

Advances in Endoscopic Imaging of the Esophagus

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KEYWORDS

- Gastroesophageal reflux disease
- Barrett's esophagus
- Squamous cell carcinoma of the esophagus
- Narrow-band imaging • Chromoendoscopy
- Autofluorescence imaging • Confocal laser endomicroscopy

Identification of esophageal pathology by standard electronic videoendoscopes depends on the presence of various mucosal lesions such as erosions, plaques, nodules, or ulcers. Certain conditions such as esophageal squamous cell dysplasia,¹ however, can be missed on standard endoscopy because they may have very subtle mucosal changes. In addition, the diagnosis of Barrett's esophagus (BE)² might not be always accurate with regular endoscopy because it has been shown that biopsy specimens from short-segment BE identify metaplasia in only 40% to 60% of patients. Furthermore, because the distribution of dysplasia and early adenocarcinoma (EAC) is patchy and focal, the accurate detection of these conditions using standard biopsy techniques is low. Gastroesophageal reflux disease (GERD) is a common disorder in the Western world;³ however, less than half of the patients who have heartburn and regurgitation have endoscopic abnormalities, making standard endoscopy a less than optimal test for the diagnosis of GERD. Thus, advanced endoscopic techniques have been developed with the intention of improving the overall accuracy of endoscopy and biopsy for the diagnosis of squamous cell dysplasia, squamous cell carcinoma (SCC), BE and associated dysplasia, and EAC. The aim of this review is to describe recent advances in endoscopic technology and to review the available literature pertaining to the clinical application of these techniques in esophageal diseases.

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ADVANCED ENDOSCOPIC TECHNIQUES

Over the past few years, endoscopic technology has continued to evolve with the aim of providing a precise and “real-time” endoscopic diagnosis. The flexible fiberoptic endoscope that was introduced in 1961 was a significant milestone in gastrointestinal endoscopy that helped with the diagnosis and management of various gastroenterologic conditions. This endoscope was later replaced by the conventional videoendoscopes that have charged-coupled device chips of 100K to 300K pixels, implying that each image is built from 100,000 to 300,000 individual pixels. This technical feature, known as pixel density, is important because it relates to the image resolution, or the ability to discriminate two closely approximated points. In the next few paragraphs, the technical aspects of some of the advanced endoscopic techniques that have shown promise for clinical application in various esophageal diseases are discussed. These techniques include high-resolution and magnification endoscopy, chromoendoscopy, narrow-band imaging (NBI), autofluorescence imaging (AFI), and confocal laser endomicroscopy.

High-Resolution and Magnification Endoscopy

As mentioned earlier, the higher the pixel density, the better the image resolution for improved detection of minute lesions. High-resolution endoscopes are equipped with charged-coupled device chips of up to 850K pixels. These instruments are capable of discriminating lesions that are 10 to 71 μm in diameter, thus providing views with greater mucosal detail.⁴ Occasionally, the term *high-resolution* is used interchangeably with *high-magnification*. Magnification endoscopy is a relatively simple technique that enlarges the video image up to 150 times using a movable lens that is controlled by the endoscopist to vary the degree of magnification. When using magnification endoscopy, a cap is fitted onto the distal tip of the endoscope, allowing the mucosa in contact with the cap to be magnified without being affected by esophageal motility. High-resolution endoscopy (HRE) thus improves the ability to discriminate lesions, whereas magnification endoscopy enlarges the image.^{5,6}

Chromoendoscopy

Chromoendoscopy involves the application of contrast agents to improve the characterization of the gastrointestinal mucosa. This technique is often used in conjunction with high-resolution and high-magnification endoscopes. The stains used for this technique can be classified as (1) vital stains such as Lugol's solution, methylene blue, and toluidine blue that are actively absorbed by the cells; and (2) contrast stains such as indigo carmine that are not absorbed but highlight the mucosal patterns by accumulating in the pits and valleys of the tissue. Lugol's solution, methylene blue, and indigo carmine are the most commonly used stains in the esophagus. Lugol's solution is absorbed by the glycogen-containing nonkeratinized squamous epithelium (normal lining of the esophagus). Esophageal columnar epithelium, SCC, and inflammatory or dysplastic squamous epithelium do not stain with Lugol's solution.⁷⁻¹⁰ Methylene blue is readily taken up by metaplastic epithelium of the esophagus. Before staining with methylene blue, the surface mucus is generally removed with 10% *N*-acetylcysteine to expose maximal surface area for staining. Spraying the mucosa with 0.5% methylene blue is followed by vigorous irrigation of the stained areas using tap water.¹¹⁻¹³ Indigo carmine does not require pretreatment with other agents and can be sprayed onto the mucosa in a one-step process.¹⁴ Although chromoendoscopy can help highlight the mucosal characteristics, it is not useful to study the vascular architecture of the esophagus.

Narrow-Band Imaging

NBI is a recent advance in endoscopic technology that is based on the principle that depth of light penetration into tissues is directly proportional to the wavelength, implying that the shorter the wavelength, the more superficial the penetration. The use of spectral narrow-band filters (red, green, and blue bands) helps with imaging of the mucosal and vascular patterns of the esophagus without the need for chromoendoscopy. The main chromophore in the esophageal tissues in the visible wavelength range is hemoglobin, which has a maximum absorptive wavelength near 415 nm and is within the wavelength range for NBI. Thus, it is expected for NBI to detect vascular structures and patterns more accurately than conventional endoscopy. NBI, like chromoendoscopy, can also be combined with high-resolution and high-magnification endoscopy. An important advantage of NBI is that it provides a clear visualization of the vascular network in the mucosa.^{15,16}

Autofluorescence Imaging

AFI is a technique based on the principle that excitation of tissue with light of a shorter wavelength leads to emission of a longer wavelength of light. In the gastrointestinal tract, a number of biologic substances, also known as fluorophores, can be stimulated by light. On excitation, the fluorophores emit fluorescent light that is spread over a range of longer wavelengths, from the green to red spectrum. Submucosal collagen is the most important contributor to tissue autofluorescence in the gastrointestinal tract. Chromophores, on the other hand, absorb light without emitting fluorescence. Hemoglobin is the predominant chromophore in gastrointestinal tissue. Thus, the fluorescent light that is detected on the mucosal surface depends not only on the exciting light but also on the biochemical composition (collagen) and perfusion (hemoglobin) of the tissue. Malignant transformation of tissue is associated with emission of relatively longer wavelengths of light (shift from green toward the red end of the spectrum).^{17,18} For example, normal squamous mucosa and Barrett's epithelium without dysplasia appear greenish, whereas areas suspicious for BE-associated neoplasia appear blue-purple.¹⁹

Confocal Laser Endomicroscopy

Confocal laser endomicroscopy is a relatively new imaging technology that integrates a confocal laser microscope in the tip of a standard videoendoscope or as a probe that can be passed through the channel of any endoscope. The aim of this technique is to provide real-time histology (in vivo histology) during the procedure. With confocal endoscopy technology, white-light microscopy and confocal microscopy can be used simultaneously, and the working channel can be used to perform targeted biopsies. During the procedure, a single-line laser delivers an excitation wavelength of 488 nm, and the maximum laser power output is 1 mW or less at the surface of the tissue. The images are collected at a rate of 0.8 to 1.6 frames per second. The optical slice thickness is 7 μm with a lateral resolution of 0.7 μm , and the field of view is 500 \times 500 μm . The range of the z-axis is 0 to 250 μm below the surface layer. One drawback of this system is that when the confocal window is placed in contact with the tissue for image collection, the white-light image is unavailable to guide confocal placement. In contrast, the confocal probe system²⁰ moves independently of the endoscope so that placement onto the tissue can be guided by the white-light image. The probe can then be retracted from the channel to obtain biopsy samples. The probe system consists of a light source, the probe that is passed through the working channel of the endoscope, and software that enables visualization of dynamic digital sequences in real time.

Depending on the depth that needs to be sectioned, there are different miniprobes available with differing working distances. As with the other system, the excitation wavelength is 488 nm, the transverse and axial resolution ranges from 2.5 to 5 μm and 15 to 20 μm , respectively. Images are collected at a rate of 12 frames per second, with a field of view ranging from 600 \times 500 μm and 240 \times 200 μm , respectively. For endomicroscopy, a fluorescent contrast agent such as fluorescein, acriflavine, tetracycline, or cresyl violet is usually used to achieve high-contrast images.

CLINICAL APPLICATIONS

Squamous Cell Carcinoma of the Esophagus

Worldwide, SCC of the esophagus (SCCE) is the most common esophageal malignancy. Some of the risk factors for SCCE include tobacco and alcohol use, long-standing history of achalasia and caustic strictures, prior history of SCC of the head and neck region, Plummer-Vinson syndrome, and tylosis.²¹⁻²⁵ In the United States, most of the cases of SCCE can be attributed to the use of alcohol and tobacco.²⁶ By the time of diagnosis, SCCE is usually advanced and is associated with high mortality and morbidity.

Early SCCE usually presents as small erosion or a flat lesion, and diagnosing this is a challenge, even for the experienced endoscopist.⁶ In a study of 389 patients who had newly diagnosed SCC of the head and neck, 55% of patients who had an irregular multiform region of Lugol's voiding mucosa had SCCE.⁸ In a recent multicenter trial of 1095 patients who were deemed high risk for SCCE, 20% of early cancers, 66% of high-grade dysplasia (HGD), and 77% of low-grade dysplasia (LGD) were detected only after adding Lugol's staining to standard endoscopy.²⁷ Using high-magnification endoscopy, observation of normal esophageal mucosa and esophageal cancer led to the description of the vascular architecture of the esophagus and identification of the intrapapillary capillary loop (IPCL).²⁸ The organization and structure of blood vessels of the esophagus is dynamic and undergoes considerable changes during inflammation and neoplasia.²⁹ Observation of the IPCL and microvascular architecture was shown to be useful for diagnosing the depth of invasion of early SCCE using high-magnification endoscopy; the rate of agreement between depth of invasion based on histology and magnified appearance approached 84%.²⁸ In a study evaluating the role of NBI with magnifying endoscopy in 41 patients who had superficial esophageal lesions, NBI achieved an accuracy of 85% compared with histopathology and helped assess the depth of invasion.³⁰ Finally, in a recent study evaluating the role of confocal laser endomicroscopy in providing an in vivo diagnosis for 43 lesions in 21 patients who had known SCCE, the sensitivity and specificity of this technique for SCCE compared with histology were 100% and 95%, respectively (**Fig. 1**).³¹

Thus, based on the available evidence, chromoendoscopy with Lugol's solution should be performed in patients at high risk for SCCE. NBI and confocal endoscopy may have a role in the further characterization of these lesions.

Gastroesophageal Reflux Disease

Endoscopic evaluation of patients who have GERD using standard endoscopy is specific for the diagnosis when erosions are present. Using standard white-light endoscopy, however, less than half of GERD patients have findings of erosive esophagitis. Recently, several studies have examined the role of these newer endoscopic modalities in GERD. In a study using Lugol's chromoendoscopy, several Lugol's unstained streaks were seen in 19 of 39 patients (49%) who had reflux symptoms but did not have esophageal erosions on standard white-light endoscopy,

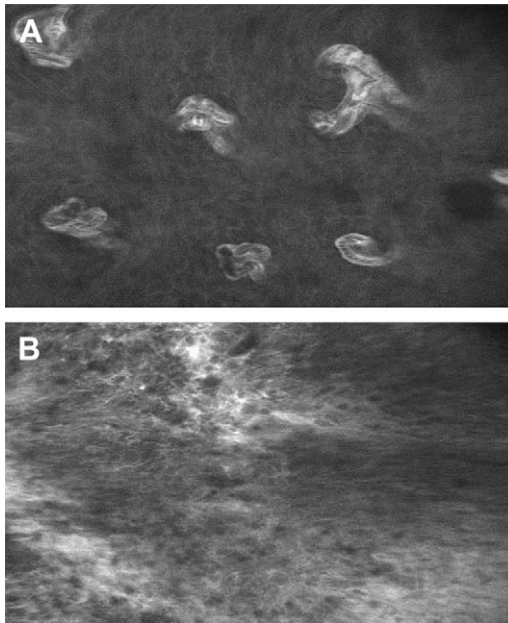


Fig. 1. Confocal images of (A) normal-appearing squamous epithelium of the esophagus and (B) SCCE showing nonhomogenous cells without regular or clear borders and leakage of fluorescein. (Courtesy of Oliver Pech, MD, Welsbaden, Germany.)

leading the investigators to speculate that the unstained streaks represented acid-induced mucosal injury to the esophagus.³² At least two studies using HRE have attempted to evaluate minimal-change esophagitis in a group of patients who had nonerosive reflux disease. A variety of features including triangular indentations, apical mucosal breaks, pinpoint blood vessels at the squamocolumnar junction, and punctate erythema above the Z-line were evaluated. Punctate erythema above the Z-line was the only finding that was seen more commonly in patients who had nonerosive reflux disease compared with control subjects.³³ None of the changes, however, was considered sensitive or specific for the diagnosis of GERD. NBI has also been evaluated in GERD patients to assess the impact of contrast change in increasing the detection of erosive esophagitis³⁴ and to recognize subtle mucosal and vascular changes. In a study of 50 patients who had GERD and 30 control subjects, patients who had GERD had increased number, dilatation, and tortuosity of IPCLs, microerosions (not seen on standard endoscopy), and increased vascularity at the squamocolumnar junction compared with control subjects. On multivariate analysis, increased number and dilation of IPCLs were the best predictors for diagnosing GERD, and the maximum, minimum, and number of IPCLs per field were significantly higher in the GERD group. In summary, chromoendoscopy and contrast-enhancing techniques (eg, NBI) appear simple, easy, and promising in a select population of GERD patients who do not have erosions. These findings, however, need to be confirmed in future prospective and blinded studies.³⁵

Barrett's Esophagus and Esophageal Adenocarcinoma

BE, a well-recognized precancerous lesion for EAC, is usually diagnosed at the time of upper endoscopy. It is revealed as columnar-lined esophagus above the

gastroesophageal junction, and biopsy results document the presence of intestinal metaplasia.³⁶ When diagnosed with BE, current guidelines recommend enrollment into a surveillance program for the detection of dysplasia or cancer.³⁷ It has been shown, however, that standard endoscopy might miss metaplasia, dysplasia, and EAC in patients who have BE because of the patchy distribution of these lesions.³⁸ Therefore, the ability to target biopsies and increase the yield of metaplastic and dysplastic areas would be of immense clinical benefit for the screening and surveillance of BE patients.

Chromoendoscopy

In one of the initial studies using methylene blue staining with magnification endoscopy ($\times 80$) in 30 patients who had columnar-appearing epithelium in the distal esophagus, five discrete patterns of mucosal surface were identified: small/round, straight, long oval, tubular, and villous. Metaplastic tissue on histopathology was predominantly seen in the areas with tubular patterns, villous patterns, or both.³⁹ In a recent prospective randomized crossover trial of 48 patients comparing the utility of methylene blue-directed biopsies with that of standard white-light endoscopy combined with four-quadrant random biopsies, both techniques were comparable for diagnosing intestinal metaplasia and dysplasia; however, the mean number of biopsies required to diagnose these conditions was significantly lower in the methylene blue group compared with the white-light group.⁴⁰ Overall, chromoendoscopy with methylene blue in BE patients has shown inconsistent results¹² and has more recently been implicated in causing genetic damage in Barrett's epithelium.⁴¹ Acetic acid has also been used in a study of 49 patients who had suspected short-segment BE, leading to the identification of four mucosal patterns: round, reticular, villous, and ridged. The yield of intestinal metaplasia in areas with the villous and ridged patterns was 87% and 100%, respectively.⁴² Using indigo carmine along with magnification endoscopy in a study of 80 patients, three types of mucosal patterns were noted: ridge/villous, circular, and irregular/distorted. The presence of the ridge/villous pattern for detecting intestinal metaplasia had a high sensitivity, specificity, and positive predictive value (97%, 76%, and 92%, respectively). Six patients who had an irregular/distorted pattern had HGD on biopsy; however, areas of LGD had mucosal patterns similar to nondysplastic areas.¹⁴ Despite having some positive results with the use of chromoendoscopy in BE patients, its use cannot be recommended in routine clinical practice, given the difficulty in achieving complete and even coating of the mucosal surface with the dye, the need for equipment for dye spraying, the inability to detect superficial vascular patterns, the inconsistent published data, and the advances in the field of electronic chromoendoscopy that eliminate the need for dye spraying.^{43,44}

Narrow-band imaging

There has been recent interest in evaluating the role of NBI in BE and early cancer, and preliminary results from these studies appear promising (Figs. 2 and 3). In a study of 51 patients who had known or suspected BE, images obtained from the NBI system were graded according to the mucosal (ridge/villous, circular, irregular/distorted) and vascular patterns (normal, abnormal) and correlated with histology in a prospective and blinded manner. The sensitivity, specificity, and positive predictive value of the ridge/villous pattern for the diagnosis of intestinal metaplasia without HGD were 93.5%, 86.7%, and 94.7%, respectively. The sensitivity, specificity, and positive predictive value of the irregular/distorted pattern for HGD were 100%, 98.7%, and 95.3%, respectively. NBI, however, was unable to distinguish areas of intestinal metaplasia from those with LGD.⁴⁵ Similarly, in a study of 63 patients who had BE, findings of

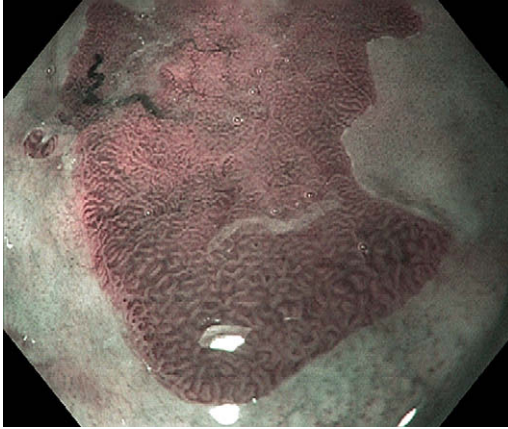


Fig. 2. NBI of nondysplastic Barrett's epithelium.

an irregular mucosal pattern, irregular vascular pattern, and the presence of abnormal blood vessels on NBI images had a sensitivity of 94%, a specificity of 76%, and a negative predictive value of 98% for HGD.⁴⁶ Is NBI (ie, electronic chromoendoscopy) better than regular chromoendoscopy? The answer to this question is not clear at this time, but a small study attempted to address this. A randomized crossover trial of 28 patients who had BE was done to compare the efficacy of indigo carmine chromoendoscopy and NBI in detecting HGD/EAC when used as adjuncts to HRE (done 6–8 weeks apart). The sensitivities for detecting HGD/EAC with indigo carmine chromoendoscopy and NBI were 93% and 86%, respectively, and were not statistically different. Although NBI and chromoendoscopy detected more lesions, these techniques were not superior to HRE in identifying patients who had HGD/EAC.⁴⁴

Autofluorescence endoscopy

A multicenter randomized crossover trial of 130 patients showed that fewer examinations were needed to diagnose additional cases of HGD/EAC using AFI-targeted biopsy plus four-quadrant biopsy compared with conventional endoscopy. The

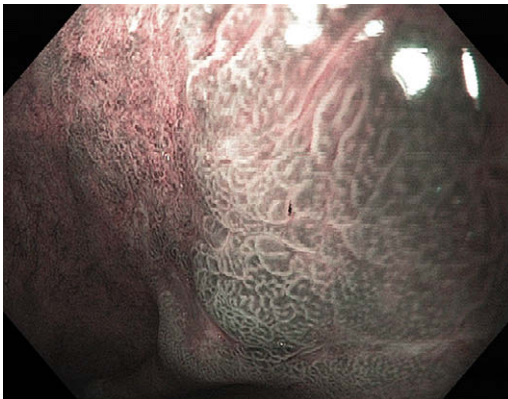


Fig. 3. Irregular mucosal and vascular patterns of Barrett's epithelium in a patient who has HGD.

sensitivity, however, was only 42% for the detection of HGD/EAC, and it was concluded that four-quadrant biopsy in addition to the endoscopic examination (AFI or conventional) was necessary. In this study, autofluorescence (Storz system, Storz Ltd., Tuttlingen, Germany) was adapted in a fiber-optic endoscope (Olympus 1T30, Olympus, Inc., Germany).⁴⁷ A feasibility study evaluating the utility of high-resolution AFI demonstrated that the use of AFI improved the detection rate of HGD in BE patients but had a positive predictive value of only 50%.¹⁹

Confocal laser endomicroscopy

There is limited literature evaluating the utility of confocal laser endomicroscopy in patients who have BE. In a study of 63 patients (GERD,²⁰ BE surveillance,³⁰ and BE with suspected neoplasia)¹³ who underwent confocal laser endomicroscopy, intestinal metaplasia could be predicted with a sensitivity and specificity of 98.1% and 94.1%, respectively. The sensitivity and specificity for detecting BE-associated neoplasia was also high at 92.9% and 98.4%, respectively.⁴⁸ Images obtained during

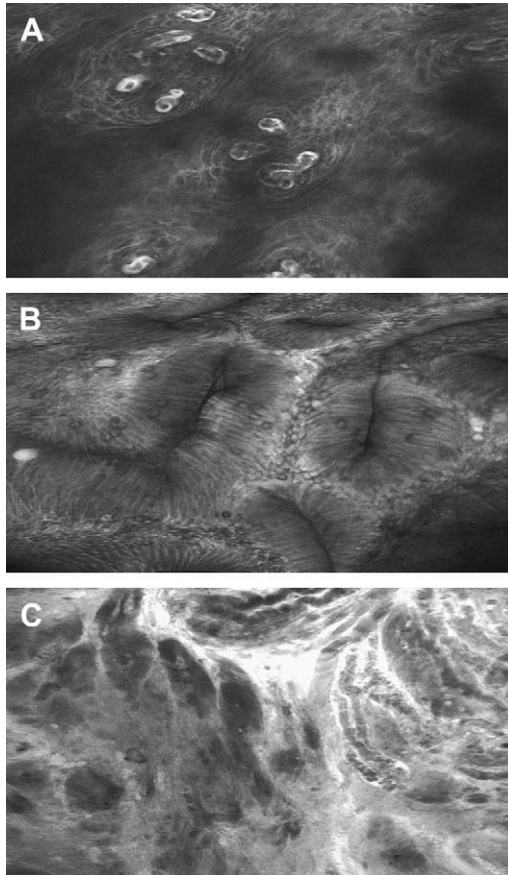


Fig. 4. Confocal images of (A) normal distal esophageal epithelium, (B) nondysplastic Barrett's epithelium with mucin in goblet cells, and (C) Barrett's adenocarcinoma with leakage of fluorescein, neovascularity, and irregular-appearing glands. (Courtesy of Ralf Kiesslich, MD, Mainz, Germany.)

the endomicroscopic examination were later assessed for interobserver agreement by a blinded investigator using the confocal Barrett classification system (Fig. 4). The mean kappa value for interobserver agreement was 0.843. A few studies using confocal laser endomicroscopy were recently presented at Digestive Diseases Week (May 2008), but full publications are awaited. Although results of these trials appear promising, further multicenter trials are awaited.

Multimodality imaging

Multimodality imaging involves the use of more than one advanced imaging modality simultaneously, with the intent of improving the detection of HGD/EAC and thus improving the sensitivity and specificity of the combined technique (eg, reducing the rate of false positivity of AFI by using NBI). The techniques that have been used in tandem are HRE, AFI, and NBI, using a prototype endoscopy system that incorporates all these techniques.

With this intention, 20 patients who had suspected or endoscopically treated HGD were initially examined by HRE and AFI, followed by NBI. AFI identified suspicious areas, and NBI with magnification was used to inspect the mucosal and vascular patterns of these areas before biopsy samples were obtained. AFI identified 47 suspicious lesions based on the color blue/violet; however, only 28 of these had HGD (false-positive rate of 40%). Subsequent evaluation by NBI led to an overall reduction in the false-positive rate (to 10%). Fourteen of the 19 false-positive areas had regular patterns on NBI.⁴⁹ In a similarly designed multicenter trial with 84 patients, AFI could identify all the HGD/EAC lesions that were seen on HRE. An additional 102 lesions were identified by AFI, of which only 19 were confirmed as HGD/EAC, giving an AFI false-positive rate of 81%. As in the previous study, the false-positive rate was reduced to 26% after examination with NBI.⁵⁰ HGD/EAC, however, was still missed in 3 patients (10%) by enhanced imaging (ie, detected only by random four-quadrant biopsy).

SUMMARY

In conclusion, the main advantages of the advanced endoscopic imaging techniques in the management of esophageal lesions are to identify abnormal mucosal and vascular patterns, to obtain targeted biopsy samples from high-yield areas, and perhaps to avoid biopsy samples from nondysplastic areas. These advantages could translate into decreased numbers of biopsies needed for the diagnosis of these conditions and a reduction in the possibility of missing these lesions on random biopsy samples obtained during standard white-light endoscopy. Image-guided endoscopic therapy (such as mucosal resection) has also been reported and is very appealing.

Although these technologies represent significant scientific advances compared with standard white-light endoscopy, certain challenges still need to be overcome, such as high interobserver variability for pattern recognition⁵¹ with NBI or chromoendoscopy, increased duration and complexity of the procedure because only small areas of the mucosa can be examined at one time with these techniques, limited access to training, lack of reimbursement, the need for additional equipment, and the lack of consensus regarding the interpretation of mucosal and vascular patterns with these techniques. It is hoped that these concerns will be addressed in the future with larger multicenter randomized trials.

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