EDITORIAL

Wide-area transepithelial sampling with 3-dimensional cytology: Does it detect more dysplasia or yield more hype?

Barrett's esophagus (BE) is a known precursor for esophageal adenocarcinoma. The best predictor of neoplastic progression is dysplasia in esophageal tissue biopsy specimens obtained at the time of endoscopy. Dysplastic changes include cytologic features (nuclear alterations that generally extend to the surface in well-oriented biopsy specimens) and architectural crowding, which accompany high-grade dysplasia (HGD) and early adenocarcinoma (EAC).

Tissue sampling during endoscopy to diagnose dysplasia or cancer arising in BE remains a challenge. The Seattle biopsy protocol was first described 25 years ago.[1] This technique uses systematic 4-quadrant large-capacity biopsy forceps to sample the entire length of BE, and it continues to be the reference standard with which other approaches to neoplasia detection are compared. The main problems with the Seattle biopsy technique include the increased procedure time for long lengths of BE, high cost of multiple specimens for pathologic examination, low overall diagnostic yield, and suboptimal sensitivity resulting from incomplete sampling of at-risk mucosa. Furthermore, there is low adherence to practice guidelines recommending the Seattle biopsy protocol, particularly in community practice settings, and this in turn results in decreased dysplasia detection.[2-5]

What alternative types of neoplasia detection techniques have been developed? The use of high-definition white-light endoscopy and careful inspection of BE mucosa with a minimum time for inspection of 1 minute for every centimeter length of BE can improve dysplasia detection.[6] Endoscopic imaging techniques like narrow-band imaging,[7] iScan,[8] acetic acid chromoendoscopy,[8] and confocal laser endomicroscopy[9,10] provide additional means of "smart" or targeted biopsy to potentially minimize the number of unnecessary samples from nondysplastic BE. Moreover, confocal laser endomicroscopy may eliminate the need for random biopsy by enabling real-time evaluation of histologic features.[9,10] However, the routine use of these ancillary methods for dysplasia detection is controversial. The American Gastroenterological Association (2011 medical position statement)[11] and American College of Gastroenterology (2016 guidelines)[12] discourage the use of these advanced imaging techniques in routine BE surveillance. More recent reviews of the scientific evidence support the application of these technologies by endoscopists who have met the American Society for Gastrointestinal Endoscopy preservation and incorporation of valuable endoscopic innovation (PIVI) thresholds for diagnostic accuracy.[13,14]

Standard brush cytology for BE surveillance has not become standard practice because of variable sensitivity and specificity, particularly with the diagnosis of low-grade dysplasia (LGD).[15-17] However, in recent years, a novel technique for sampling BE mucosa has been developed. In the current issue of *Gastrointestinal Endoscopy*, Vennalaganti et al[18] in 16 medical centers report the results of a tandem study in which all BE patients undergoing routine surveillance or referred for management of dysplasia underwent high-definition white-light endoscopy with 4-quadrant random biopsy and wide-area transepithelial sampling (WATS) with an abrasive brush in random order. The technique involved 2 WATS brushes per 5-cm BE length applied vigorously to induce mucosal bleeding to obtain transepithelial samples from flat BE without suggestive lesions. Patients with lesions >10 mm were excluded from the study. The specimens from WATS sampling were analyzed by a central laboratory pathologist (CDx Diagnostics, Suffern, New York). However, in addition to the manual review of the slides, computer-aided 3-dimensional analysis using neural networks also efficiently provided 200 suggestive areas for the WATS pathologist to review. A blinded pathologist interpreted the dysplasia grade for each tissue sampling technique. The study demonstrated a modest absolute increase in the number of patients (23/160, 14.4%) with HGD/esophageal adenocarcinoma when the WATS sampling technique was added to random biopsy, regardless of the order of sampling.

> Should WATS sampling be preferentially used in referral centers? Or should any busy gastroenterologist who does not use advanced imaging techniques be a WATS user?

What are the potential advantages and disadvantages of the WATS esophageal sampling technique? For long BE,

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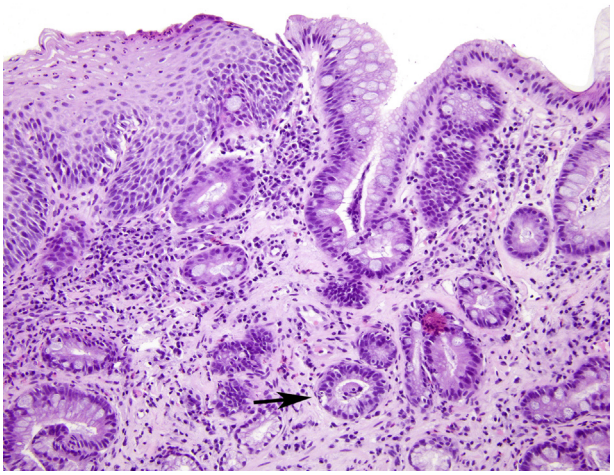

Figure 1. Barrett's esophagus, negative for dysplasia, standard preparation (H&E, orig. mag. ×20). This example displays prominent lamina propria with chronic inflammation and reactive changes. The nuclei at the base of the biopsy are larger and rounder than those toward the surface. There is reactive squamous epithelium at the upper left, the surface of which is lightly encrusted with acute inflammatory cells. The *arrow* indicates a deep gland/pit showing cytologic alterations that are readily recognized as reactive in the context of the architectural features of the sample.

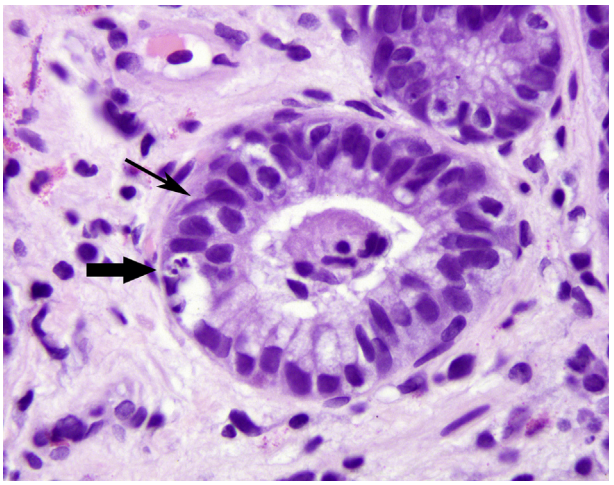

Figure 2. Barrett esophagus, negative for dysplasia, standard preparation, higher magnification of the indicated gland seen in Figure 1 (H&E, orig. mag. ×100). At very high magnification, the nuclei of the indicated gland appear enlarged and hyperchromatic (*thin arrow*), and an apoptotic body is indicated by the thick arrow. This gland is clearly not dysplastic in the context of the architecture of the sample, but were it viewed as a "naked" gland, it could easily be interpreted as dysplastic.

the wide area sampling might potentially decrease sampling variability and false-negative surveillance procedures. The WATS specimens might mimic "minibiopsy specimens" because of deeper sampling of the epithelium, potentially the entire thickness. However, it is unclear how many of the specimens actually acquire full-thickness samples. The WATS specimen is optically imaged in 50 1-μm slices, which are then integrated together to form a 3-dimensional image. Hence, glandular morphology, which is missing from standard esophageal brushings, might provide improved dysplasia assessment. However, there is minimal lamina propria and muscularis mucosae in WATS epithelial specimens to assess, the presence of which can be key for differentiating HGD from invasive cancer. Furthermore, the architectural features of nonneoplastic versus neoplastic BE glands and the differences between surface epithelium and deep glands cannot be evaluated, unlike in standard biopsy specimens. Evaluating such architectural features is a key element in assessing severe columnar neoplasia. Hence, the WATS specimen diagnosis is limited to a combined "HGD/EAC" diagnosis, and even that may be open to interpretation. This is concerning because the treatment of a patient with unlocalized EAC differs from that of a patient with HGD.

The images of the WATS-sampled BE in the current study are concerning to an expert pathologist, which raises the question whether an upgraded WATS sample diagnosis can be "correct" compared with the reference standard biopsy diagnosis. In Figure 2 from the article by Vennalaganti and colleagues,[18] image A is perfectly diagnosed as BE negative for dysplasia, whereas image B may be reasonable for LGD, except that nondysplastic glands at the bases of the pits can have the same appearance. However, image C, labeled as HGD, was taken at a very high magnification and does not appear to show dysplasia at all. The surface mucin displays the "4 lines" of nondysplastic Barrett's mucosa, and the depicted mitosis may simply reflect the fact that the gland is from the deeper aspect of the tissue. However, this can be neither confirmed nor refuted when the WATS technique is used. Figure D cannot be diagnosed as adenocarcinoma in isolation. There is a real risk for overdiagnosis of dysplasia. We provide an example of a case readily diagnosed as negative for dysplasia according to standard methods but that might result in an overdiagnosis by the evaluation of deep glands out of context (Figs. 1-3).

The study investigators are to be congratulated for completing a prospective, relatively large, well-designed, and appropriately powered study. However, we must also consider the study limitations. First, this study involved predominantly academic referral centers with enrichment of HGD/EAC, and the results cannot be generalized to a nonenriched community setting, where most BE patients undergoing surveillance have no dysplasia. It is important to note that the majority (91%) of the 23 patients with diagnoses of HGD/EAC by WATS sampling alone already had a prior diagnosis of dysplasia at study entry. Hence, the true incremental benefit of WATS sampling with regard to increasing the dysplasia grade is uncertain. Second, the performance characteristics of WATS are unknown and not formally studied. Only the WATS-positive HGD/EAC slides were blindly reviewed by