

## Chromoendoscopy for Barrett's esophagus in the twenty-first century: to stain or not to stain?

*"I cannot pretend to be impartial about the colors. I rejoice with the brilliant ones, and am genuinely sorry for the poor browns."*

Winston Churchill (1874-1965)

Chromoendoscopy (also known as chromoscopy or vital staining) has been a controversial topic in the field of GI endoscopy for the last decade. This is not a novel technique; gastroenterologists have applied tissue stains to GI mucosa to improve endoscopic visualization and characterization of potentially neoplastic lesions for at least 30 years. The purpose of tissue staining is to characterize mucosal abnormalities that are not readily apparent or to provide fine detail of mucosal neoplasm. For example, in the esophagus, Lugol's solution can dramatically outline the boundaries of a squamous-cell esophageal cancer that does not take up the stain before biopsy or mucosal resection. In the colon, a red spot on the mucosa can be transformed into an early depressed colon cancer with the aid of indigo carmine and magnification endoscopy. A subtle colonic mucosal irregularity in a patient with chronic ulcerative colitis may be transformed to a discrete neoplastic-type lesion with methylene blue (MB) or indigo carmine staining. Yet, despite numerous studies from investigators around the world, chromoendoscopy has not yet become part of routine clinical care, except in endoscopy units with special interest in the technique. Possible barriers to the dissemination and adoption of this relatively inexpensive and readily available endoscopic technique in the United States include the perceived lack of efficacy, the lack of routine training in gastroenterology fellowship programs, the perceived time and technical difficulty of the technique, and the lack of a specific Current Procedural Terminology (CPT) code for billing and reimbursement for the additional time and effort that is added to the procedure.

The detection of intraepithelial neoplasia arising in Barrett's esophagus (BE) continues to be a challenge in the twenty-first century. Numerous techniques, including chromoendoscopy, have been developed over the years to improve the visualization of early neoplastic changes, but none have yet been consistently proven to be better than

standard "white-light" videoendoscopy and a "random" 4-quadrant biopsy approach to tissue sampling. It is unfortunate that such a costly, time-consuming, "hit-or-miss" approach is still the criterion standard for endoscopic surveillance of BE for American gastroenterologists in 2006.<sup>1</sup> It is even more unfortunate that, in actual practice, many endoscopists do not routinely perform the modified Seattle biopsy protocol.<sup>2</sup>

MB chromoendoscopy was first introduced 10 years ago as a technique for potentially improved diagnosis and

Ultimately, the ultra-rapid "in vivo" diagnosis through highly accurate advanced endoscopic imaging techniques will allow immediate clinical decision making and application of endoscopic therapy of neoplastic lesions (such as EMR), all at the same visit.

surveillance of BE.<sup>3</sup> Numerous studies published on the use of MB staining for detecting specialized columnar epithelium showed variable results. Seven studies<sup>4-9</sup> reported high sensitivity (91%-98%) and variable specificity (43%-97%), whereas 4 small studies<sup>10-13</sup> reported unsatisfactory results (sensitivity, 53%-72%; specificity, 32%-51%). Differences in the study design, the technique of MB staining, the interpretation of staining patterns, and the endoscopist's experience with vital staining have contributed to the inconsistencies in the results. To improve the technique, endoscopists have used high-magnification endoscopy, together with MB staining to improve the characterization of the esophageal mucosal pit pattern and to increase the specificity for detection of BE to 92% to 100%.<sup>14,15</sup>

Even more controversial is the role of MB chromoendoscopy for improving the diagnosis of dysplasia in BE. Two studies<sup>4,16</sup> showed MB-directed biopsy to be significantly better than random biopsy for the diagnosis of dysplasia, but 2 others<sup>8,12</sup> did not confirm these results. In the current issue of *Gastrointestinal Endoscopy*, Lim et al<sup>17</sup> report the results of another study that compared MB chromoendoscopy with random biopsy for the detection of dysplasia in BE. The investigators randomly assigned patients with

a history of Barrett's dysplasia to either MB-directed biopsy or random biopsy before repeating the alternative technique within 6 months. Of the 30 patients who completed the study, a random biopsy found dysplasia in 17 patients, whereas MB-directed biopsy of unstained areas found dysplasia in only 9, regardless of what technique was used first. The investigators concluded that MB chromoendoscopy is "less sensitive in detecting dysplasia," and they discouraged its use during routine surveillance of BE.

The study results of Lim et al<sup>17</sup> might seem to shift the evidence even further toward the negative for MB chromoendoscopy, but readers might also conclude that it adds to the confusion and controversy because of limitations of their study. First of all, 5 of the 35 originally randomized patients (14%) did not complete the study, and 4 of these dropouts were randomized to receive random biopsy first. It is also unclear whether the pathologists were unaware of the endoscopic biopsy technique used. Furthermore, the long interval between the first and second biopsy procedures (median, 90 days; interquartile range, 85-125 days) also makes the interpretation of the data difficult. Could the study results have been confounded by the regression or progression of dysplasia over the lengthy interval between the 2 study procedures? Variability in tissue sampling and pathologic diagnosis is particularly true of low-grade dysplasia, which comprised the majority of the dysplasia "missed" by MB in the study. The study results would be easier to interpret if the time between the 2 biopsy procedures was limited to a few weeks rather than months. More importantly, the investigators modified the standard MB technique, and only unstained mucosa was sampled. The standard technique for MB chromoendoscopy for the detection of Barrett's dysplasia involves a biopsy of both stained and unstained epithelium. *Ex vivo* and *in vivo* studies of Barrett's epithelium after the application of MB have shown that non-ulcerated high-grade dysplasia and carcinoma almost uniformly do not take up stain, whereas low-grade dysplasia may appear stained with MB approximately a third of the time.<sup>5</sup> Sampling of only unstained mucosa would predictably lead to preferential nonsampling of low-grade dysplasia. Finally, the prior training and experience of the endoscopist performing MB chromoendoscopy was not described in this paper. Like other techniques in endoscopy, interpretation of the results of chromoendoscopy is operator dependent, regardless of what vital stain is used. The technique of chromoendoscopy is simple, but the interpretation of staining results remains a challenge.

In the current study of MB-directed biopsy vs random biopsy, Lim et al<sup>17</sup> mentioned the concern that has been raised by Olliver et al<sup>18</sup> regarding the potential oxidative deoxyribonucleic acid (DNA) damage to MB-stained columnar epithelial cells photosensitized by white light. Although widely quoted in support of arguments against MB chromoendoscopy, the study by Olliver et al<sup>18</sup> did not prove that the DNA changes apparently induced by MB staining are permanent, clinically significant, or increase cancer

risk. There has not been any reported increased incidence of malignancy in patients undergoing surveillance with MB chromoendoscopy for BE or chronic ulcerative colitis. Furthermore, the known interaction between MB and white light has been used for purposeful destruction of mucosal cancer cells in the urinary bladder,<sup>19</sup> similar to photodynamic therapy of BE. One could take the opposite stance and postulate that MB plus white light should, in fact, be specifically studied for the possibility of ablation of Barrett's mucosa.

The concern regarding the safety of MB chromoendoscopy adds to the controversy of "to stain or not to stain" in the esophagus when one considers the published data and recommendations for the use of MB chromoendoscopy in the colon. Based on the favorable results of a randomized controlled trial showing improved detection of colorectal intraepithelial neoplasia in ulcerative colitis,<sup>20</sup> the use of chromoendoscopy by "appropriately trained endoscopists" was endorsed at a consensus conference sponsored by the Crohn's Disease and Colitis Foundation of America.<sup>21</sup> Does the recommendation for the use of chromoendoscopy during surveillance procedures for neoplasia in chronic ulcerative colitis reflect a "double standard" (that is, MB is okay for the colon but bad for the esophagus) for the interpretation of the safety of MB? And, how would one define an endoscopist appropriately trained in chromoendoscopy? The learning curve for chromoendoscopy has not been defined, as is the case with most other endoscopic techniques. There are few medical centers in the world that routinely perform MB chromoendoscopy and teach their trainees. Hence, "on-the-job" training appears to be the most practical approach to learning chromoendoscopy outside centers with special interest. With the lack of formal training in chromoendoscopy, the outcomes of vital staining for the diagnosis of GI neoplasia will predictably be varied and operator dependent.

With all the conflicting published data on chromoendoscopy, should we now totally abandon the idea of vital staining for improved detection of intraepithelial neoplasia in BE? Many would say "yes." Exciting advances in the field of endoscopic imaging could decrease or eliminate the need for dye spraying. We are now in a new era of "digital chromoendoscopy," with the recent development of "high-tech" imaging modalities that differ in their approach to improving the diagnosis of mucosal disease (Table 1).

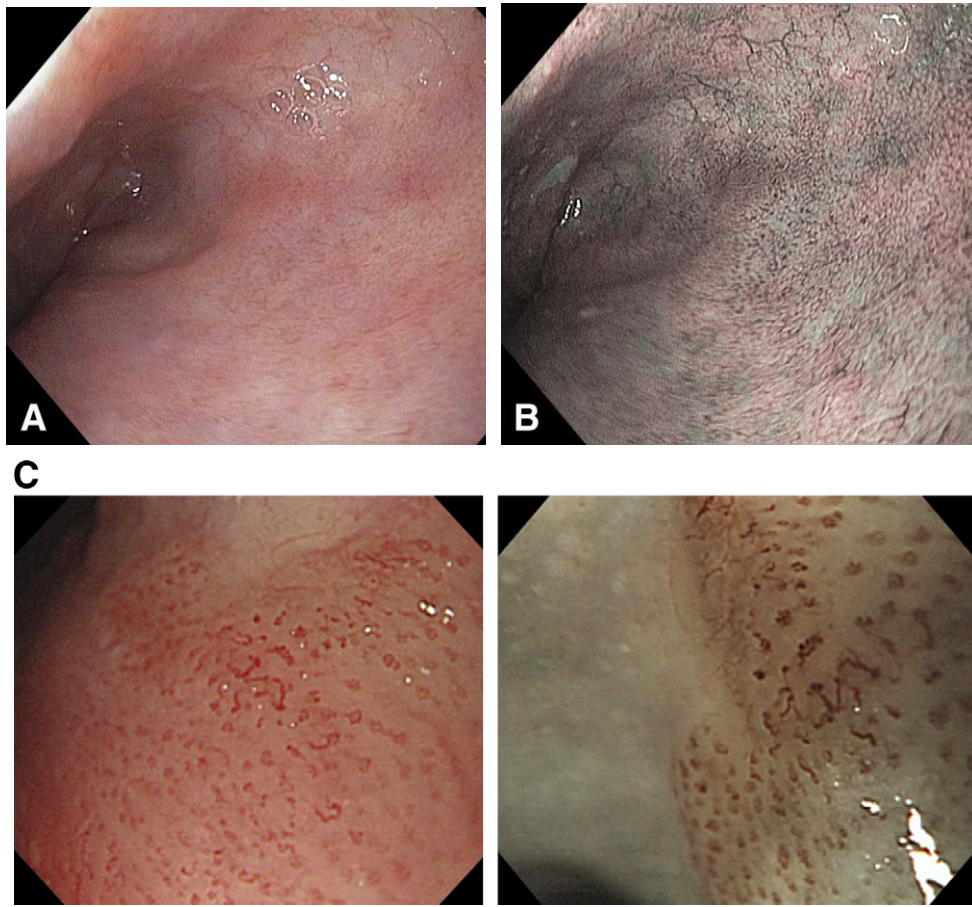
Given the impossible task of acquiring tissue samples from the entire GI mucosal area at risk, it is clear that we need some endoscopic imaging technology that combines 2 processes during screening or surveillance endoscopy. First, we need a technique that is good for scanning large areas of mucosa for possible neoplasia in an accurate and efficient fashion. Then, we need a technique that can provide minute detail of a suspicious area for further characterization (Table 1). For example, scanning techniques include high-resolution endoscopy (HRE), narrow-band imaging (NBI), and autofluorescence imaging (AFI). NBI plus HRE

**TABLE 1. Novel endoscopic technologies for improving visualization of GI disease**

<b>Technology</b>	<b>Basis for improved imaging</b>	<b>Improves general survey of mucosa (scanning)</b>	<b>Improves visualization of surface morphology (point detection)</b>	<b>Provides tissue characterization other than surface morphology</b>
High-magnification endoscopy	Enlargement of image up to 150-fold, usually used in conjunction with chromoendoscopy (magnification chromoendoscopy).		X	
HRE	Improved quality of image because of very high number of charged coupled device that increases pixel density of image and increases discrimination of detail; can be combined with optical and electronic high magnification; must be used with high-density monitor to fully appreciate improved resolution.	X	X	
OCT	Analogous to B-scan US, emitted light scatters in tissue, then is reflected back to the emitter and processed to create high-resolution (10 micron), cross-sectional, or linear images of surface epithelium as well as submucosal structures (probe based).		X	X
Autofluorescence endoscopy	Blue light excites the naturally occurring fluorescence of tissues (autofluorescence), and the system produces real-time pseudocolor images that combine red-to-green fluorescence intensities compared with surrounding tissue; probe or endoscope systems under evaluation (only endoscope-based systems permit "scanning" of GI mucosa for possible neoplastic change).	X		X
NBI	Optical filters narrow the band width of transmitted light through optical fibers, allowing preferential imaging of deeper structures, such as submucosal blood vessels; can be combined with high-resolution and high-magnification endoscopy.	X	X	X
Endocytoscopy	Probe or endoscope with ultra-high magnification capability, allowing up to ×1000 magnification and visualization of surface cellular and subcellular structures in the surface epithelium, including nuclei, nucleoli, and cytoplasm.		X	X
Confocal autofluorescence endomicroscopy	Miniaturized laser confocal microscope fitted onto the tip of a conventional videoendoscope provides high-resolution (lateral resolution 0.7 microns) optically sectioned fluorescence digital images of cellular and subcellular structures in the epithelium and up to 200 microns deep from the surface.		X	X

can provide the endoscopist with enhanced views of neoplastic mucosal irregularities, as well more detailed examination of the submucosal capillary pattern when combined with magnification (Fig. 1).<sup>22</sup> In a recent study,

NBI plus HRE was as accurate as indigo carmine high-resolution chromoendoscopy (86% vs 93%, respectively) for detection of high-grade dysplasia or early cancer.<sup>22</sup> AFI enables broad scanning of the mucosal surface of the esophagus

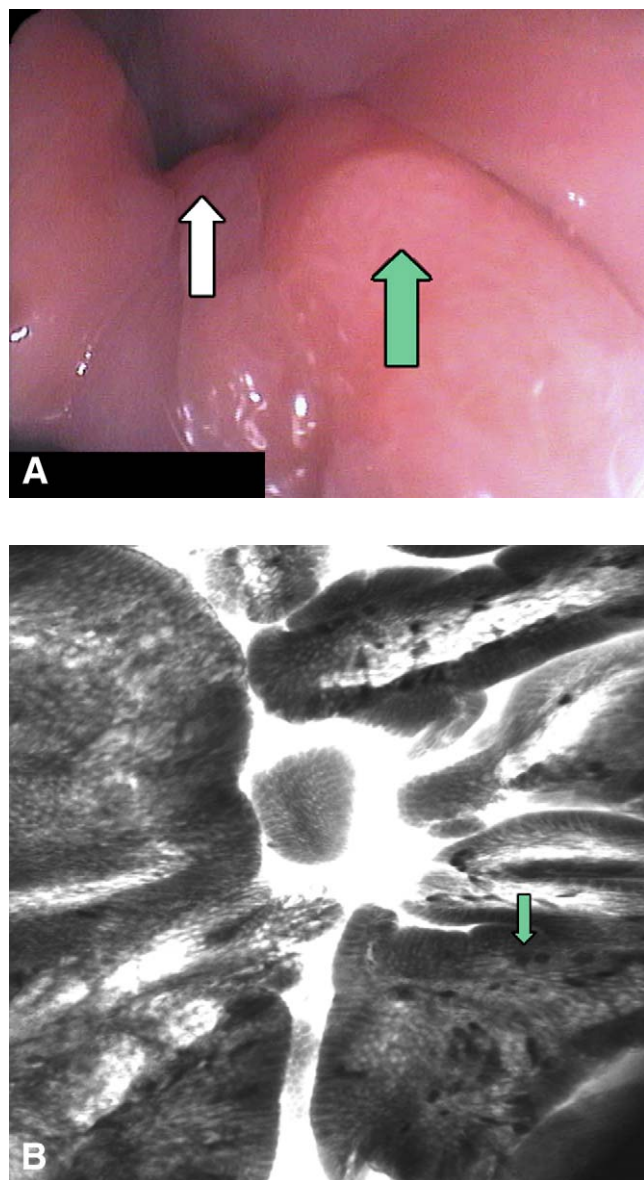


**Figure 1.** **A**, Standard white-light endoscopy image of BE. **B**, High-resolution image (NBI mode) of the same area in BE, showing enhanced mucosal detail and superficial submucosal blood vessels. **C**, High-magnification views of normal squamous epithelium adjacent to a small invasive squamous-cell carcinoma with distorted and dilated IPCLs (standard white light, left image; NBI mode right image; orig. mag.  $\times 80$ ) (courtesy of Haruhiro Inoue, Yokohama, Japan).

that is based upon inherent differences in the autofluorescence properties of neoplastic and non-neoplastic tissues. Neoplastic Barrett's mucosa tends to appear blue-violet, whereas nondysplastic BE appears green.<sup>23</sup> Rapid advances in instrumentation have also led to the development of endoscopes that combine imaging modalities, such as standard white light, NBI, AFI, high magnification, and HRE, which may allow sequential scanning, followed by point detection with 1 endoscope. Hence, with the flick of a knob or a switch, NBI and AFI may provide accurate visualization of "inapparent" or subtle mucosal abnormalities associated with high-grade intraepithelial neoplasia and early cancer, without the "hassle" factor and "mess" of chromoendoscopy. These technologies have the potential for making our current method for surveillance by using simple "white-light" videoendoscopy and random biopsies obsolete.

The candidate techniques that can provide enhanced detail of GI epithelium include optical coherence tomography (OCT), NBI plus HRE or high-magnification endoscopy (Fig. 1B and C), endocytoscopy, and endomicroscopy

(Table 1). Endoscopic OCT is based upon the interaction of light with superficial tissues; it is an optical analog of US. It can provide images of mucosa and submucosal structures. The limited data on the current generation of OCT probes suggest that the performance characteristics for detection of dysplasia in BE (accuracy, 78%; primarily because of low sensitivity) may still be suboptimal for clinical use.<sup>24</sup> On the other hand, NBI plus high magnification for visualization of distorted, dilated, or corkscrew intrapapillary capillary loops (IPCL; markers for the neovascularization that accompany neoplastic change) (Fig. 1C)<sup>25,26</sup> may provide sufficient visual clues to the endoscopist for the detection of superficial squamous-cell cancers, but it still needs to be compared with Lugol's chromoendoscopy. Finally, techniques that enable extremely high magnification ( $\geq \times 1000$ ) of mucosal tissue and subsurface structures, such as endocytoscopy<sup>27</sup> and laser confocal fluorescence endomicroscopy (Fig. 2),<sup>28</sup> may change the way that we examine GI mucosa and sample tissue, and treat early neoplastic lesions in the future. Could these techniques provide us with true "optical biopsy specimens" and decrease or



**Figure 2.** **A**, Standard white-light endoscopy image of columnar mucosa in the distal esophagus (*green arrow*), with the squamocolumnar junction shown distally (*white arrow*). **B**, Confocal endomicroscopic image of the columnar mucosa indicated in (**A**), showing typical villiform intestinal-type mucosa with dark goblet cells (*green arrow*) (courtesy of Ralf Kiesslich, Mainz, Germany). Biopsy specimens directed to this area confirmed the presence of intestinal metaplasia (BE).

eliminate the need for mucosal biopsy specimen processing and interpretation? Ultimately, we would wish that the ultra-rapid “in vivo” diagnosis and the high accuracy of advanced endoscopic imaging techniques will allow immediate clinical decision making and application of endoscopic therapy of neoplastic lesions (such as EMR), all at the same visit. While this approach may seem quite radical, it is a highly efficient and an attractive one that needs to be further studied because of its potential for making a dramatic impact on practice efficiency and the cost of GI endoscopy.

What will be challenges for the early detection of GI neoplasia in the future? Presently, improvements in “white-light” videoendoscopic imaging (such a very high number of charge-coupled devices in high-resolution endoscopes) has already enhanced the gastroenterologist’s ability to detect subtle neoplastic lesions,<sup>22</sup> even without chromoendoscopy, high magnification, or other techniques.<sup>22</sup> In the hands of experienced endoscopists, HRE alone showed an accuracy rate of 79% for diagnosis of subtle high-grade intraepithelial neoplasia or early adenocarcinoma in BE, with no significant increase in the accuracy with the addition of indigo carmine chromoendoscopy plus HRE or NBI plus HRE.<sup>22</sup> We need to study the incremental benefit of these novel advanced imaging techniques to the endoscopic diagnosis provided by HRE alone. We need randomized validation studies to be performed after pilot and prospective cohort studies describe the potential for new imaging technologies. These studies should include the investigation of the reliability and the cost-effectiveness of novel imaging techniques compared with standard endoscopic techniques. Unfortunately, these are difficult to organize and fund. Endoscope manufacturers need to increase partnerships with clinical investigators to promote high-quality studies and, by doing so, provide justification for new and expensive “toys” for imaging the GI tract. We also need consensus on terminology and techniques for novel imaging technologies. Finally, the issues of training and credentialing also need to be addressed or the adoption of new imaging techniques by GI practitioners will be slow or unsuccessful.

The future shows great promise for enhanced endoscopic diagnostics that may one day directly influence therapy. Electronic digital “chromoendoscopy” and other advanced endoscopic imaging technologies may indeed one day completely replace vital staining. For future gastroenterologists, chromoendoscopy may become just a page in the history of GI endoscopy.

## DISCLOSURE

*None of the authors have any conflict of interest to report.*

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