Methylene blue chromoendoscopy for detection of short-segment Barrett’s esophagus

Prateek Sharma, MD, Margarita Topalovski, MD, Matthew S. Mayo, PhD, Allan P. Weston, MD
Kansas City, Missouri

Background: The yield of intestinal metaplasia (IM) with randomly obtained biopsy specimens in patients with short lengths of columnar-appearing mucosa in the distal esophagus is low (30%-50%). Vital staining would be beneficial if it identified more patients with short-segment Barrett’s esophagus (SSBE). Our aim was to compare the confirmation of IM in patients with suspected SSBE (columnar-appearing mucosa <3 cm in length) by using methylene blue (MB)-directed versus random biopsies.

Methods: Consecutive patients undergoing EGD in whom columnar-appearing mucosa less than 3 cm in length was visualized underwent MB staining. Stained areas within suspected SSBE segments were targeted for biopsies. All biopsy specimens were stained with H & E with alcian blue at pH 2.5 and evaluated by a single pathologist. A historical control group (different from patients undergoing MB staining) consisted of patients with less than 3 cm of columnar-appearing mucosa in whom biopsy specimens were obtained randomly without MB staining.

Results: The MB group included 75 patients (mean age 63.8 ± 10.9 years) with a mean length of columnar-appearing mucosa of 1.2 cm (range 0.5-2.5 cm). The control group included 83 patients (mean age 60.5 ± 12.9 years) with a mean length of columnar-appearing mucosa of 1.16 cm (range 0.5-2.5 cm). IM (i.e., confirmed SSBE) was detected in 61% of the MB group versus 42% of the control group (p = 0.0237). Patients in the MB group required significantly fewer biopsies (4.3 ± 1.5 vs. 5.1 ± 12.3, p = 0.0162). Confirmation of IM by length was as follows: less than 1 cm (irregular Z line), MB 17.4% versus control 25% (p = 0.73); 1 to less than 2 cm, MB 77% versus control 45% (p = 0.03); 2 to less than 3 cm, MB 90% versus control 58% (p = 0.02).

Conclusions: MB chromoendoscopy significantly increases the detection of IM and requires fewer biopsies in patients with suspected SSBE with greater than 1 cm of columnar-appearing mucosa. It does not appear to be beneficial in patients with irregular Z lines (<1 cm). (Gastrointest Endosc 2001;54:289-93.)

Until recently, the term Barrett’s esophagus was used to describe extensions of columnar-lined epithelium of greater than 3 cm into the tubular esophagus.1,2 It has become apparent that columnar-lined distal esophagus is a mosaic of different types of columnar epithelium of which only intestinal epithelium appears to portend an increased risk of dysplasia and malignancy. Furthermore, recent studies indicate that both short and long segments of columnar mucosa that contain intestinal metaplasia (IM) are associated with increased risk.3-5 Hence, recently published practice guidelines define Barrett’s esophagus as columnar-lined epithelium of any length that can be recognized at endoscopy and confirmed histopathologically as containing IM.6

Short-segment Barrett’s esophagus (SSBE) now refers to columnar mucosa less than 3 cm in length that contain IM.7 However, in patients with short segments of columnar mucosa in whom biopsy specimens are obtained randomly, IM is confirmed in only 30% to 50%, presumably because IM is either absent or is present in a patchy distribution.8,9 The hallmark of the histologic diagnosis of Barrett’s esophagus (columnar-lined distal esophagus with IM) is the presence of goblet cells, which are present in the normal small intestine and colon but not in normal gastric or esophageal epithelium. Methylene blue (MB) is a vital dye that is taken up by goblet cells. When MB is applied to the esophagus at endoscopy, increased blue staining of the mucosa is suggestive of the presence of specialized IM, that is, Barrett’s esophagus.10,11 The use of MB chromoendoscopy in patients with short segments of columnar-appearing mucosa would be beneficial if it resulted in increased detection of specialized IM.
The aim of this study was to compare the detection of IM in patients with suspected SSBE by using MB chromoendoscopy versus random biopsies.

PATIENTS AND METHODS

Patients
Consecutive patients presenting to the Kansas City Veterans Affairs Medical Center for EGD in whom segments of columnar mucosa less than 3 cm in length were detected in the distal esophagus (suspected SSBE) were enrolled in the study. Patient demographics were noted. Written informed consent was obtained from all patients. The investigative protocol was approved by the Human Subjects Committee of our medical center as part of an ongoing study of the prevalence of SSBE. All EGDs and procurement of endoscopic biopsies were carried out by 2 endoscopists (P.S., A.P.W.).

During EGD, the relationship between the gastro-esophageal junction (GEJ) and squamocolumnar junction (SCJ) was carefully noted. The GEJ was defined as the pinch at the end of the tubular esophagus coinciding with the proximal margin of the gastric folds. The length of Barrett's esophagus was taken as the distance from the incisors to the GEJ minus the distance from the incisors to the most proximal level of displaced SCJ. The columnar epithelium was carefully inspected for the presence of erosions, nodules, and plaques.

MB group
The following protocol was used in patients in the MB group. The distal esophagus was washed with 10% acetylcysteine (Mucomyst, Apothecon, Princeton, N.J.) by using a spray catheter (GT-7, Wilson Cook Medical, Inc., Winston-Salem, N.C.) to dissolve the mucus layer and clear the esophagus of saliva and gastric secretions. Next, a 0.5% solution of MB (American Regent Laboratories, Inc., Shirley, N.Y.) was sprayed on the columnar-lined portion of the distal esophagus until dark blue staining was achieved. After 1 to 2 minutes, the distal esophagus was irrigated vigorously with tap water until there was no further loss of staining within the columnar mucosa (Fig. 1A-C). The volumes of Mucomyst and MB used varied according to the length and circumference of the columnar epithelium. All EGDs were performed with videoendoscopes (GIF100 or GIF130, Olympus America, Inc., Melville, N.Y.) and biopsy specimens were obtained with a standard forceps (Radial Jaw 3—1599 Microvasive Endoscopy, Boston Scientific Corp., Natick, Mass.).

By using criteria agreed on by the 2 principle investigators before study initiation, areas within the columnar-lined esophagus were further classified as stained or unstained. Stained areas were further classified as diffuse (>75% of the columnar mucosa) or focal.13 Biopsy specimens were obtained from all stained and unstained areas and placed in separate containers.

Control group
The control group consisted of patients (different from the MB group) with short extensions (<3 cm) of columnar...

Figure 1. A, Endoscopic view of distal esophagus in 76-year-old man; 4 narrow 2-cm extensions of columnar-appearing mucosa are evident. B, Endoscopic view of diffuse, intense staining of entire distal esophagus after spraying methylene blue. C, Endoscopic view of persistent areas of methylene blue staining (arrow) within the columnar mucosa after irrigation of distal esophagus with water. Biopsy specimens obtained from the stained area indicated by the arrow revealed intestinal metaplasia, confirming the diagnosis of short-segment Barrett's esophagus.
mucosa in whom biopsy specimens were obtained randomly without chromoendoscopy. These patients had undergone EGD with biopsies before the initiation of the MB staining protocol. In patients with circumferential columnar-lined esophagus, specimens were obtained in 4 quadrants at 2-cm intervals beginning in the distal esophagus. In patients with narrow or small extensions of columnar mucosa, at least 2 biopsy specimens were obtained from each centimeter of columnar mucosa. EGD was performed with videoendoscopes (GIF100, GIF130, Olympus) and biopsy specimens were obtained with a standard forceps (Radial Jaw 3-1599, Microvasive).

**Histology**

All biopsy specimens were placed in formalin and then embedded in paraffin. Sections cut from paraffin blocks were stained with H & E in combination with alcian blue stain at pH 2.5. Specimens were examined for the presence of IM, which was diagnosed if blue staining goblet cells were present. All specimens were interpreted by a single pathologist (M.T.) who was blinded to the study protocol. The presence of dysplasia was assessed with standard criteria as no dysplasia, low-grade dysplasia/indeterminate dysplasia, high-grade dysplasia, and adenocarcinoma.

**Statistics**

Frequencies and percentages were used to summarize categorical variables. Means and SD were used to summarize quantitative variables. Fisher exact test was used to compare proportions between the MB and control groups. Exact binomial 95% CIs were calculated for the proportion of individuals who had positive biopsy specimens in the MB and control groups. Two-sample t tests were used to compare the means between the MB and control groups. A 5% significance level was used for all comparisons is indicating statistical significance.

**RESULTS**

The MB group comprised 75 patients (all men; 95% white, mean age 63.8 ± 10.9 years). The mean length of columnar-lined esophagus was 1.2 cm (range 0.5-2.5). The mean volumes of reagents used per patient were as follows: Mucomyst 17 mL (range 8-35 mL), MB 7.2 mL (range 2-16 mL), water for irrigation 198 mL (range 60-300 mL). The numbers of patients with columnar-lined esophagus of various lengths are shown in Table 1. Over three fourths (76.8%) had a diffuse MB staining pattern. In 6 patients there was no staining: 4 in the group with less than 1 cm of columnar epithelium and 2 in the group with 1 to 2 cm of columnar epithelium. The control group consisted of 83 patients (99% men, 90% white, mean age 60.5 ± 12.9 years). The mean length of columnar-lined esophagus was 1.16 cm (range 0.5-2.5 cm). No patient in either the MB or the control group had erosive esophagitis or other visible lesions at endoscopy.

The overall detection rate of IM in the MB group was 61% versus 42% in the control group (p = 0.0237, Fisher exact test). The confirmation of IM in relation to the length of columnar mucosa is shown in Table 2, along with 95% CIs. In patients with less than 1 cm of columnar epithelium and 2 in the group with 1 to 2 cm of columnar epithelium. The control group consisted of 83 patients (99% men, 90% white, mean age 60.5 ± 12.9 years). The mean length of columnar-lined esophagus was 1.16 cm (range 0.5-2.5 cm). No patient in either the MB or the control group had erosive esophagitis or other visible lesions at endoscopy.

The number of biopsy specimens required per patient to confirm SSBE (i.e., to detect IM) was significantly less in the MB group (4.3 ± 1.5) compared with the control group (5.1 ± 2.3) (p = 0.016; 2-sample t test).

**DISCUSSION**

The rapidly rising incidence of esophageal adenocarcinoma has focused attention on the only known precursor for this cancer, Barrett’s esopha-
gus. This condition is suspected at endoscopy when columnar-lined mucosa is present in the distal esophagus and is confirmed by the demonstration of IM in mucosal biopsy specimens. The diagnosis of Barrett's esophagus is dependent on random biopsies for the detection of IM. Hence, the use of techniques that can identify IM would be beneficial with respect to the diagnosis of SSBE. Moreover, if areas of IM could be specifically identified, biopsies could be targeted that would reduce time and cost in comparison to the practice of obtaining multiple specimens in random fashion. One candidate technique for targeting IM is the use of vital stains, that is, chromoendoscopy.

Techniques for detection of IM should be readily available, widely applicable, technically feasible, and reproducible. With respect to these parameters, chromoendoscopy has yielded conflicting results. Canto et al. evaluated the use of MB chromoendoscopy in 14 patients with Barrett's esophagus (both long-segment and SSBE) and 12 control patients. The overall accuracy of MB staining for the detection of IM was 95%. However, other investigators have not been able to confirm these results. These inconsistencies may be due to differences in methodology for MB staining, including the concentration and volume of MB used, the volume of water used for irrigation, and the use of either a syringe or spray catheter to wash away excess dye. The dwell time for MB (i.e., time between MB spraying and water irrigation) may also influence staining characteristics within Barrett's epithelium. Other important factors include interobserver variability in classifying stained areas, the number of endoscopists involved, and operator experience.

The results of the present study indicate that MB chromoendoscopy significantly increases the detection of IM in patients with suspected SSBE, especially those with greater than 1 cm of columnar-appearing mucosa in the distal esophagus. It does not appear to be beneficial in patients with irregular Z lines (<1 cm of columnar mucosa). In these patients, biopsy specimens obtained randomly may be as accurate as MB-targeted specimens. Our results also show that MB staining reduced the number of specimens required for the diagnosis of SSBE.

One drawback of the present study is the use of a historical control group made up of patients with suspected SSBE in whom esophageal biopsy specimens had been obtained randomly. A better study design would have been randomization of a single group of patients to MB staining followed by random biopsies, or vice versa, after a fixed time interval. However, a strength of this study is the large number of patients in both the MB and the control groups.

The presence of dysplasia within Barrett's esophagus may affect MB staining. A heterogenous pattern of staining within areas of Barrett's epithelium harboring high-grade dysplasia has been described in one pilot project. In a recent study, Canto et al. evaluated 43 patients with Barrett's esophagus undergoing EGD with either MB-targeted biopsies or random biopsies in a randomized sequence. The mean number of specimens obtained with MB was significantly lower than the number of random specimens for the detection of IM. Furthermore, dysplasia or cancer was diagnosed in 44% of patients by MB staining compared with 20% by the random biopsy specimen method, a significant difference. Further prospective studies are needed to confirm these results before MB staining can be widely used for endoscopic surveillance in patients with Barrett's esophagus.

In conclusion, the results of our study indicate that MB staining appears to be significantly better than randomly obtained biopsy specimens for the detection of IM in patients with greater than 1 cm of columnar-appearing mucosa in the distal esophagus. If these results are confirmed by other investigators, MB chromoendoscopy will prove to be clinically useful in terms of increased identification of patients with SSBE. Before MB chromoendoscopy can be recommended generally for clinical practice, the technique must be standardized and its potential benefits confirmed in further studies. With further improvements in technology, such as magnification endoscopy, staining techniques, the characteristics of dyes, and perhaps optical methods for detection of IM and dysplasia, random biopsies may be rendered obsolete.

REFERENCES

7. Sharma P, Morales TG, Sampliner RE. Short segment


