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ORIGINAL ARTICLE

Computer-Assisted Brush-Biopsy Analysis for the Detection of Dysplasia in a High-Risk Barrett's Esophagus Surveillance Population

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Abstract
Background Barrett's epithelial dysplasia, the direct precursor to esophageal adenocarcinoma, is often unapparent and frequently missed during surveillance of Barrett's esophagus with four-quadrant forceps biopsy protocol.
Aim To determine whether the detection of dysplasia is improved by adding computer-assisted brush biopsy (EndoCDx©) to four-quadrant biopsy protocol.
Methods Patients with a history of Barrett's esophagus with dysplasia scheduled for endoscopic surveillance were recruited from four academic medical centers. Patients underwent brush biopsy followed by four-quadrant biopsy every 1–2 cm. The results from brush and forceps biopsy were reviewed independently by pathologists blinded to the other's results.
Results Among 151 patients enrolled (124 men, 27 women; mean age: 65), 117 (77.5%) had forceps and brush-biopsy specimens adequate for interpretation. The mean number of forceps biopsies was 11.9 (median 10, range 2–40) and brush biopsies was 2.0 (median 2, range 1–4). The overall yield of forceps alone was 25.2% ($n = 38$). Brush biopsy added an additional 16 positive cases increasing the yield of dysplasia detection by 42% (95% CI: 20.7–72.7). The number needed to test (NNT) to detect one additional case of dysplasia was 9.4 (95% CI: 6.4–17.7). There were no significant differences in results among different centers, between standard versus jumbo forceps, or between forceps biopsies taken every 1 cm versus every 2 cm.
Conclusions These data suggest that computer-assisted brush biopsy is a useful adjunct to standard endoscopic surveillance regimens for the identification of dysplasia in Barrett's esophagus.

Keywords Barrett's esophagus · Esophageal cancer · Cytology · Brush biopsy · Endoscopy

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Introduction

Esophageal adenocarcinoma (EAC) develops in approximately 0.5–1% of patients with Barrett's esophagus annually. Adenocarcinoma is thought to arise through the progression of metaplastic, dysplastic, to invasive cancerous changes in the distal esophagus [1]. The steady rise in the incidence of EAC and its continued poor prognosis have spawned research focused on the early detection of dysplasia in Barrett's esophagus (BE).

Unfortunately, dysplasia in BE remains an elusive target. Unlike the colon, where early neoplasms are typically polypoid and endoscopically evident, dysplasia in BE is often inconspicuous, flat, and patchy in distribution [2]. Currently, the discovery of Barrett's dysplasia relies on

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four-quadrant biopsies taken every 1–2 cm throughout the Barrett's segment. This, however, is time-consuming, and studies evaluating patients undergoing esophagectomy for high-grade dysplasia have found adenocarcinoma in 43–57% of patients that was undetected at surveillance endoscopy [3]. Moreover, surveys have shown that only half of all gastroenterologists comply with the current practice guidelines [4]. A number of novel imaging techniques have been introduced (magnification chromoendoscopy, confocal microscopy, etc.) to enhance current surveillance methods but their interpretation is often subjective, the examinations are time-consuming, and their usage is predominantly confined to major academic centers.

The computer-assisted brush-biopsy technique was developed as an attempt to overcome the limited sampling of four-quadrant biopsy protocol. Unlike traditional cytology, which obtains only superficial exfoliated cells, this brush-biopsy technique uses an abrasive sampling instrument that obtains a sample of the entire thickness of the squamous or glandular epithelium being tested down to the lamina propria. The resultant specimen is a disaggregated combination of intact tissue fragments (microbiopsies), cell clusters, and individual cells. Microscopic examination of this complex tissue sample is aided by a multiple focal plane, neural network-based computer-assisted scan of each slide, which highlights potentially abnormal cells for presentation on a video monitor to a pathologist, who also examines the specimen using the manual microscope.

The technology was initially piloted in 1999 for use in the detection of oropharyngeal dysplasia and carcinoma (OralCDx®). EndoCDx® involves a modification of the same transepithelial brush-biopsy technology for use in the esophagus. The brush-biopsy device is enclosed in a 2.5-mm sheath that is passed through the operating channel of a standard endoscope. The neural network computer system was reprogrammed to detect esophageal abnormality.

The results of a recent multicenter trial with a similar protocol but applied to a relatively low-risk predominantly screening population of 1,183 subjects from eight community GI centers is reported in a companion paper [5]. In that study, the addition of the computer-assisted brush-biopsy protocol increased the overall detection of BE by 39.8% (95% CI: 33%–46%, $n = 507$) and increased the detection of dysplasia by 87.5% (95% CI 44%–133%, $n = 30$).

In order to extend these observations from a predominantly screening population evaluated in community-based practices to a high-risk, surveillance population, all of whom had previously documented BE dysplasia or at least a diagnosis of indefinite-for-dysplasia, we undertook a study at four academic centers with special interest in BE. A single gastrointestinal pathologist with expertise in BE blindly interpreted the forceps biopsies from all four centers, thus standardizing the histopathologic grading. Our goal was to determine the increased yield for the detection of dysplasia (LGD, HGD) or cancer by the addition of the brush biopsy (BB) to the standard forceps biopsy (FB) protocol.

Methods

During the period 2004–2008, subjects over the age of 18 scheduled for endoscopic surveillance for BE were recruited in four academic medical centers: The Mount Sinai Medical Center, The MD Anderson Cancer Center, The Hines-Illinois VA Medical Center, and Baylor College of Medicine-Houston VA Medical Center. We selectively enrolled subjects with a known prior history (recent or remote) of BE with dysplasia/neoplasia (indefinite-for-dysplasia [IND], low-grade [LGD], high-grade dysplasia [HGD] or intramucosal adenocarcinoma [IMCA]) and no grossly evident lesion. Patients with a visible lesion requiring targeted biopsy prior to brushing were excluded. Institutional Review Board (IRB) approval was obtained from all sites prior to study initiation. A total of 151 subjects were enrolled in the trial.

Standardized clinical requisition forms were completed at all the sites to obtain clinical, demographic, and endoscopic information, including age, gender, weight, Barrett's segment length, hernia presence and size, procedure duration, endoscopic appearance (ulceration, stricture, etc.), type of forceps (jumbo or standard) used, and the interval/number of biopsies taken (every 1 vs. 2 cm). Standardized EndoCDx kits were used by all investigators and consisted of: (1) two brush-biopsy devices, (2) two bar-coded glass slides, (3) an alcohol/carbowax fixative pouch for sample preservation, (4) 5 cc of alcohol, and (5) preaddressed packet for submitting the contents.

Endoscopic Procedure

Investigators were provided with a video demonstration and written instructions on how to perform the brush biopsy. The brush biopsies (mean of two per patient) were performed prior to the forceps biopsies (mean 12 per patient) in order to avoid obscuring the visual field and artifact from excessive bleeding caused by the forceps. The brush, in its enclosed sheath, was passed through the working channel of the endoscope and placed against the surface of the mucosa. Sampling of any visualized columnar mucosa was performed by maintaining pressure against the mucosa, and rotating the brush circumferentially along the epithelial surface. Pinkish-red tissue or pinpoint bleeding at the brush-biopsy site was evidence of

proper technique. Up to 4 cm of the columnar-lined mucosa was sampled with a single brush.

The cellular material collected on the brush was then transferred to a bar-coded glass slide and immersed in fixative. The procedure was then repeated using a second, new brush and the bristle portion of the brush clipped off into the vial of alcohol. 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. Following the two brush biopsies, standard four-quadrant forceps biopsies of the esophagus were obtained at 1–2 cm intervals, based upon the prior pathologic grade.

Pathologic Evaluation

The brush-biopsy specimens were processed at CDx Laboratories. The slide was stained in accordance with a modified Papanicolau method. Concomitantly, cellular material was harvested from the alcohol-preserved brush tip by mechanical abrasion and two cell blocks were prepared from the centrifuged fluid. One was stained with Hematoxylin and Eosin (H&E) and the other with Alcian Blue. All stained slides were scanned by the EndoCDx computer system, which consists of a neural network-based image processing system that is designed to highlight potentially abnormal cells to the pathologist. Images of potentially abnormal cells identified by the computer system were displayed on a high-resolution color video monitor for review by the CDx pathologist (S.F.) who performed both standard microscopic examination and a computer-generated review before rendering a final diagnosis of either (1) no Barrett's esophagus (2) Barrett's metaplasia/negative for dysplasia (3) Barrett's indefinite-for-dysplasia, and (4) dysplasia (low-grade dysplasia/high-grade dysplasia/intramucosal adenocarcinoma). The forceps biopsies were placed in formalin per standard of care and submitted for standard histopathologic evaluation at the primary site. Slides were then mailed to the central study pathologist (N.H.) for a standardized diagnosis based upon the following histopathologic grading of Barrett's esophagus: (1) no Barrett's esophagus (Neg), (2) Barrett's metaplasia (IM), (3) Barrett's indefinite-for-dysplasia (IND), (4) Barrett's low-grade dysplasia (LGD), (5) Barrett's high-grade dysplasia (HGD), and (6) intramucosal adenocarcinoma (CA).

Diagnostic criteria for both brush and forceps biopsies: Barrett's metaplasia (IM) was defined as esophageal-derived columnar mucosa containing goblet cells. Biopsies classified as negative for dysplasia contained uncrowded glands lined by epithelial cells with cytologically bland nuclei characterized by smooth nuclear contours, pale chromatin and inconspicuous or absent nucleoli. Reduced nuclear-to-cytoplasmic ratios along the surface compared to the deeper glands resulted in a maturation gradient. Reactive changes elicited by inflammation included mildly increased nuclear-to-cytoplasmic ratios, nucleolar prominence and mitotic activity, but without significant hyperchromasia or nuclear membrane irregularity. Biopsies classified as indefinite-for-dysplasia (IND) featured mild to moderate atypia of the epithelium lining the deep glands, i.e., hyperchromasia, increased nuclear-cytoplasmic ratios, irregular nuclear contours and plentiful mitoses, but preservation of the maturation gradient. Mild glandular crowding was present in some cases. Biopsies classified as positive for low-grade dysplasia (LGD) featured loss or near-loss of the surface maturation gradient resulting from extension of nuclear atypism (hyperchromasia, crowding, irregular contours, and increased nuclear-cytoplasmic ratios) from the glands to the surface. Nuclear parallelism and cellular polarity were maintained. The glands were sometimes crowded but retained intervening lamina propria. Biopsies classified as positive for high-grade dysplasia (HGD) exhibited, in addition to the features of low-grade dysplasia, loss of nuclear parallelism, marked nuclear atypism and, in some cases, marked glandular crowding or fusion. Intramucosal carcinoma (CA) was defined by penetration of epithelium through the basement membrane, manifested by single or small cell clusters, incomplete glands or a syncytial glandular pattern.

A typical example of a BB from a BB+/FB- case exhibiting the pathognomonic and cytoplasmic characteristics of low grade dysplasia and high grade dysplasia are shown in Figs. 1 and 2, respectively.

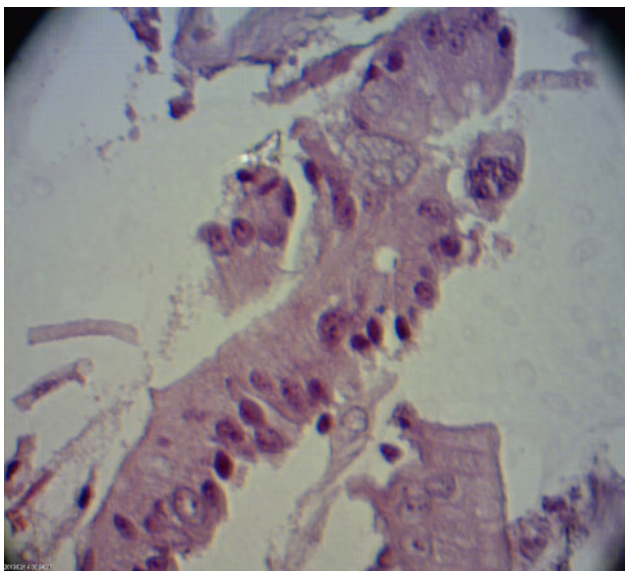

Fig. 1 Example of a BB from a BB+/FB- case exhibiting the moderate degree of nuclear hyperchromasia, increased nuclear-to-cytoplasmic ratio, and loss of polarity characteristic of low-grade dysplasia

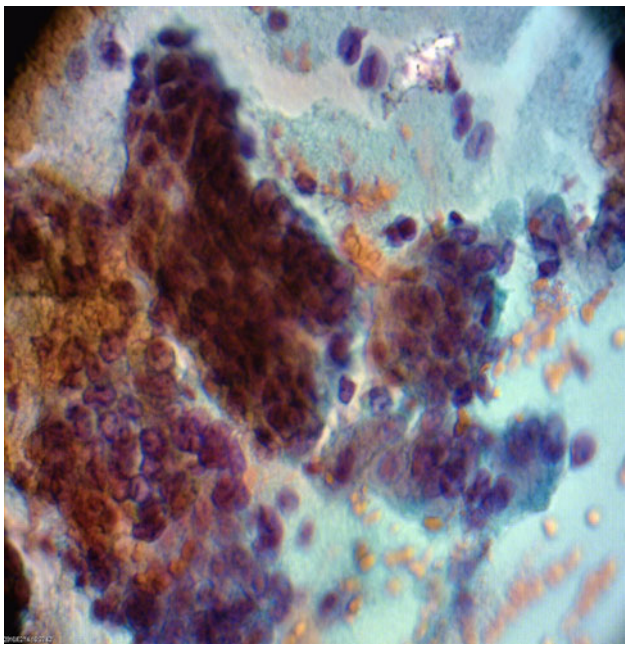

Fig. 2 Example of a BB from another BB+/FB- case exhibiting tissue fragments with multiple layers of dysplastic cells characterized by the marked degree of nuclear hyperchromasia, irregularly thickened nuclear membranes, highly increased nuclear to cytoplasmic ratio, overlapping nuclei, and marked loss of nuclear polarity characteristic of high-grade dysplasia

Statistical Analysis

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 calculated 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.

Results

A total of 151 subjects (82% male, mean age: 65) with a confirmed prior history of Barrett's esophagus with either indefinite-for-dysplasia, low-grade dysplasia, high-grade dysplasia or intramucosal adenocarcinoma and no visible nodule or lesion were enrolled in the study. Table 1 shows the clinical, demographic, and endoscopic features of the 151 subjects enrolled.

All 151 subjects underwent both brush-biopsy and standard endoscopic forceps biopsy protocols. Among the 151 subjects enrolled, an adequate, paired set of FB and BB specimens were available on 117 subjects. In 34 subjects, an "inadequate sample" was noted on either FB ($n = 21$) or BB ($n = 13$) due to a lack of Barrett's epithelium on either modality. There were no cases where BE was absent on both FB and BB, thus all 151 cases were evaluable and included in the final analysis. In Table 2, the BB and FB reads for all 151 subjects are shown. The readings are cross-tabulated in four categories: no dysplasia, indefinite-for-dysplasia, positive for any definite dysplasia (LGD/HGD/CA) and inadequate sample.

In Table 3, results are compared for no dysplasia, indefinite for dysplasia or inadequate ("NEGATIVE") versus any DEFINITE DYSPLASIA (LGD/HGD/CA). The readings were concordant on both BB and FB in 112/151 subjects or 74.2%. The yield of FB alone was 25.2% of all patients tested with an additional 16 cases detected by the BB. This increment represents an increased yield of detected definite dysplasia or carcinoma of 42.1% (95% CI: 20.6–61.5). The absolute increase of 16 cases of definite dysplasia or carcinoma detected, or 10.6% of all patients

Table 1 Clinical, demographic, and endoscopic characteristics of enrolled subjects with BE ($n = 151$)

Subject characteristics	Number ($n = 151$)
Male gender	82.4% ($n = 124$)
Age (mean)	65.0 (range: 46–87)
Race	
White	83.7% ($n = 126$)
African-American	0.8% ($n = 1$)
Hispanic	6.0% ($n = 10$)
Other	9.5% ($n = 14$)
Prior pathologic grade of Barrett's	
IND	9.5% ($n = 14$)
LGD	75.2% ($n = 114$)
HGD	14% ($n = 21$)
IMCA	1.3% ($n = 2$)
Barrett's segment length (mean)	4.6 (range 0–14 cm)
Hiatal hernia present (%)	75.2% ($n = 88$)
Mean # forceps biopsies (FB)	11.9 (range: 2–40)
Mean # brush-biopsies (BB)	2.0 (range: 1–4)

Table 2 Biopsy (BB) and forcep's biopsy (FB) reads: Barrett's metaplasia (IM), indefinite for dysplasia (IND), dysplasia (LGD/HGD/CA), and inadequate (no BE)

Forcep's biopsy	Brush biopsy (BB read)			
	Metaplasia	Indefinite	Dysplasia	Inadequate
Metaplasia	12	6	5	5
Indefinite for dysplasia	40	11	9	4
Dysplasia	11	8	15	4
Inadequate	18	1	2	0

Table 3 Brush-biopsy (BB) and forcep's biopsy (FB) reads: no dysplasia/indefinite/inadequate (−) versus LGD/HGD/CA (+)

Forceps biopsy read	Brush biopsy read (BB read)	
	−	+
−	97	16
+	23	15

Added yield = 16/38 = 42.1%, NNT = 151/16 = 9.4

tested, results in a NNT of 151/16 or 9.4 (95% CI: 5.6–16.6). Thus, for every 9.4 patients tested, one additional case of definite dysplasia/carcinoma was detected as a result of adjunctive use of the brush biopsy.

In sub-analyses, no significant differences were noted among centers or endoscopists, between standard versus jumbo forceps, or between closer or wider biopsy intervals (1 vs. 2 cm). Although the identification of high-grade intraepithelial neoplasia was not the primary endpoint of this trial, a total of nine cases of HGD or CA were detected by either method. Of these, five were detected on both FB and BB; one was detected on BB alone; and three were detected on FB alone.

Discussion

In this multicenter evaluation of subjects with a prior history of Barrett's dysplasia, the addition of a computer-assisted brush-biopsy protocol (an average of two BBs per patient) to standard forceps' biopsy protocol (an average of 12 FBs per patient) increased the yield of detected abnormality defined as LGD/HGD/CA by 42.1%. The detection of abnormality in an additional 10.6% of all patients tested results in a NNT of 9.4.

All of the study endoscopists were from tertiary-care centers with a high-volume population of Barrett's esophagus. In every case, four-quadrant biopsy protocol was strictly adhered to and, in many cases, biopsies were taken every 1 cm using jumbo forceps. While biopsy interval and forceps size did not confer a statistically significant difference in this study, every subject had a minimum of four biopsies taken every 2 cm. Since all of the subjects in this academic surveillance study had a history of dysplasia, the endoscopists were far more likely to perform a more rigorous visual and biopsy examination, potentially diminishing the incremental benefit of the brush biopsy. Thus, these results, while significant, may be less pronounced than those in a community-based setting.

One question that may arise is whether the interpretation of the BB was appropriate. That is, were the BB+/FB- cases true positives? While the computer-assisted brush-biopsy method tested here provides an alternative method for obtaining and evaluating specimens, these brush-biopsy specimens themselves are interpreted and reported based on standard diagnostic criteria that are considered pathognomonic for disease (Fig. 1). Indeed, retrospective evaluation of the 16 subjects that were BB+/FB- revealed clear cytopathologic evidence of dysplasia. In a post-hoc analysis, follow-up endoscopies were reviewed on 11 of the 16 patients that were BB+/FB- 3–18 months after their study procedure. Of these 11 subjects, five (45%) indeed had a follow-up diagnosis of IND, LGD, or HGD despite the negative forceps' examination on the prior endoscopy.

Another question that may arise is whether subjects classified as indefinite-for-dysplasia should have been classified in the dysplasia category? Since indefinite is not a guarantee of dysplasia and its diagnosis may, in fact, be confounded by inflammatory changes, we chose to focus our study on the incremental yield of the BB for a diagnosis of true dysplasia or neoplasia (LGD, HGD, CA). Nevertheless, if the "indefinite" forceps biopsy report is grouped with unequivocally abnormal findings, the yield of "positive" readings on FB increases from 67.5 to 76.8%. Consequently, the incremental yield from the addition of BB falls from 42.1 to 13.7%. Nonetheless, even by this alternate analysis, the absolute increase in detection resulting from addition of BB remains almost unchanged (10.6% compared to 9.8%). In other words, the NNT (9.4 vs. 10.8) is quite similar for both methods of analysis.

Not unexpectedly, the independent detection yield for dysplasia of an average of only two BBs taken per patient was lower than the independent detection yield of the standard four-quadrant biopsy protocol with an average of nearly 12 FBs taken per patient. While this suggests that four-quadrant biopsy cannot be abandoned, the goal of this study was not to asses the performance of the BB independently, but rather as an adjunctive supplement to the standard FB protocol in a high-risk surveillance population of known dysplastics. We did not seek to determine whether the number of forceps biopsies could be reduced by the addition of the brush. Given the recent advances in endoscopic imaging technology, one potential avenue of future research would be to determine whether the BB can be combined with advanced imaging techniques to enhance diagnostic yield. Indeed, one can imagine using the brush to *selectively* sample areas that are abnormal on widefield imaging technologies such as narrow-band or autofluorescence imaging. While not evaluated within the context of this study, such an approach could dramatically alter existing surveillance practices.

Thus, in 151 patients with Barrett's esophagus and a prior diagnosis of dysplasia, the addition of a computer-assisted brush-biopsy protocol to standard endoscopic surveillance procedure increased the detection of dysplasia by 42.1% (=16/38) (NNT: 9.4). This increased yield

suggests that computer-assisted brush biopsy may be valuable as an adjunct to standard endoscopic surveillance programs in high-risk subjects with Barrett's esophagus.

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