

Inter-Observer Agreement among Pathologists Using Wide-Area Transepithelial Sampling With Computer-Assisted Analysis in Patients With Barrett's Esophagus

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- OBJECTIVES:** The histopathological diagnosis of Barrett's esophagus (BE)-associated dysplasia has poor inter-observer agreement. The wide-area transepithelial sampling (WATS) procedure uses a minimally invasive brush biopsy technique for acquiring wide-area sampling of BE tissue followed by computer-assisted analysis. In this study, our aim was to assess inter-observer agreement among pathologists in the diagnosis of Barrett's-associated dysplasia using the WATS computer-assisted analysis technique.
- METHODS:** WATS slides with varying degrees of BE dysplasia were randomly selected and distributed to four pathologists. Each pathologist graded the slides as nondysplastic, low-grade dysplasia (LGD), or high-grade dysplasia/esophageal adenocarcinoma (HGD/EAC) and completed a standardized case report form (CRF) for each slide.
- RESULTS:** In all, 149 BE slides were evaluated in a blinded manner by 4 pathologists. The slides included the following: no dysplasia ($n=109$), LGD, and HGD/EAC ($n=40$). The overall mean kappa value for all 3 diagnoses for the 4 observers was calculated at 0.86 (95% confidence interval (CI) 0.75–0.97). The kappa values (95% CI) for HGD/EAC, IND/LGD, and no dysplasia were 0.95 (0.88–0.99), 0.74 (0.61–0.85), and 0.88 (0.81–0.94), respectively.
- CONCLUSIONS:** The diagnosis of BE and associated dysplasia using the WATS technique has very high inter-observer agreement. This appears to be significantly higher as compared with previously published data using standard histopathology.

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Barrett's esophagus (BE) is a condition in which columnar epithelium with intestinal metaplasia replaces the squamous epithelium that normally lines the esophagus (1). BE can progress to esophageal adenocarcinoma (EAC) through varying grades of dysplasia. EAC is the one of the fastest growing cancers in the United States (2). Studies have shown that patients who had early diagnosis of EAC had substantially better outcome (3,4). Patients with BE are enrolled in surveillance programs for the detection of dysplasia and/or early cancer and undergo four-quadrant biopsies every 1–2 cm apart (5). Endoscopy along

with random biopsies every 1–2 cm apart is used for BE surveillance in most centers. The histopathological diagnosis of BE-associated dysplasia has poor inter-observer agreement even among experienced gastrointestinal pathologists (6–8). Published estimates for the inter-observer agreement (kappa values) in the reading of dysplasia in BE have varied between 0.15 and 0.69 (6,9).

The wide-area transepithelial sampling (WATS) procedure is a new technique for acquiring wide-area tissue sampling using a minimally invasive brush biopsy technique. Unlike standard soft

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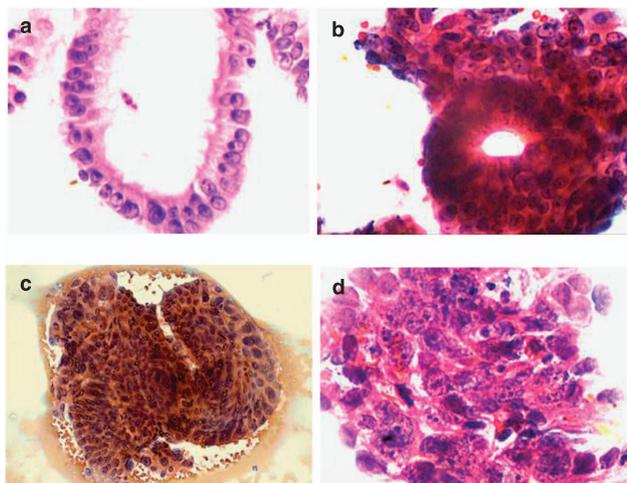


Figure 1. Computer-generated images using wide-area transepithelial sampling (WATS) technique. (a) Non-dysplastic Barrett's esophagus (NDBE); (b) low-grade dysplasia (LGD); (c, d) and high-grade dysplasia (HGD).

cytology brushes, WATS brushes are comparatively abrasive and designed to consistently sample deeper layers (including muscularis mucosae) of the more firmly attached glandular epithelium. Therefore, the histology samples of the WATS specimens are much thicker and have unique three-dimensionality. The analysis of the WATS sample is aided by a high-speed computer scan that identifies potentially abnormal cells, cell clusters, and abnormal glandular cells on a high-resolution video monitor for pathologists to review (**Figure 1**). Approximately 200 "most suspicious" cells are then flagged by the computer as a starting point for analysis. These abnormal cells are then visualized by pathologists for the detection of BE and low/high-grade dysplasia. Two prospective trials by Johanson *et al.* (10) and Anandasabapathy *et al.* (11) have demonstrated a 39.8 and 42% increase in the detection of intestinal metaplasia and dysplasia by using WATS as an adjunct to standard four-quadrant forceps biopsy. However, the inter-observer agreement among pathologists using the WATS samples is not known. Our aim was to assess inter-observer agreement among pathologists in the diagnosis of BE and low-grade dysplasia (LGD) and high-grade dysplasia (HGD) using the WATS computer-assisted analysis technique.

Methods

Patients and WATS specimens

WATS brushing samples obtained in patients with BE, who underwent surveillance endoscopies in their respective medical centers (55 centers), were identified from a central database. The specimens were obtained from patients with documented BE undergoing surveillance endoscopy. The samples were then transported to a central laboratory (CDx Diagnostics, Suffern, NY) for analysis. The study was approved by the Institutional Review Board.

WATS procedure and technique

WATS specimens were obtained before performing forceps biopsies. After the esophagus was intubated and mucosa was visualized, the first brush was passed through the operatory channel of the scope. The bristles of the brush were placed against the surface mucosa. While maintaining firm pressure, the brush was rotated and repeatedly passed back and forth over the biopsy site until pinpoint bleeding was observed. Patients underwent two WATS biopsies of every 5 cm segment of BE. The brush was removed from the endoscope and after the contents on the brush were smeared on to the glass slide, the portion of brush with bristles was cut and placed inside a vial containing the transport medium. Approximately 100,000 cells are present between the vial and the smear (on the slide).

The cells on the slide (smear) were fixed using the fixative provided with the kit. The slides and the vial with the brush tip were then transported to the central laboratory (CDx Diagnostics), where a cell block was made from the cells collected in the vial. Cells on the slide and the cell block were then separately analyzed by the computer.

Study specimens, inclusion, and exclusion criteria

Only specimens from patients with documented BE were included in the study. All samples were obtained from CDx diagnostics repository. The samples were randomly selected from the repository to provide a spread of all diagnoses including non-dysplastic BE, LGD, HGD, and cancer. The baseline diagnosis was made by histopathology. A second pathologist confirmed all cases with dysplasia. Specimens with very scanty cellularity or extensive air-dried artifacts were excluded, as these are the basis for specimen sample inadequacy.

Each slide was labeled 1–149 and did not have any other identifiable marker or number on the slide. No clinical information about the cases was provided to the pathologists. All slides were randomly distributed to each pathologist. Each pathologist was identified only by a number (1–4) assigned to them before the study, and recorded their diagnoses on a form.

Analysis of WATS specimen

All study specimens were examined by pathologists who had special training in analysis of WATS samples aided by a high-speed computer scan. The advanced computer analysis system is specifically designed to detect metaplastic and dysplastic cells. This is accomplished through detection of abnormal cellular morphology. Images of abnormal cells identified by the computer system are individually displayed on a high-resolution color video monitor for review by a pathologist who is specially trained in computer-assisted analysis. The computer does not provide a diagnosis; rather, it assists in the search and identification of abnormal areas, which are then visually assessed and interpreted by the pathologist who renders a final diagnosis.

Statistical analysis

A sample size of 146 slides using 4 reviewers was calculated to be sufficient to maintain a power of 80% with an alpha of 0.05.

Table 1. Kappa values among pathologists in the diagnosis of BE and dysplasia using WATS technique

Overall (95% CI)	HGD/EAC (95% CI)	IND/LGD (95% CI)	NDBE (95% CI)
0.86 (0.75–0.97)	0.95 (0.88–0.99)	0.74 (0.61–0.85)	0.88 (0.81–0.94)

BE, Barrett's esophagus; CI, confidence interval; EAC, esophageal adenocarcinoma; HGD, high-grade dysplasia; IND, indeterminate for dysplasia; NDBE, non-dysplastic Barrett's esophagus; WATS, wide-area transepithelial sampling.

Using the published range of kappa values for histopathological diagnosis, we assumed a minimal acceptable level of reliability (pO) of 0.799 and an expected rho value (p1) of 0.85. The kappa values were graded on the basis of Landis and Koch scale (kappa values: 0.41 to 0.60 indicate moderate agreement, 0.61 to 0.80 indicate substantial agreement, and above 0.80 indicate nearly perfect agreement) (12). STATA version 10 (College Station, TX) was used for statistical analyses.

Results

A total of 149 BE slides were evaluated in a blinded manner by 4 pathologists, who are board-certified in Anatomic Pathology and with 2, 20, 30, and 35 years of experience, respectively. Patients included men (n=82, 55%) and women (n=67, 45%) with a mean age of 55 years (age range 21–88 years). The slides included the following: no dysplasia (n=109), LGD (n=18), and HGD/EAC (n=22).

The overall mean kappa value for all three diagnoses for the four observers was calculated at 0.86 (95% confidence interval (CI) 0.75–0.97). The kappa values (95% CI) for HGD/EAC, IND/LGD, and no dysplasia were 0.95 (0.88–0.99), 0.74 (0.61–0.85), and 0.88 (0.81–0.94), respectively (Table 1). Inter-observer agreement between any two pathologists ranged between 0.83 and 0.89. Percentage agreement was calculated at 88.6% (95% CI: 82.36–93.21) (132/149) among 4 pathologists. There were 17 specimens with discordance. In 14 specimens (nondysplastic Barrett's: n=6; LGD: n=6; HGD: n=2), three pathologists agreed on the same diagnosis, but not the 4th pathologist, and in three specimens (nondysplastic Barrett's vs. LGD) only two pathologists agreed on the diagnosis (Figure 2).

Discussion

The diagnosis of BE and associated dysplasia using the WATS technology had very high kappa values. This is the first study conducted to evaluate inter-observer variation in diagnosing BE and esophageal dysplasia using this technology. The inter-observer agreement was nearly perfect in patients with no dysplasia (κ=0.88) or HGD dysplasia/cancer (κ=0.95). The kappa value for LGD (0.74) was substantial.

Histopathological analysis of standard forceps biopsies is the current accepted practice for diagnosing BE and esophageal dysplasia. Kerkhof *et al.* (8) showed that the inter-observer agreement for diagnosing dysplasia between experts and non-experts, as well as between experts, was fair (κ=0.24 and 0.27

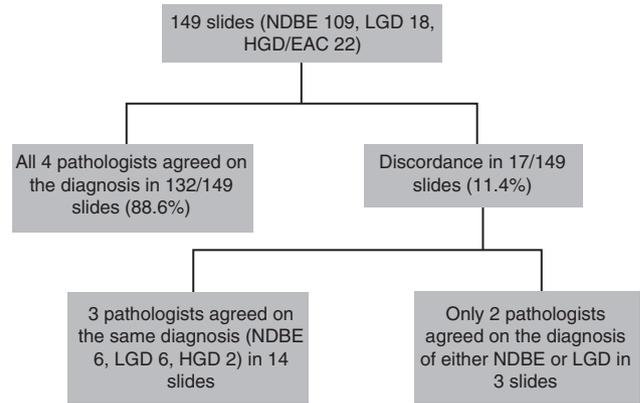


Figure 2. Discordant slide flowchart.

respectively), and inter-observer agreement was 0.62 and 0.58 for differentiating HGD from ND/LGD. Montgomery *et al.* (6) also showed that the inter-observer agreement for HGD and LGD was substantial (κ=0.65) and fair (κ=0.32), respectively. Although we did not directly compare histopathology with WATS readings, these values appear to be much lower than what we determined in the current study.

In the traditional histopathological diagnosis of BE and dysplasia in BE, low inter-observer agreement among even expert gastrointestinal pathologists was attributed mainly owing to subjectivity in identifying subtle morphological changes between baseline atypia and LGD along with the presence of persistent esophageal inflammatory changes owing to chronic gastroesophageal reflux disease (13,14). Moreover, pathologists may evaluate different areas of the same slide, accounting for low inter-observer agreement (low kappa values). WATS uses a computer neural network that chooses 200 most suspicious areas from the brush samples and projects the same cells on a computer monitor to every pathologist evaluating the sample. The pathologists must examine the entire slide, not just the 200 areas presented by the computer before they make a final diagnosis. Therefore, all pathologists look at the same set of cells, which may potentially explain the high inter-observer agreement between different pathologists.

The clinical utility of WATS has been evaluated in two pilot studies. In a large multicenter prospective trial of 1,266 patients being screened for BE and dysplasia, the addition of WATS to the standard four-quadrant forceps biopsy protocol increased the overall detection of intestinal metaplasia by 39.8% (95% CI 32–48%) (11). In a second multicenter study of 151 patients, all in a high-risk BE surveillance program, the addition of WATS^{3D} to the standard four-quadrant forceps biopsy protocol added an additional 16 cases of dysplasia, increasing the yield of esophageal dysplasia detection by 42% (95% CI: 20.7–72.7) (10). A large multicenter prospective study is currently underway to further evaluate the role of the WATS technology for the increased detection of HGD and cancer.

One of the advantages of using WATS is that endoscopists are already well versed with performing esophageal brushing. The technique for performing WATS brushings is not significantly different from performing brushings—for e.g., for suspected

esophageal candida infection. However, WATS differs from traditional brush biopsy in specimen handling and analysis after the brush biopsy. WATS may also prove to be a useful tool in reliably diagnosing low or indeterminate grades of dysplasia, as these two diagnoses have the lowest rates of agreement. If future studies show that WATS is able to improve the diagnostic accuracy of random four-quadrant biopsies, it may be helpful for screening or surveillance of patients with BE.

The role of cytological sampling in the screening and diagnosis of BE is being increasingly investigated in other studies as well (15,16). In a preliminary prospective study in 2010, Kadri *et al.* (17) compared the accuracy of a nonendoscopic esophageal cell sampling device coupled with immunohistochemistry for Trefol factor 3 with standard endoscopy with biopsies in 501 patients with gastroesophageal reflux disease; however, owing to low prevalence of BE in the population, the accuracy of cytological sampling coupled with immunohistochemistry could not be evaluated, but the results of sensitivity and specificity are promising.

Our study has several limitations. All the specimens had intestinal metaplasia with or without dysplasia. All four pathologists who participated in the study had special training in the analysis of WATS specimens. Currently, WATS samples are only evaluated by pathologists at CDx diagnostics, and therefore the study results cannot be generalized. In this study, diagnosis of BE and Barrett's-associated dysplasia by WATS brush biopsies were not directly compared with standard forceps biopsies.

In summary, the diagnosis of BE and associated dysplasia using WATS aided by computer analysis has high inter-observer agreement and appears to be significantly higher compared with previously published data using histopathology specimens. The diagnosis of LGD in BE, which has been a challenge even among expert pathologists and WATS, showed a kappa value of 0.74. WATS with computer-aided analysis is a promising tool, and in the future it may aid traditional biopsy protocol to improve the diagnosis of BE and associated dysplasia.

CONFLICT OF INTEREST

Guarantor of the article: Prateek Sharma, MD.

Specific author contributions: Study conception and design; analysis and interpretation of the data; drafting of the manuscript; and critical revision of the manuscript for important intellectual content: Prashanth Vennalaganti; study concept and design; statistical analysis; interpretation of data; and drafting of the manuscript: Vijay Kanakadandi; critical revision of the manuscript for intellectual content: Seth A. Gross, Kenneth K. Wang and Sravanthi Parasa; analysis and interpretation of the data; drafting of the manuscript; and critical revision of the manuscript for important intellectual content: Neil Gupta; study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; and statistical analysis: Prateek Sharma.

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Study Highlights

WHAT IS NEW HERE

- ✓ Diagnosis of Barrett's esophagus and associated dysplasia using wide-area transepithelial sampling (WATS) aided by computer analysis has high inter-observer agreement.
- ✓ The diagnosis of low-grade dysplasia in Barrett's esophagus, a challenge even among expert pathologists, showed a kappa value of 0.74.
- ✓ WATS with computer-aided analysis is a promising tool to improve the diagnosis of Barrett's esophagus and associated dysplasia.

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