

Acetic acid allows effective selection of areas for obtaining biopsy samples in Barrett's esophagus

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Objective The aim of this study was to determine whether macroscopic changes resulting from acetic acid application on the surface of columnar-lined esophagus allow regular, nonmagnifying, endoscopic identification of areas presenting dysplasia and/or cancer in Barrett's esophagus.

Patients and methods A total of 100 patients (mean age, 53 years; range, 27–86 years) under surveillance because of short-segment ($n=71$) and long-segment ($n=29$) Barrett's esophagus, with no alterations of columnar-lined esophagus on standard endoscopy, were enrolled. After endoscopic examination, 3% acetic acid was sprayed on columnar-lined esophagus. The subsequent appearance of the mucosa was classified as:

(1) Normal pattern: uniform reticulum along the entire columnar-lined esophagus.

(2) Abnormal pattern: reticulum presenting areas of rough or irregular appearance.

Biopsy samples were obtained from areas of normal and abnormal patterns, and the results of the corresponding histological studies were compared. All endoscopies were performed by the same investigator.

Results The endoscopic appearance, after acetic acid application, corresponded to a normal pattern in 85% of cases and an abnormal pattern in 15%. The percentage of dysplasia and adenocarcinoma in biopsy specimens was significantly higher in patients with rough or irregular areas (86.7%) than in those with normal uniform reticulum (0%) ($P < 0.001$). Sensitivity for the identification of areas of dysplasia or adenocarcinoma was 100% (95% confidence interval: 71.7–100%). Specificity was 97.7% (95% confidence interval: 91.2–99.6%).

Conclusions This prospective study shows that acetic acid test is useful for standard, nonmagnifying, endoscopic detection of dysplasia and cancer in Barrett's esophagus. *Eur J Gastroenterol Hepatol* 19:187–193 © 2007 Lippincott Williams & Wilkins.

European Journal of Gastroenterology & Hepatology 2007, 19:187–193

Keywords: acetic acid, Barrett's esophagus, chromoendoscopy, endoscopy

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Received 8 February 2006 Accepted 25 August 2006

Introduction

Barrett's esophagus (BE) is an acquired condition related to gastroesophageal reflux. It is characterized by replacement of normal, squamous esophageal epithelium by columnar epithelium with specialized intestinal metaplasia (SIM) [1–5]. The presence of these changes, irrespective of length or extension of the columnar-lined esophagus (CLE), establishes the diagnosis [3–9]. The most important risk of BE is the development of high-grade dysplasia (HGD), which in turn may lead to adenocarcinoma, first intramucous and advanced later. Although annual incidence of adenocarcinoma in patients with established BE diagnosis may be lower than that reported previously [10–12], regular endoscopic surveillance of BE with multiple biopsy obtention is advised [3–7].

Diagnosis of BE is currently established through endoscopy and biopsy: endoscopy allows macroscopic identification of CLE, of a more intense red shade than

squamous epithelium. The endoscopic appearance may also suggest to the examiner complications of BE in the form of dysplasia, with or without cancer. The study of biopsy samples confirms diagnosis, showing the presence of SIM, with or without complications. The obtention of multiple biopsy samples (specific techniques, such as jumbo forceps and four samples at 1–2 cm intervals have been suggested) may present some technical difficulty, as it is performed over a segment in constant motion (breathing, nausea), the exit direction of the biopsy forceps is tangent to the wall, and, in addition, mucus and bleeding from biopsy sites interfere in the vision field. A practical consequence of this is that patterns for surveillance of BE are highly variable [13].

For the past several years, many investigators, of our group and others, have observed that gastric intestinal metaplasia absorbs methylene blue (MB), a property

which makes MB useful for its endoscopic identification [14–17]. In 1996, Canto *et al.* [18] applied MB vital staining techniques for selective identification of SIM in BE. Despite initial good expectations [19–21], this methodology was soon questioned, owing to its technical problems and inability to distinguish between SIM and dysplasia [22,23].

An alternative method for the identification of dysplasia arises from Hinselmann [24] studies using the colposcope he described, 80 years ago. He observed that acetic acid (AA), which he used in these procedures to remove excess mucus from the cervix, produced a reaction that allowed identification of cellular changes [25]. This effect, termed acetowhite epithelium [26], proved, a few years ago, useful for recognizing remnant islets of columnar epithelium after endoscopic therapy of BE [27]. AA, alone or combined with indigo carmine, also permitted, with the aid of endoscopic magnification, the detection of SIM in CLE [28–30].

In our practice, during standard, nonmagnifying endoscopy, we have repeatedly observed particular reactions of CLE, after exposure to AA, used to clear excess mucus before other contrast agents were applied. These reactions, interacting with AA and CLE, appeared to relate to the presence or absence of severe dysplasia or adenocarcinoma, as shown by the study of biopsy samples.

In the background of previous scientific reports, we therefore decided to carry out a prospective study, to assess whether AA may be useful to allow effective identification of areas of dysplasia and/or adenocarcinoma in BE using standard, nonmagnifying endoscopy.

Patients and methods

An observational prospective study was designed to determine the sensitivity and specificity of the AA test to identify dysplasia and/or adenocarcinoma, by standard upper endoscopy associated with chromoscopy, in patients with BE.

One hundred upper endoscopies performed on 100 patients under follow-up were included in this study. After an initial endoscopic examination, 3% AA was sprayed onto the CLE, starting at 2 cm proximal to the squamocolumnar junction, and using an Olympus spray catheter (PW-5L) (Olympus Spain, Barcelona, Spain). The volume of AA depended on the length of the CLE (approximately 1 ml for each centimeter of CLE). About 15 s later, excess AA was washed off with 10–30 ml of water. Then, the resulting mucous surface pattern of

CLE was evaluated and classified according to the following criteria:

- (1) Normal pattern: uniform and diffuse reticular pattern, in which the mucous reticulum and the whitish reaction are homogeneous and appear at the same level.
- (2) Abnormal pattern: reticular pattern with rough areas (some of these with slightly elevated mucous surface) or irregular areas (thicker areas with cracks).

Biopsy specimens were obtained by the standard follow-up protocol. Specimens were also taken from rough or irregular areas when present. The study was approved by the institutional review board of our hospital, and all patients provided written consent for the procedure.

Histopathologic diagnosis

All specimens were immediately placed in 10% formalin solution, embedded in paraffin and sectioned. Tissue sections were stained with hematoxylin and eosin, and also periodic acid-Schiff–alcian blue. Biopsies were reported using the following five-tiered classification of histological diagnosis in BE: negative for dysplasia, indefinite dysplasia, low-grade dysplasia, HGD, and carcinoma [31–33].

Statistical analysis

A descriptive analysis of the variables obtained was performed. Sensitivity, specificity and predictive values, with 95% confidence interval (CI), were calculated to determine the diagnosis potential of AA to detect dysplasia and/or cancer. Fisher's exact test with a bilateral approach was used to compare the percentage of cases of dysplasia or cancer according to the endoscopic appearance of CLE after AA application. The statistical analysis was carried out using SPSS 12.0 for Windows (SPSS Inc., Chicago, Illinois, USA) and Epilnfo (Experts Exchange LLC, Atlanta, Georgia, USA) software.

Results

One hundred patients under BE follow-up were included in this study. All the endoscopies were performed by one investigator between July 2001 and February 2005. Out of the 100 patients, 69 were men and 31 were women. Median age was 53 years, and patients' age ranged from 27 to 86 years. Short-segment BE was seen in 71 patients and long-segment BE was observed in 29 patients. After AA application, CLE presented a uniform appearance in 85 cases. An abnormal pattern was observed in 15 patients. The effect of AA lasted for about 2–3 min. No complications owing to the use of this technique were registered. The characteristics of patients are set out in Table 1. Figures 1–3 show images of the different endoscopic appearances of CLE after application of AA.

Table 1 Principal characteristics of the patients

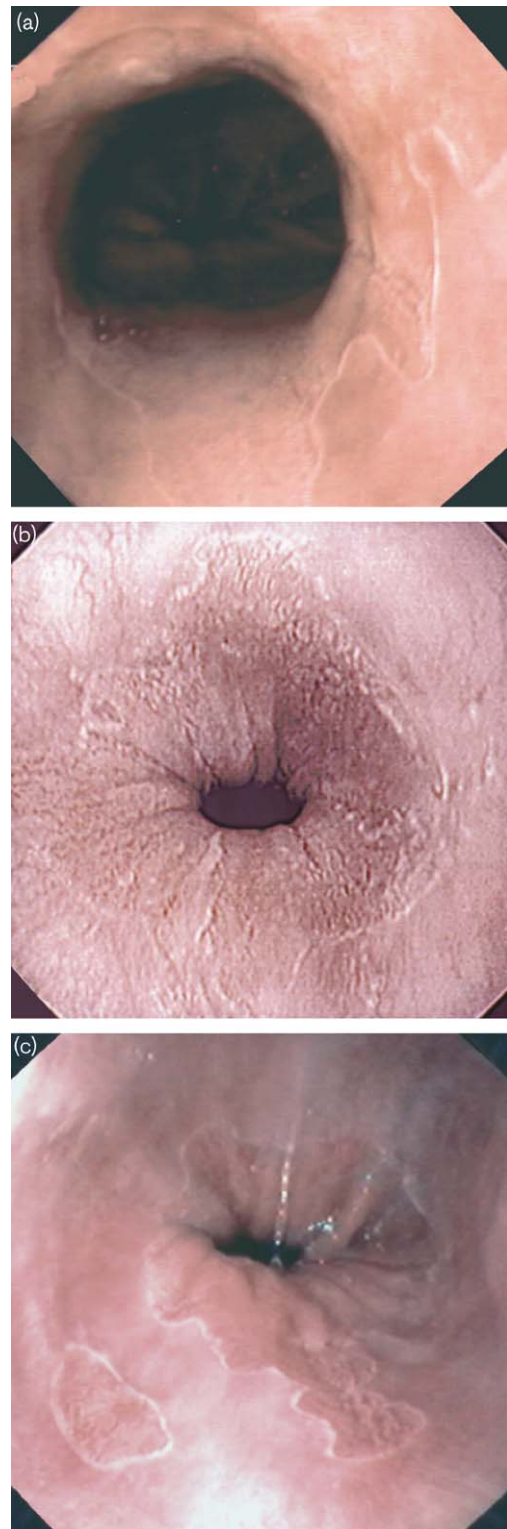
Total patients	100
Mean age (years)	53 (27–86)
Men	69
Women	31
Barrett's esophagus	
Short segment	71
Long segment	29
After acetic acid application	
Normal pattern	85
Abnormal pattern	15

Histopathologic examination of the biopsy samples identified dysplasia or cancer in 13 out of the 100 examinations performed. The percentage of cases of dysplasia or adenocarcinoma was significantly higher in patients in which AA revealed a rough or irregular appearance (86.7%), when compared with those with a uniform pattern (0%) ($P < 0.001$). The results of the biopsies in regard to findings after using AA are shown in Table 2. The sensitivity of the AA test to identify areas with dysplasia or adenocarcinoma was 100% (95% CI: 71.7–100%) and specificity was 97.7% (95% CI: 91.2–99.6%). Rough or irregular areas were observed in all cases of dysplasia and adenocarcinoma. The diffuse uniform pattern was seen in all patients without dysplasia. Two patients showing an abnormal pattern presented no dysplasia or cancer, but were diagnosed of other anomalies (esophagitis and ulcer). The positive predictive value of the AA test to detect areas with dysplasia or adenocarcinoma was 86% (95% CI: 58.4–97.7%) and its predictive negative value was 100% (95% CI: 94.6–100%).

Discussion

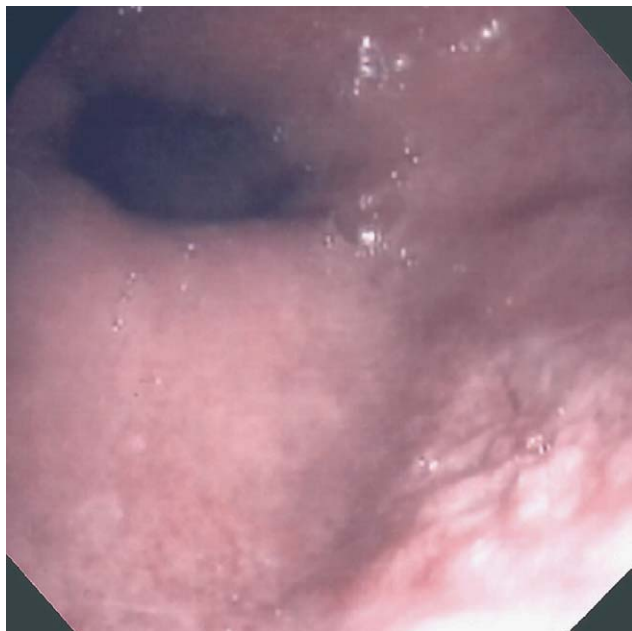
Diagnosis and effective endoscopic follow-up of BE are, though laborious in some cases [3–10], very important as CLE may lead to cancer [10–12]. As Sampliner [4] explained, 'The goal of surveillance in patients with BE is the detection of dysplasia and early cancer. Dysplasia occurs in the background of metaplasia, a fundamental and distinctive change in the epithelium of the esophagus from one differentiated cell type to another. Dysplasia represents the final step of neoplasia, and is characterized by cytological changes and architectural changes. Dysplasia is the best current indicator of the risk of cancer'. Endoscopic biopsy is the only known approach to reliably establish diagnosis of dysplasia and early cancer in a nonsurgically treated BE.

Which is the ideal number of biopsy specimens to obtain, to ensure best results in the detection of dysplasia or early cancer? No ready answer is available to this question. Some follow-up protocols advocate taking up to four specimens per centimeter of a short-segment BE, or every 2 cm in patients with long-segment BE [4,6]. Notwithstanding, technical reasons already mentioned make this a frequently very difficult goal to achieve.

Fig. 1

(a) Effect of the acetic acid on patients with Barrett's esophagus and no dysplasia. The acetowhite reaction, which occurs almost immediately and lasts for about 3–4 min, affects both epithelia and is more marked in the area of epithelial transition. (b, c) In columnar-lined esophagus without dysplasia, a homogeneous, uniform and diffuse reticular pattern may be seen at the same level.

Fig. 2



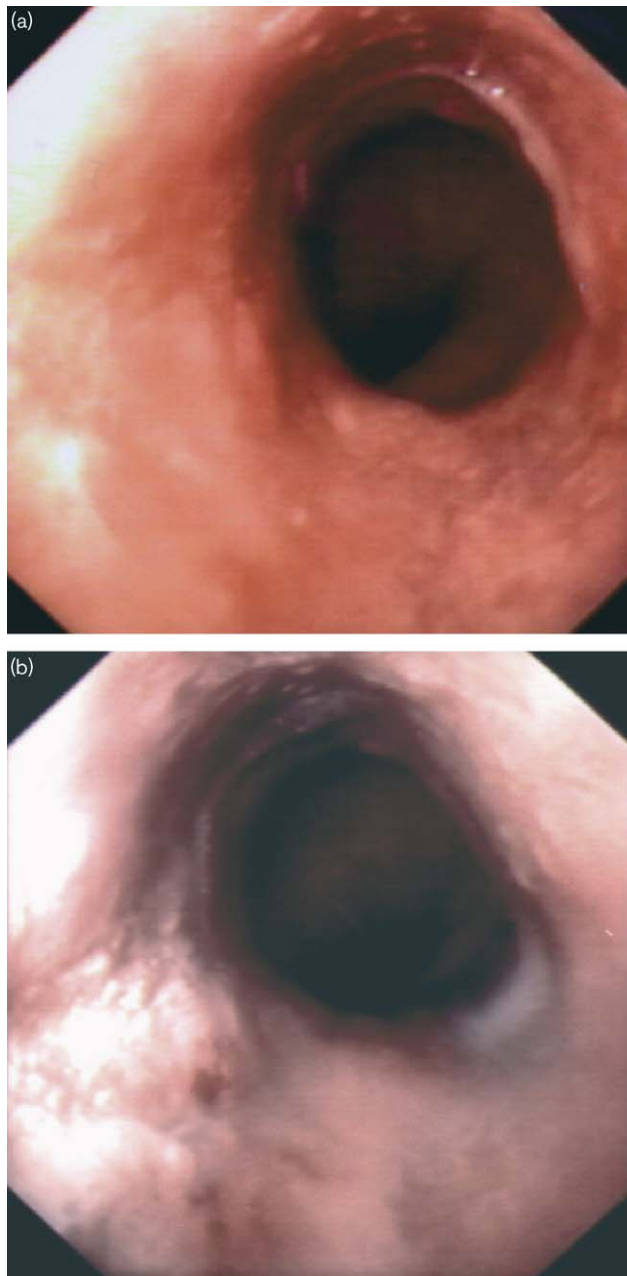
A rough area appeared as a result of the application of acetic acid on columnar-lined esophagus in this patient. High-grade dysplasia was found in the biopsy specimens.

Although we may consider these protocols adequate, a simple mathematical calculation will show that most of the esophageal cancers will not be histologically studied: if we hypothetically calculate 20-mm diameter for the esophageal lumen, the lateral surface of a 10-mm tall CLE cylinder will be 628 mm^2 ($2 \times 3.1416 \times 20/2 \times 10$). Each biopsy specimen has a maximum 9 mm^2 (3×3), and thus four biopsy specimens every centimeter will represent a surface of 36 mm^2 (9×4). Hence, the area of mucosa of that cylinder in which specimens are not taken is 592 mm^2 ($628 - 36$), that is, 95% of CLE.

Considering this, we may deduce that a key to a reliable surveillance in patients with BE could be provided by an endoscopic system allowing selection of areas with suspected dysplasia or early cancer, thus permitting targeted biopsy sample obtention. This has been the aim of all techniques differing from standard endoscopy, including vital staining [18–23], endoscopic magnification [28,29] and other more sophisticated techniques available only to advanced researchers [34–37].

Techniques such as vital staining and endoscopic magnification have not resolved this problem. MB has been successfully used for detection of SIM [18]. Although identification of areas of dysplasia and early cancer, through differences in intensity and uniformity of staining, mainly in ex-vivo studies, has been considered

Fig. 3



(a) Esophagus at 32 cm in patient with long-segment Barrett's esophagus. (b) The same area after acetic acid was applied. An irregular white area can be seen. Adenocarcinoma was found in the biopsy specimens of this area.

an important advance by some authors [20], other researchers have not considered it so [38].

In 1978, our group found that gastric mucosa with dysplasia and cancer sometimes presents absorption of MB, the appearance of which may be indistinguishable from that observed in areas with intestinal metaplasia

[16]. Staining with MB has some minor problems: time needed for exploration, a reduced endoscopic light intensity and a blurred field of vision.

Magnification techniques have two main difficulties: they are not available in all the endoscopy units, and are laborious and difficult to use in large areas of CLE. Besides, interesting communications using magnification endoscopy by itself, so far, correspond only to identification of SIM and to the description of different endoscopic patterns of CLE [28]. Magnification endoscopy alone has not proved useful for detecting HGD or early cancer.

Our results show that a simple methodology is all that is needed to obtain good results regarding prebiopsy selection of mucosal areas with suspected dysplasia or cancer. As shown in Table 2, dysplasia or cancer was not found in any of the 85 endoscopies in which the pattern of the esophageal mucosa after AA application was identified as uniform. That is, a uniform pattern corresponded to the absence of dysplasia in 100% cases. On the contrary, out of the 15 cases in which rough or irregular areas were observed, only two of them were free of dysplasia or cancer (one of them had an ulcer in CLE and the other one had associated esophagitis, so the presence of a rough area might be a result of mucosal inflammation). Three of the four cases with irregular areas had adenocarcinoma, and the other one presented HGD. The statistical significance of these data regarding the presence of dysplasia or cancer in rough or irregular areas as compared with the uniform pattern is $P < 0.001$. Sensitivity (100%) and specificity (97.7%) of this method to detect dysplasia and cancer are as well very high. Noteworthy is also the fact that positive predictive value of this test was 86.7%, and negative predictive value was 100%.

The mechanism of action of AA in CLE is not yet fully understood. The explanation may be similar to that accepted for interaction of AA and uterine cervix,

although dysplasia and cancer develop into a CLE in one case, and into a squamous epithelium in the other.

Application of AA on the mucosa results in a reaction which brings out differences between squamous and columnar epithelium, because acetowhite reaction is different in each case. When reaching subepithelial tissue, AA may produce capillary congestion and swelling, and consequently turgescence of papillae of glandular mucosa. In addition, AA reacts with intranuclear proteins that change chromatin condensation, and it may also react with cytoplasmic proteins (cytokeratins), producing a change in its spatial configuration. Accordingly, the acetowhite reaction affects those areas with higher nuclear concentration more intensely, as is the case of gland crypts, regenerating areas or areas with atypical epithelium [30,39]. These are transient effects. The concentration of AA used in this study is about half of that of current cuisine vinegar, and in the conditions we have used there appears to be no question over its safety.

Although it has been generally accepted that the acetowhite reaction is useful to detect neoplastic areas in the squamous stratified epithelium of the esophagus, there are also reasons to believe that in esophagus, an adenocarcinoma in BE acquires the cytokeratins of columnar cells, while retaining those of the squamous epithelium [30]. On the other hand, in the uterine cervix, the intensity and rate of the acetowhitening phenomenon are proportionate to the density of the nuclei and the permeability of the tissue to AA. When densely packed nuclei are accessible to AA, an observable acetowhitening effect will occur. When epithelial cells are intact, highly differentiated and acting as an effective barrier to AA, little or no acetowhitening is observable [39]. If we translate these observations to the CLE, we can infer that the pattern will be uniform when no dysplasia or cancer is present. With an increasing epithelial vascularization owing to cell changes, and an increased nuclear concentration in the area caused by dysplasia or cancer, that pattern will change, and become slightly rough first and irregular later.

Our findings are not in contradiction with those reported by Guelrud *et al.* [28] for combining magnification and chromoscopy with the AA test. These authors identified SIM in all cases and classified it in specific mucous patterns using magnification (up to $\times 35$). They did not describe the appearance of dysplasia or of cancer.

Our study provides evidence of the acetowhite reaction effect on areas of dysplasia, an effect long used in gynecology and that is visible without magnification. AA has been long used during colposcopy to identify suspicious lesions [40]. In fact, Hinselmann [25], the father of the colposcope, already drew attention, in 1938,

Table 2 Histopathologic findings according to endoscopic pattern after AA application

No. of patients	After AA application	Dysplasia			Adenocarcinoma	Total
		Negative	LGD	HGD		
100	Normal pattern	85	0	0	0	85
	Abnormal pattern	2 ^a	5	5	3	15

Dysplasia was detected in 10 patients. Nine cases presented rough areas and one had irregular areas.

Adenocarcinoma was detected in three cases, all with irregular areas.

Dysplasia or cancer was not seen in any of the cases with normal pattern. $P < 0.001$.

AA, acetic acid; HGD, high-grade dysplasia; LGD, low-grade dysplasia.

^aOne patient, esophageal ulcer; one patient, associated esophagitis.

to this peculiar effect of AA on cervix, an area of epithelial transitions, as is the case of those occurring between normal squamous epithelium and CLE in esophagus. The acetowhite reaction is very obvious in the area of epithelial transition of the cervix, and also when the squamous epithelium contains undifferentiated, immature, neoplastic squamous cells [26]. In recent years, gynecologists have verified that the effect of the acetowhite reaction allows identification of HGD and cervical cancer by the naked eye [41–43], and that visual magnification does not improve the interpretation of results [44,45]. Recently, Amano *et al.* [46] have reported that the combination of crystal violet chromoendoscopy and pit pattern diagnosis in BE patients, studied using standard, nonmagnifying endoscopes, provides results showing good correlation between villous pit pattern and dysplasia, as well as between irregular pit pattern and carcinoma. Despite the fact that their methodology differs from ours in that we use AA, the results obtained by these investigators are in good agreement with ours.

In conclusion, according to our results, and despite the fact that our study is based on a small sample, the AA appears to be very effective for detection of areas with dysplasia and cancer in BE during standard endoscopy, with naked eye. Further controlled, randomized and blind studies are required before the use of this test can be advised to substitute standard 'blind' biopsy or other chromoendoscopy techniques, in the surveillance of BE.

Acknowledgement

The authors express their special thanks to Sonia Pértega for statistical analysis.

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