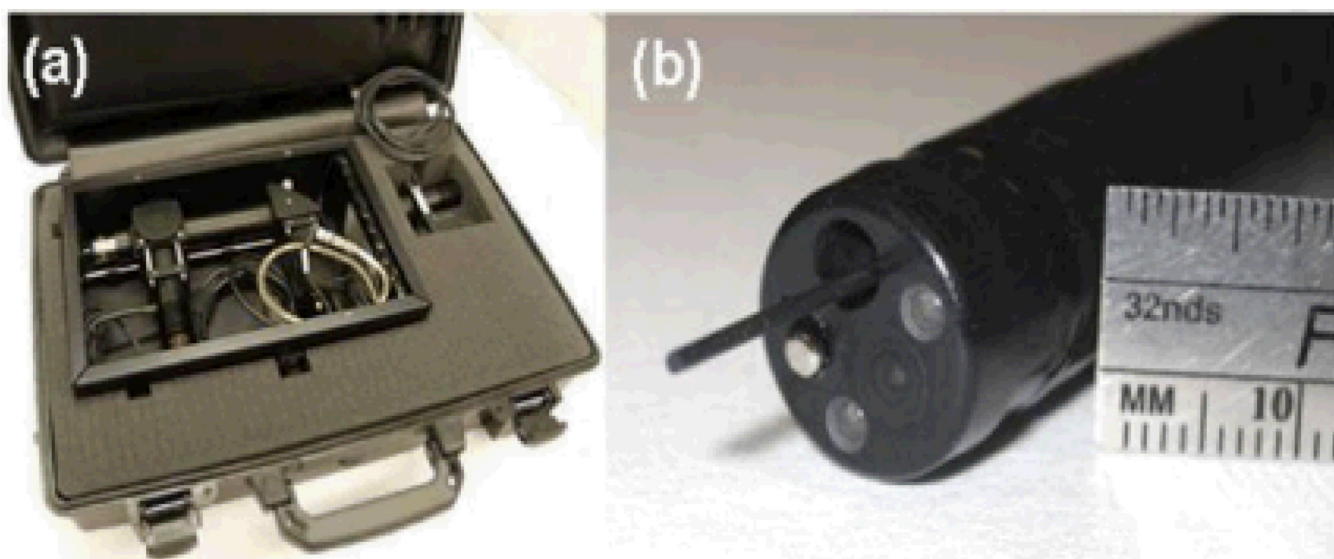
Figure 2.
Photo of the (a) high-resolution microendoscope (HRME) in a standard size briefcase and the (b) HRME probe inside the accessory channel of a standard endoscope.
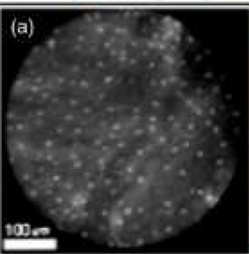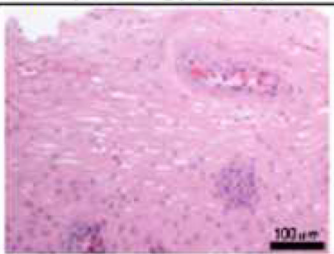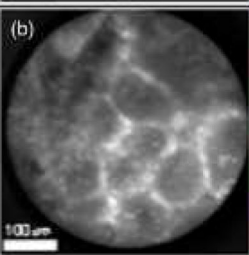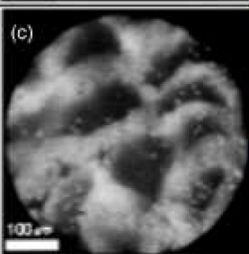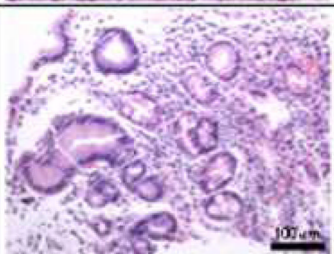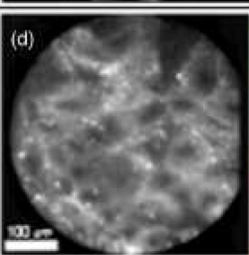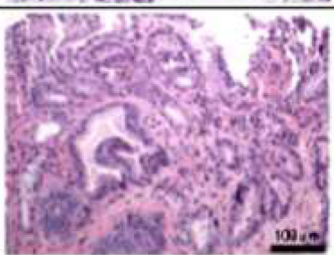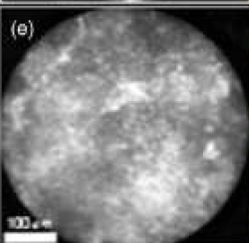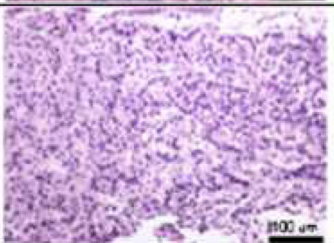
Tissue Diagnosis	HRME Description	Example Images (HRME [left] and Histopathology [right])
Squamous Mucosa	Bright and evenly spaced, small, and equally sized nuclei with ample cytoplasm	
Gastric Cardia	Thin epithelial layer with large rounder lumens; web-like or honeycomb appearance	
Barrett's Metaplasia	Elongated glands with wider epithelial layer compared to gastric cardia	
High-grade Dysplasia	Crowded heterogenous glands with small, irregular luminal spaces and crowded nuclei	
Adenocarcinoma	Loss of glandular architecture; large, dense, overlapping, and pleomorphic nuclei	

Figure 3.
High-resolution microendoscope (HRME) image classification criteria of (a) normal squamous mucosa, (b) normal gastric cardia, (c) Barrett's metaplasia, (d) high-grade dysplasia, and (e) adenocarcinoma. Note: The brightness of the image does not correlate with the disease state.
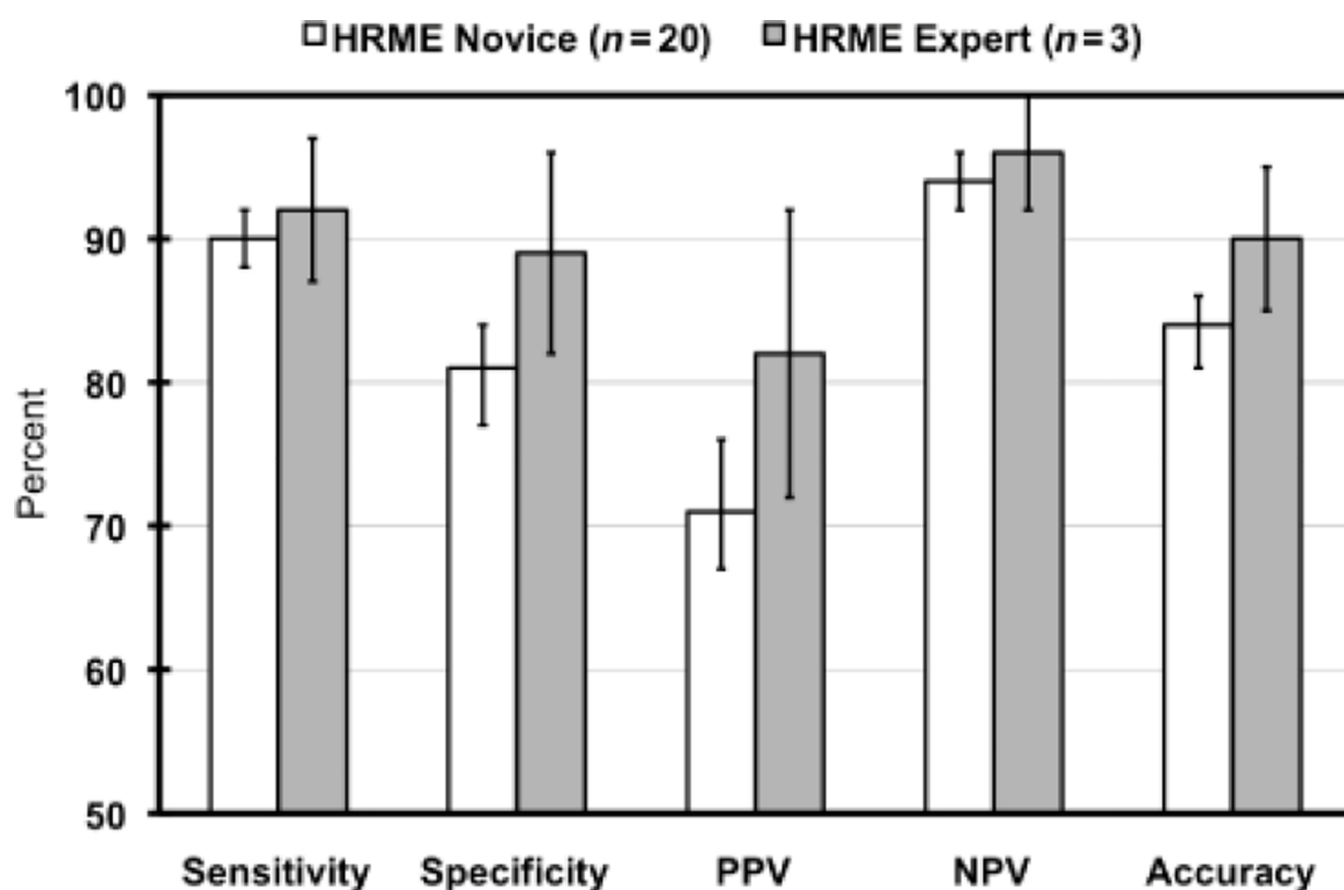
Figure 4.
Rater accuracy of classification of Barrett's-related neoplasia high-grade dysplasia (HGD/ cancer) versus Barrett's and normal squamous and gastric mucosa. NPV, negative predictive value; PPV, positive predictive value.
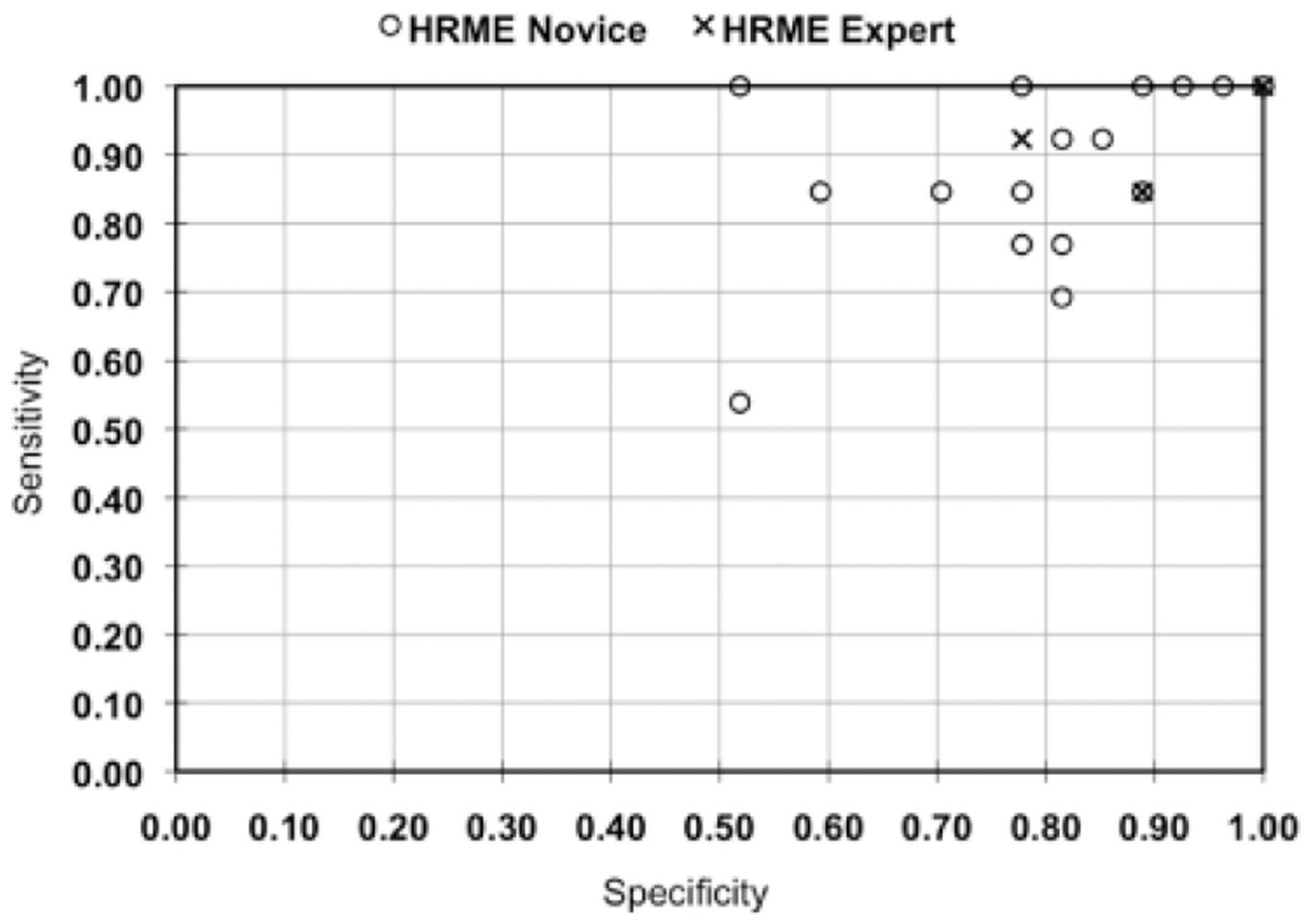
Figure 5.
Sensitivity and specificity of classification of neoplasia high-grade dysplasia (HGD/cancer)
versus non-neoplasia (Barrett's metaplasia, low-grade dysplasia [LGD], normal squamous
and gastric mucosa) for each rater.

Table 1

Technical specifications of high-resolution microendoscopy (HRME) and confocal laser endomicroscopy

Characteristic	Confocal microendoscopy	HRME
Contrast agent	Fluorescein 10% (intravenous), acriflavine 0.05% (topical)	Proflavine 0.01% (topical)
Imaging depth	60–250 μm	50 μm
Resolution	0.7–1.0 μm	4.5 μm
Field of view	0.05–0.28 mm^2	0.44 mm^2
Frame rate	1.2–12 frames/second	12 frames/second

Table 2

Distribution of HRME image sites in training and test sets, grouped by pathologic category established by pathology

Pathology category	HRME images		
	Training	Test	Total
Normal squamous mucosa	2	9	11
Normal gastric mucosa	3	9	12
Barrett's intestinal metaplasia/LGD	3	9	12
High-grade dysplasia/EAC	3	13	16
Total	11	40	51

EAC, esophageal adenocarcinoma; HRME, high-resolution microendoscope; LGD, low-grade dysplasia.