Dig Dis Sci
DOI 10.1007/s10620-010-1497-6

ORIGINAL ARTICLE

Computer-Assisted Analysis of Abrasive Transepithelial Brush Biopsies Increases the Effectiveness of Esophageal Screening: A Multicenter Prospective Clinical Trial by the EndoCDx Collaborative Group

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Received: 27 August 2010 / Accepted: 10 November 2010

Abstract
Background The sensitivity of screening for Barrett's esophagus (BE) and esophageal dysplasia (ED) is hampered by the limited amount of tissue that can be sampled by forceps biopsy (FB).
Aim The aim of this study was to evaluate computer assisted analysis of an abrasive, transepithelail brush biopsy as an adjunct to FB to increase detection of BE and ED.
Methods This was a multicenter prospective trial of patients being screened for BE and ED. Each patient had two brush biopsies (BB) and then random four-quadrant FB every 1–2 cm of the esophagus. All BB were examined with computer assistance by pathologists at CDx Laboratories (Suffern, NY), and all FB were examined by the investigators' local pathologists.
Results Of 1,266 patients enrolled, 363 were diagnosed with BE by FB alone and 146 additional cases of BE were identified by adding BB. The addition of BB to FB increased the overall detection of BE by 39.8% (95% CI 32–48%). This added detection of BE in 11.5% of all patients tested with the BB (146/1266) resulted in a number of patients needed to test (NNT) to obtain each additional positive finding of Barrett's esophagus of 8.7. Among a subset of 848 patients with gastroesophageal reflux disease and no prior history of BE, the addition of BB to FB identified an additional 105 patients with BE increasing the overall detection of BE by 70.5% (95% CI 54–90%). Dysplasia was diagnosed in 16 patients by FB alone, with an additional 14 cases detected by adding BB. The addition of BB to FB thus increased the detection of ED by 87.5%.
Conclusion These results suggest that adjunctive computer-assisted analysis of an abrasive brush biopsy has the potential to substantially improve the detection of Barrett's esophagus and dysplasia in screening populations.

Keywords GERD · Barrett's esophagus · EGD · Surveillance · Brush biopsy

Introduction

Barrett's esophagus (BE), a potentially serious consequence of chronic gastroesophageal reflux disease, is diagnosed by biopsy findings of specialized columnar epithelium, which is characterized by acid mucin-containing goblet cells. The importance of BE lies in its being the precursor of nearly all cases of esophageal adenocarcinoma [1]. Patients with Barrett's esophagus have a risk of esophageal adenocarcinoma 30–60 times that of the general population [2]. Other risk factors for malignant esophageal transformation include increasing age, increasing length of Barrett's segment, low grade or high grade esophageal dysplasia, male sex, obesity and tobacco use [3, 4].

The incidences of both BE and esophageal adenocarcinoma have increased dramatically in recent years such that adenocarcinoma is now the predominant form of esophageal cancer in the United States [5]. In fact, the incidence of esophageal adenocarcinoma has increased 4–10% per

Members of the EndoCDx Collaborative Group are given in the Appendix.

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Published online: 04 December 2010

year among US men since 1976, more rapidly than for any other type of cancer [6]. Despite advances in diagnosis and therapy, esophageal adenocarcinoma remains aggressive and usually lethal with a median survival of less than 1 year [7]. It has been postulated that neoplastic progression in BE occurs in a subset of patients with an acquired genomic instability who generate abnormal clones of cells that ultimately become an early carcinoma [8]. Therefore, it may be useful to combine histology with flow cytometry to identify a subset of patients with BE who require more frequent endoscopic surveillance for the early detection of dysplasia and carcinoma [9, 10].

The sequence from intestinal metaplasia to dysplasia to cancer is currently monitored by careful endoscopic observation with multiple random forceps biopsies [11]. However, the standard protocol of four-quadrant biopsy at 1–2 cm intervals has significant sampling limitations. Since intestinal metaplasia, dysplasia and carcinoma are not uniformly distributed within the columnar-lined mucosa, random forceps biopsies may miss abnormalities located between the sampled areas, resulting in low sensitivity [12]. In fact, by the time biopsy specimens show high-grade dysplasia in patients with BE, approximately one third of patients already have an unsuspected, invasive cancer, even with rigorous surveillance using a jumbo biopsy protocol [13, 14].

In view of this problem, we designed a study to assess the value of a computer-assisted analysis of an abrasive, transepithelial brush biopsy (EndoCDx®, CDx Laboratories, Suffern, NY) as an adjunct to standard endoscopic evaluation with multiple random forceps biopsies in the detection of BE and dysplasia. Specifically, the objective of the study was to determine if and by how much the detection of BE and esophageal dysplasia can be increased by the addition of EndoCDx to a standard esophageal biopsy protocol.

Methods

A prospective multicenter, community-based trial utilizing EndoCDx testing was conducted at eight sites. During the study interval (2004–2005), all patients over age 18 who were scheduled for mucosal forceps biopsies for screening or surveillance of BE and dysplasia were eligible for enrollment. Patents with symptoms of gastroesophageal reflux and suspected BE as well as those with known BE undergoing surveillance for dysplasia were enrolled. Institutional Review Board (IRB)-approved consent was signed by all patients before participating.

EndoCDx kits were supplied to investigators and consisted of a brush biopsy instrument (a proprietary abrasive brush which obtains a transepithelial specimen), two precoded glass slides, an alcohol/carbowax fixative pouch, a vial containing approximately 5 cc of alcohol and a preaddressed packet for submitting the contents. Investigators completed a test requisition form which included demographic data such as the patient's age and gender, clinical esophageal findings including the length of suspected or known Barrett's, the presence of stricture or inflammation, and total number of forceps biopsies and brush biopsies performed.

Investigators were provided with written instructions on how to perform the brush biopsy. Briefly, the brush (Fig. 1) was passed through the working channel of the endoscope, placed against the surface of the mucosa, and while maintaining firm pressure, rotated and repeatedly passed back and forth in all four quadrants over the entire esophageal abnormality (Fig. 2). Pinkish red tissue or

Fig. 1 The EndoCDx brush biopsy instrument. The EndoCDx brush is a proprietary abrasive brush biopsy instrument which penetrates the entire epithelium (transepithelial) and was specifically designed to consistently sample deeper layers of the more firmly attached glandular epithelium found in Barrett's esophagus

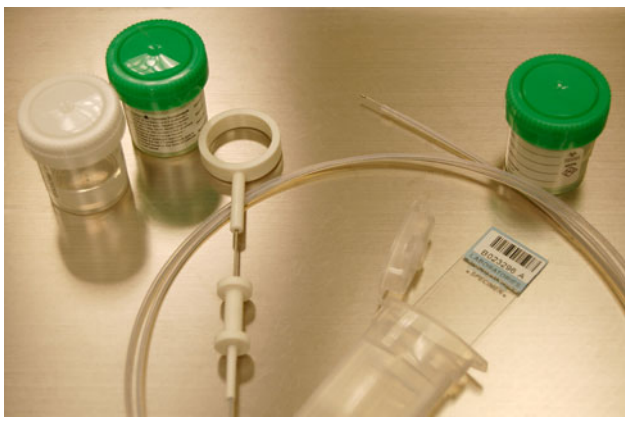

Fig. 2 The EndoCDx kit. All the components needed to perform an EndoCDx brush biopsy are included in a kit which contains a sterile brush, a bar-coded glass slide pre-labeled to match the label on the test requisition form, and liquid fixatives for collecting the brush tip for a cell block

pinpoint bleeding at the brush biopsy site was evidence of proper technique.

The cellular material collected on the brush was then transferred to a bar-coded glass slide and rapidly flooded with fixative to avoid air-drying. A second brush biopsy sample of the esophageal abnormality was then obtained and a second glass slide specimen was prepared. Finally, the bristle portion of the brush was clipped off into the liquid-filled vial. After approximately 15 min, the dry slides were placed in a plastic slide container and together with the vial and bar-coded requisition form, sent in the preaddressed mailing container. Immediately after the brush biopsies were performed, the investigator obtained standard four-quadrant forceps biopsies of the esophagus at 1–2 cm intervals.

All EndoCDx specimens were analyzed at CDx Laboratories in Suffern, NY, and all forceps biopsy specimens were analyzed by the investigators' local pathologists. Patients with inadequate brush biopsy or forceps biopsy results or those with missing pathology reports were excluded from the study. Forceps biopsy results that were histologically characterized as "suggestive of" or "compatible with" Barrett's esophagus were classified as Barrett's esophagus. Pathologists analyzing the EndoCDx specimens and forceps biopsies were blinded from each other's results.

At CDx Laboratories, one of the slides was stained in accordance with a modified Papanicolau method and the second slide with Alcian blue. Concomitantly, cellular material was harvested from the brush tip into alcohol via mechanical abrasion. A cell block was prepared from the fluid and stained with hematoxylin and eosin.

The brush biopsy method creates a disaggregated tissue specimen containing a three-dimensional array of intact tissue fragments ("microbiopsies"), individual cells, and cell clusters. Laboratory interpretation of this complex specimen is aided by the EndoCDx computer system, which consists of a neural network-based image processing system, to scan the stained slides, and generate images of suspect cells. Review and interpretation of the computer-generated images, in conjunction with a standard microscopic evaluation of each of the slides, were performed by a pathologist utilizing standard pathogneumonic criteria for disease to make the diagnosis.

The results of the FB cross classified by BB were arrayed as multinomial outcomes of the total number of cases. The proportionate increase in yield provided by adding BB to FB was estimated as the ratio of the number of cases that were negative on FB but positive on BB, to the number of cases that were positive on FB. Confidence intervals for this ratio were estimated using Fieller's theorem. Results were calculated with Mathematica software, version 6.1 (Wolfram Research, Inc., USA).

Results

There were 1,266 patients included in the study, of which 1,183 were adequate by both FB and BB. Their demographic features and esophageal findings are summarized in Table 1.

The detection rates of BE are shown in Table 2. Among the 1,183 patients, BE was diagnosed in 363 patients by FB and in 340 patients by BB. Of the 340 patients with BE detected by the BB, 146 had negative FB results. Thus, the addition of two brush biopsies to the standard multiple forceps biopsy protocol increased the detection of BE by 39.8% (146/363, 95% confidence interval 32–48%). This added detection of BE in 11.5% of all patients tested with the BB (146/1,266) resulted in a number of patients needed to test (NNT) to obtain each additional positive finding of Barrett's esophagus of 8.7.

While dysplasia was a relatively uncommon finding in this predominantly screening population, detection rates of dysplasia by the two techniques are shown in Table 2. Esophageal dysplasia was diagnosed in 16 patients by forceps biopsy and in 19 patients by brush biopsy. Of the 19 patients diagnosed with esophageal dysplasia by brush biopsy, 14 had negative forceps biopsy results. Thus, the addition of two brush biopsies to the standard multiple forceps biopsy protocol increased the detection of

Table 1 Profile of study patients and esophageal findings ($n = 1{,}183$)

Characteristic	Value
Male:female	54%:46%
Mean age	58 y
Age range	18–90 y
Average suspected Barrett's esophagus (BE) length	2.5 cm
Presence of esophageal stricture	4%
Presence of esophageal inflammation	48%

Table 2 All cases: Increased detection of Barrett's esophagus or dysplasia ($n = 1{,}183$)

Forceps biopsy (FB)	Brush biopsies (BB)		
	Negative	Barretts	Dysplasia
Neg	670	139	7
Barrett's	166	178	7
Dysplasia	3	8	5

	Relative increase	Absolute increase (%)	Needed to test (NNT)
Barrett's+	39.8% (CI 32–48%)	11.5	8.7
Dysplasia	87.5%	1.1	90.4

Table 3 Screening patients: Increased detection of Barrett's esophagus or dysplasia ($n = 792$)

Forceps biopsy (FB)	Brush biopsies (BB)		
	Negative	Barretts	Dysplasia
Neg	538	101	4
Barrett's	85	57	3
Dysplasia	1	2	1
	Relative increase	Absolute increase (%)	Needed to test (NNT)
Barrett's+	70.5% (CI 54–90%)	12.4	8.1
Dysplasia	175%	0.8	121

Table 4 Surveillance patients: Increased detection of Barrett's esophagus or dysplasia ($n = 391$)

Forceps biopsy (FB)	Brush biopsies (BB)		
	Negative	Barretts	Dysplasia
Negative	132	38	3
Barrett's	81	121	4
Dysplasia	2	6	4
	Relative increase	Absolute increase (%)	Needed to test (NNT)
Barrett's+	18.8% (CI 13–25%)	9.8%	10.2
Dysplasia	58%	1.7%	60

esophageal dysplasia by 87.5% This added detection of BE in 1.1% of all screening patients tested with the BB (14/1,266) resulted in an NNT to obtain each additional positive finding of dysplasia of 90.4.

Tables 3 and 4 show the increased yield by BB among screening patients with no prior history of BE or dysplasia (792 patients) and among those patients with prior history of BE or dysplasia (391 patients), respectively. Among the 792 screening patients without prior history of BE or dysplasia, adding an average of two brush biopsies to the standard multiple forceps biopsy protocol increased the detection of BE by 70% (95% CI 54–90%, $n = 254$).

Discussion

Current strategies for improving survival in patients with esophageal adenocarcinoma have focused on cancer detection at an early and potentially curable stage and prevention of its development by the detection of earlier stage dysplasia [12].

Most efforts have focused on enhanced techniques for endoscopic imaging and tissue sampling, combined with molecular markers to identify patients at high risk for malignant transformation [15]. Endoscopic imaging techniques such as high-resolution endoscopy, narrow band imaging, and autofluorescence endoscopy are being investigated for detection of early esophageal lesions [16]. Indigo carmine chromoendoscopy may be a useful adjunct to high-resolution endoscopy for mucosal lesions, but not for submucosal lesions [17, 18]. However, the large numbers of routine biopsies needed for identifying additional foci of dysplasia or cancer were not reduced by the use of other chromoendoscopy agents such as dye staining with methylene blue because of low sensitivity [19].

Screening for dysplasia may also be enhanced by new cytologic, spectrographic, and tomographic methods [20]. Optical spectroscopy using diagnostic molecular and/or microstructural information contained in light-tissue interactions has the potential to enhance the detection of Barrett's esophagus in real time [21]. Since most early neoplastic lesions of the esophagus are focal and not visible to the endoscopist, it is not surprising that sampling error exceeds 95% using a standard four-quadrant biopsy protocol [20].

In this study, brush biopsy samples were analyzed with the aid of a computerized digital image analysis system designed to detect abnormal glandular cells that might otherwise have been missed with manual microscopic screening. The significant increase in detection of BE and dysplasia in this study is likely due to the *combination* of an abrasive brush with a computerized digital image analysis system. Similar innovations applied to oral exfoliative cytology have resulted in a highly accurate method of detecting oral dysplasia and carcinoma [22, 23].

While many techniques that increase the detection of abnormality also increase false positive results, this is not the case for the BB technique we investigated. While the computer-assisted component of the test highlights potentially abnormal cells to the pathologist, these cellular images from the brush biopsy specimen, as visualized both on the computer monitor and by manual microscopy, are interpreted and reported based purely on standard diagnostic criteria that are considered pathognomonic for disease. This is illustrated in the representative images from BB+/FB− cases shown in Figs. 3 and 4.

It must be noted that while BB detected cases of BE and dysplasia not reported on FB, the converse was also true, i.e., FB found cases of BE and dysplasia not revealed by BB. Of the 30 total patients with dysplasia detected by either biopsy technique, the brush biopsy detected 63% of these cases while the forceps biopsy detected 47% of them. Thus the brush biopsy detected 14 patients with dysplasia not found by the forceps biopsy, while the forceps biopsy detected 11 patients with dysplasia not detected by the brush biopsy. The results are similar when the data for BE

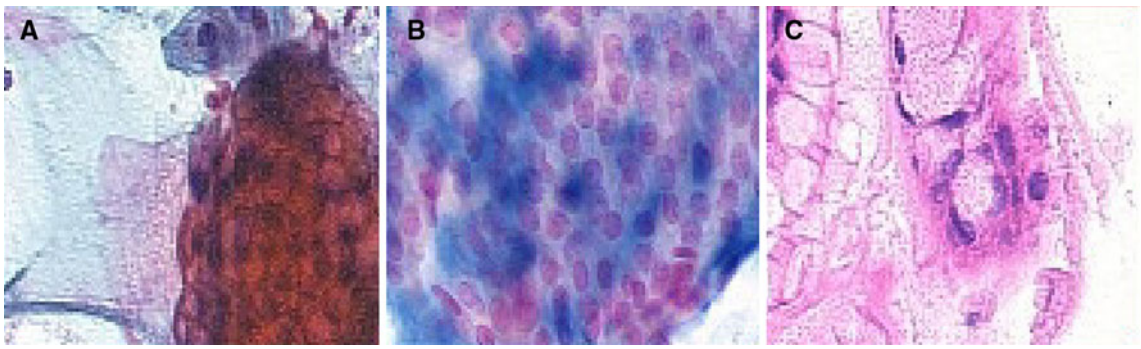

Fig. 3 Barrett's esophagus as shown on the brush biopsies (BB) of a BB+/FB− case (*FB* forceps biopsy). **a**, **c** Brush microbiopsies demonstrating intestinal metaplasia. Goblet cells, characterized by large central clear vacuoles compressing the nucleus against the cell membrane are noted. **b** The vacuoles of the goblet cells are strongly Alcian blue positive

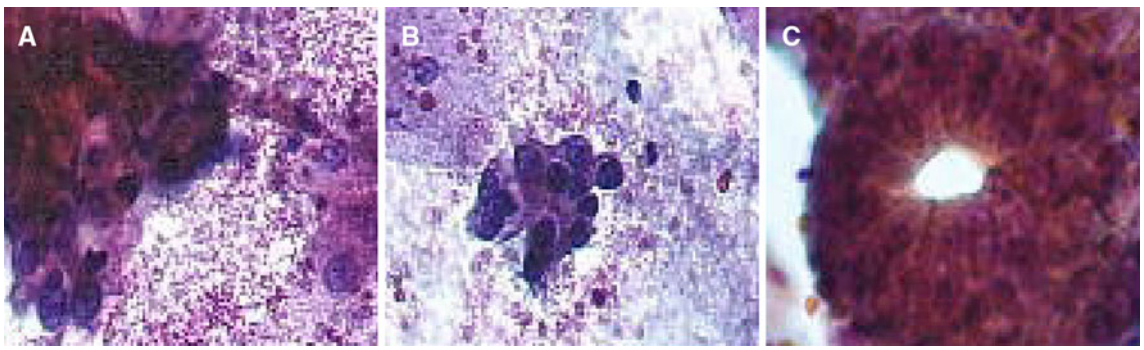

Fig. 4 Dysplasia as shown on the brush biopsies (BB) of a BB+/FB− case (*FB* forceps biopsy). **a** A brush microbiopsy showing epithelial cells exhibiting loss of surface maturation, nuclear atypism (hyperchromasia, increased nuclear-cytoplasm ratios), nuclear crowding and overlapping as well as loss of nuclear parallelism. **b** A small tissue fragment from the same slide with dysplastic epithelial cells showing nuclear atypism (hyperchromasia, increased nuclear-cytoplasm ratios), nuclear crowding and overlapping as well as loss of nuclear parallelism. **c** Also from the same slide, an intact gland can be seen comprised of highly atypical epithelial cells characterized by nuclear atypism (i.e. marked hyperchromasia and increased nuclear-cytoplasm ratios), nuclear crowding and overlapping as well as loss of nuclear polarity. These changes are consistent with high grade dysplasia

are examined. There were 139 cases of BE only detected by the brush biopsy, and 166 cases of BE only detected by the forceps biopsy. This study was not designed to determine if BB can substitute for, or is preferable to FB, and therefore our results should not be used to draw any conclusions about which technique is superior. Our results however, clearly demonstrate that *adjunctive* use of computer-assisted analysis of an abrasive brush biopsy significantly improves detection of Barrett's esophagus and dysplasia.

Unless GERD patients who truly have Barrett's esophagus are properly identified during endoscopic screening, they will likely be lost to follow-up and not undergo surveillance for identifying dysplasia. The increased detection of BE resulting form adjunctive use of the computer-assisted brush biopsy in this predominantly screening population may afford clinicians the opportunity to significantly close this potential gap in current esophageal screening practice.

Acknowledgments The study was funded by a grant by CDx Laboratories.

Appendix

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