Communication from the ASGE Technology Committee

TECHNOLOGY STATUS EVALUATION REPORT

Advances in the diagnosis and surveillance of Barrett's esophagus (with videos)

Prepared by: THE ASGE TECHNOLOGY COMMITTEE

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Background and Aims: Most patients diagnosed with esophageal adenocarcinoma do not carry a known diagnosis of Barrett's esophagus (BE), suggesting that an improved approach to screening may potentially be of benefit. The use of dysplasia as a biomarker and random biopsy protocols for its detection has limitations. In addition, detecting and appropriately classifying dysplasia in patients with known BE can be difficult.

Methods: This document reviews several technologies with a recently established or potential role in the diagnosis and/or surveillance of BE as well as risk stratification for progression to esophageal adenocarcinoma.

Results: Two technologies were reviewed for imaging or tissue sampling: (1) wide-area transepithelial sampling and (2) volumetric laser endomicroscopy. Four technologies were reviewed for molecular and biomarker technologies for diagnosis and risk stratification: (1) Cytosponge, (2) mutational load, (3) fluorescence in situ hybridization, and (4) immunohistochemistry.

Conclusion: Several technologies discussed in this document may improve dysplasia detection in BE in a wide-field manner. Moreover, the addition of different biomarkers may aid in enhanced risk stratification to optimize approaches to surveillance or treatment for patients with BE. (Gastrointest Endosc 2019;90:325-34.)

The American Society for Gastrointestinal Endoscopy (ASGE) Technology Committee provides reviews of existing, new, or emerging endoscopic technologies that have an impact on the practice of GI endoscopy. Evidence-based methods are used, with a MEDLINE literature search to identify pertinent clinical studies on the topic and a MAUDE (Food and Drug Administration Center for Devices and Radiological Health) database search to identify the reported adverse events of a given technology. Both are supplemented by accessing the "related articles" feature of PubMed and by scrutinizing pertinent references cited by the identified studies. Controlled clinical trials are emphasized, but in many cases data from randomized controlled trials are lacking. In such cases, large case series, preliminary clinical studies, and expert opinions are used. Technical data are gathered from traditional and Web-based publications, proprietary publications, and informal communications with pertinent vendors. Reports on emerging technology are drafted by 1 or 2 members of the ASGE Technology Committee, reviewed and edited by the committee as a whole, and approved by the governing board of the ASGE. When financial guidance is indicated, the most recent coding data and list prices at the time of publication are provided. For this review, the MEDLINE database was searched through September 2017 for relevant articles by using keywords such as "Barrett's esophagus," "dysplasia detection," " biomarkers," "mutational load," "fluorescence in situ hybridization," "immunohistochemistry," and " genetic mutations," among others. ASGE Technology Committee reviews are scientific reviews provided solely for educational and

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informational purposes. ASGE Technology Committee reviews are not rules and should not be construed as establishing a legal standard of care or as encouraging, advocating, requiring, or discouraging any particular treatment or payment for such treatment.

Barrett's esophagus (BE) has been defined as a change from normal esophageal squamous epithelium to specialized intestinal metaplasia.[1] It is estimated that 5.6% of adults in the United States have BE.[2] BE is a major risk factor for esophageal adenocarcinoma (EAC), the incidence of which continues to rise.[3] Interventions to impact the increasing incidence of EAC are limited because of 2 factors: (1) many patients with BE may remain undiagnosed until a symptomatic cancer arises,[4] and (2) traditional surveillance approaches are imperfect at identifying which patients with BE will progress to cancer.[5]

Most patients diagnosed with EAC do not carry a known diagnosis of BE, suggesting that an improved approach to screening may be of benefit.[6] However, endoscopic screening for BE in patients with GERD and 1 risk factor (eg, male sex, age >50 years, hiatal hernia, white ethnicity, elevated body mass index, or smoking) is limited in that it is cost prohibitive and lacks conclusive evidence that it changes the incidence of EAC.[7] Currently, cancer risk stratification in BE is based on the detection of dysplasia in random biopsy specimens taken every 1 to 2 cm over the length of the BE.[1,8] However, detecting and appropriately classifying dysplasia can be difficult. Dysplasia may be focal, and most biopsy techniques sample a fraction of the BE.[7] Further, there is wide interobserver variability in classifying dysplasia, even among expert pathologists.[7] Moreover, endoscopic surveillance of patients with known BE may not improve mortality from EAC,[5] although it is associated with increased cost. Thus, current needs in the evaluation of BE include improvements in screening approaches, the ability to detect dysplasia, and in the reliability of biomarkers. This document reviews several technologies with a recently established or potential role in the diagnosis and/or surveillance of BE as well as risk stratification for progression to EAC.

ENDOSCOPIC TECHNOLOGIES FOR IMAGING OR TISSUE SAMPLING

Wide-area transepithelial sampling

Current guidelines recommend upper endoscopy with random 4-quadrant biopsy specimens obtained every 1 to 2 cm for the detection of neoplasia in BE.[1,9] Random biopsies may miss early lesions[10] because this technique samples only about 5% of the Barrett's epithelium.[7] Further, this technique is time consuming, with resultant poor adherence to this protocol.[11,12] Wide-area transepithelial sampling (WATS) with computer-assisted 3-dimensional (3-D) analysis (WATS3D; CDx Diagnostics, Suffern, NY, USA) is a sampling technique that combines an abrasive brush biopsy followed by computer-assisted pathology analysis and represents an alternative to existing approaches. WATS3D has been cleared by the U.S. Food and Drug Administration (FDA) and is available commercially. Applicable Current Procedural Terminology (CPT) codes for use with this device include esophagoscopy with biopsy (43204) and EGD with biopsy (43235). The costs associated with WATS3D and the other devices and tests in this review are summarized in Table 1.

The WATS specimen is obtained through the use of a specially designed, highly abrasive brush that produces a hybrid cytology and/or histology tissue sample. The 19-mm long Tynex (DuPont, Wilmington, DE, USA) nylon and stainless steel wire braid brush is positioned on the distal end of a 160-cm long, 2.6-mm diameter Teflon (DuPont, Wilmington, DE, USA) sheath catheter (Fig. 1). This catheter may be advanced through the channel of a standard adult gastroscope. The brush is advanced out of the sheath, positioned against the surface of the mucosa, and repeatedly manipulated by the endoscopist in a to-and-fro manner to sample the entire Barrett's esophagus field (Video 1, available online at www.giejournal.org). After pinpoint bleeding is observed (indicating adequate biopsy of an area), the brush is retracted into the catheter, and the catheter is removed from the channel of the endoscope. Each segment is brushed twice. Two brushes are supplied with each kit; 1 brush is used to make a slide and the other brush is placed in a cell block. Each kit is intended to sample a segment of BE that is ≤5 cm in length. If a longer segment needs to be sampled, multiple kits should be used. A 95% alcohol/5% polyethylene glycol fixative pouch is included in the kit for applying the fixative to the slide after the brush sample is smeared onto the slide. The slide and cell block are then returned to a central commercial pathology laboratory for processing and interpretation.

Each brush sample typically contains both intact tissue fragments ("micro-biopsies") in addition to individual cells. On average, approximately 100,000 cells are present for analysis.[13] The dedicated brush biopsy instrument yields a tissue specimen that may be 50 to 100× thicker than the standard 2-micron–thick tissue section made with a microtome. This unusual thickness makes it difficult to analyze the specimen by using a standard microscope that has only a 3-micron depth of field. The WATS3D computer-analysis system was designed to overcome this limitation and allows the pathologist to locate and visualize abnormalities in all focal planes of this unusually thick specimen. A 3-D image of cells is created for the specimen on the slide, which may facilitate visualization of dysplastic cells as compared with standard traditional histology specimens cut with a microtome. In addition, the computer software highlights and marks suspicious areas that may be consistent with dysplasia, assisting verification by a trained pathologist on the manual microscope. All abnormalities identified by WATS3D are

TABLE 1. List prices for devices and tests discussed in this review

Device and/or test	List price (U.S. $)*	Comment
WATS 3D (CDx Diagnostics)	742†	Kit provided free of charge
VLE		
NvisionVLE (NinePoint Medical) imaging system with real-time targeting (marking console)	235,000	
NvisionVLE marking probe	1595	Single-use
NvisionVLE inflation system	175	Single-use
BarreGen mutational load (Interpace Diagnostics)	4500	
Fluorescence in situ hybridization	308-800‡	This test is available from multiple laboratories
Tissue Cypher (Cernostics, Inc)	2350	

WATS 3D, Wide-area transepithelial sampling with computer-assisted 3-dimensional analysis; *VLE,* volumetric laser endomicroscopy.
*List prices often are higher than actual prices paid. Laboratory service agreements often provide negotiated contract fees. Medical devices often are provided at discounted rates through negotiated contracts between medical centers and the medical device companies.
†The testing procedures required for analysis of a specific WATS specimen are ordered by the examining anatomic pathologist and are dependent on the actual specimen received. The fee billed to the patient's insurance by the laboratory on most specimens for the most basic components of this overall analysis is $742/case. The cost of all tissue sampling materials and shipment of the WATS specimen back to the laboratory is included in the price.
‡Multiple companies and tertiary-care hospitals offer this test. The price listed is a range of the prices typically encountered.

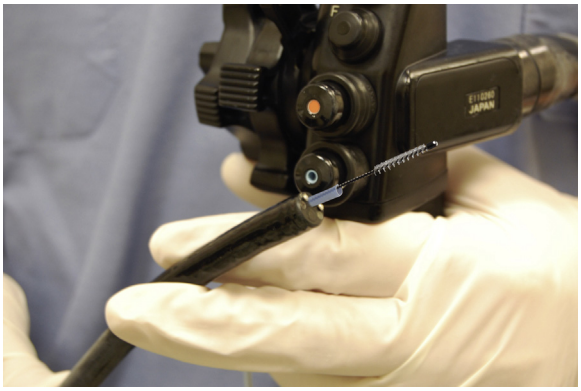

Figure 1. Wide-area transepithelial sampling 3-dimensional biopsy brush. Image obtained and used with permission from CDx Diagnostics.

diagnosed and reported by using standard pathology criteria for BE and dysplasia. In addition, all samples undergo immunohistochemical evaluation for potentially relevant biomarkers including CDX2, MUC2, p53, and AMACR.

Multiple prospective trials have evaluated the incremental yield of WATS3D beyond protocol biopsies for the detection of dysplasia during BE surveillance. A prospective multicenter trial evaluated 151 patients with a history of dysplastic BE undergoing surveillance endoscopy by performing WATS followed by standard 4-quadrant biopsies.[14] The addition of WATS to standard biopsy forceps yielded 16 additional cases of dysplasia, an incremental yield of 42% (95% confidence interval [CI], 21%-73%). Similarly, a prospective trial conducted at 16 academic centers evaluated 160 patients undergoing surveillance endoscopy, of whom 21% had known dysplasia.[15] Patients were randomized to WATS3D followed by protocol biopsies or protocol biopsies followed by WATS3D. The addition of WATS3D yielded an additional 23 cases of high-grade dysplasia (HGD) and/or EAC (absolute increase 14% [95% CI, 8%-21%]).

In an interobserver study of WATS3D slides with varying degrees of dysplasia, the kappa values for 4 blinded pathologists with prior WATS training were 0.95 (0.88-0.95), 0.74 (0.61-0.85), and 0.88 (0.81-0.94) for HGD and/or EAC, indefinite for dysplasia and/or low-grade dysplasia (LGD), and no dysplasia, respectively.[13] WATS3D also has been evaluated as an adjunctive modality to improve the detection of BE in a prospective study of 8 community practices with 1266 patients with GERD undergoing screening endoscopy. The addition of WATS3D to forceps biopsies significantly improved the detection of BE compared with forceps biopsies alone. An additional 146 cases were detected, for an incremental yield of BE detection of 39.8% (95% CI, 32%-48%).[16]

These studies evaluated WATS3D as an adjunctive tool to Seattle protocol biopsies.[14,15,16] No published reports have described the use of WATS3D as a stand-alone modality for BE screening or dysplasia surveillance. Also, it is unclear whether a WATS3D specimen with a positive result in the setting of negative results on protocol biopsies represents a true or false positive because no studies have evaluated WATS3D against an external criterion standard such as esophagectomy specimens. Finally, compared with standard biopsy specimens, evaluation of the architectural features of neoplastic versus non-neoplastic glands and differences between surface epithelium and deeper glands is hindered or not possible with WATS, limiting the differentiation of HGD versus EAC.[17]

Volumetric laser endomicroscopy

Volumetric laser endomicroscopy (VLE) is an advanced imaging technology that became commercially available in the United States in 2013 (NvisionVLE; NinePoint Medical Inc, Bedford, Mass, USA). VLE is an FDA-cleared

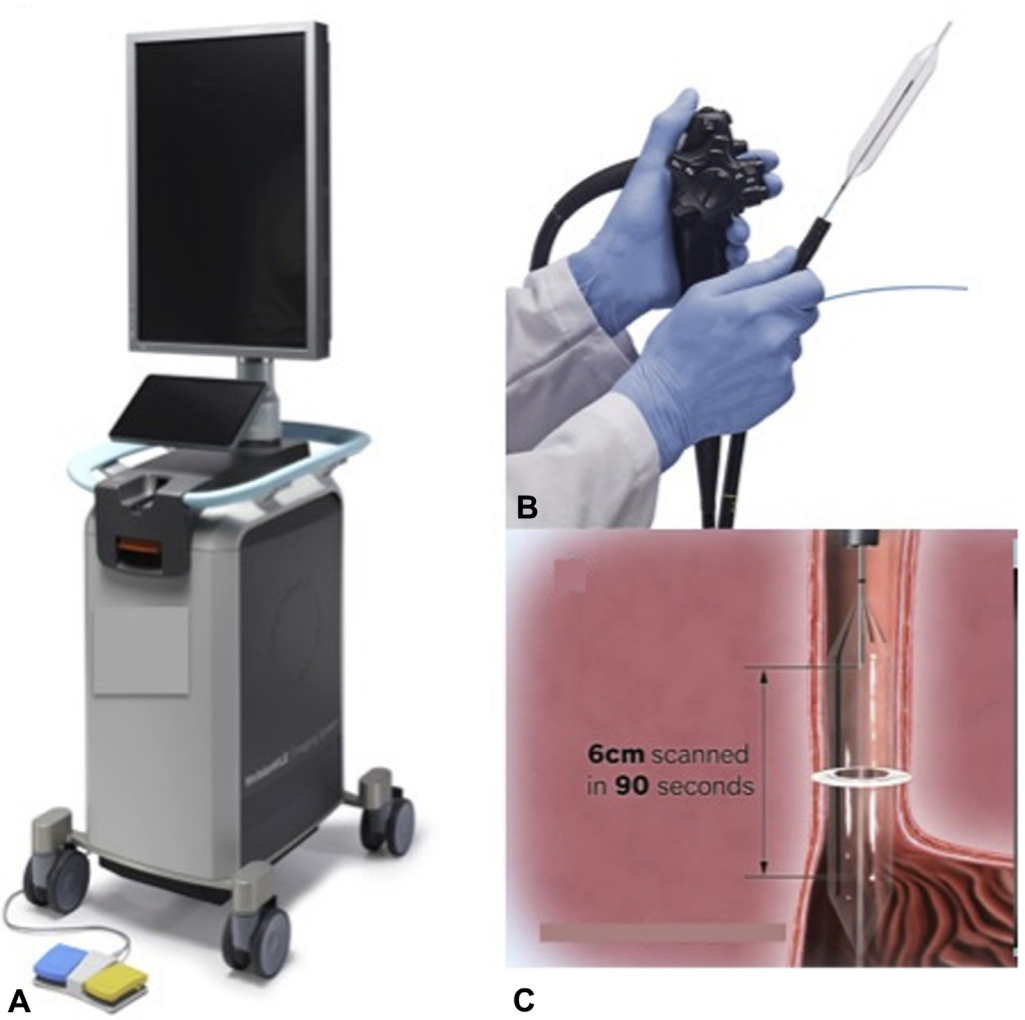

Figure 2. The components of the Nvision volumetric laser endomicroscopy (VLE) system including **A,** the VLE console **B,** the probe within the inflated balloon **C,** a cartoon depicting the VLE scan within the esophagus. Image obtained and used with permission from NinePoint Medical.

second-generation optical coherence tomography technology. Applicable CPT codes include esophagoscopy with optical endomicroscopy (43206) and EGD with optical endomicroscopy (43252). It uses infrared light to produce real-time high-resolution cross-sectional imaging of the esophagus. The NvisionVLE system can scan a 6-cm length of the esophagus in approximately 90 seconds, providing surface and subsurface wide-field cross-sectional imaging with an axial resolution of 7 μm and a depth of 3 mm.[18,19] The VLE imaging system consists of a console, monitor, and optical probe contained within a Mylar (DuPont Tejjin Fims, Chester, VA, USA) balloon on an 8F, 260-cm catheter (Fig. 2). The distal end of the catheter connects to the console. The probe is available in 14-mm, 17-mm, and 20-mm diameter balloons that are 6 cm in length. The balloon is positioned such that the distal margin of the balloon is located 1 cm distal to the gastroesophageal junction. This allows a single scan to image the gastric cardia, the gastroesophageal junction, and the distal esophagus. The balloon is inflated to 15 psi, although, depending on anatomy, the balloon inflation pressure may be modified accordingly. The inflated balloon allows for centering of the probe while helical scanning occurs. Imaging is performed during automatic retraction of the probe from the distal to proximal end of the balloon over a 90-second period, creating real-time 360° images. Twelve hundred cross-sectional scans are generated over the 6-cm segment. VLE scans are viewed by using a software interface that allows real-time viewing of cross-sectional, transverse, and longitudinal views. There is a registration line on the balloon and the VLE images that allows for orientation of VLE images with endoscopic imaging.

A recent upgrade to the imaging platform includes the ability to perform superficial laser marking of the esophageal epithelium when suspicious areas are identified on VLE to provide more precise targeting for biopsies or endoscopic resection (Video 2, available online at www.giejournal.org).[20] A safety and efficacy study was performed evaluating VLE with a prototype laser marking device in 16

patients with BE, with 222 laser marks placed.[21] The study showed that laser marking was safe and efficacious, with an 85% positional accuracy rate of the laser marks.

Scoring systems for optical coherence tomography and VLE have been developed to help detect neoplasia (HGD and intramucosal cancer) in BE.[22,23] These scoring systems were developed by VLE scanning ex vivo endoscopic resection specimens and correlating VLE features with histology.[24-26] A potential advantage of VLE is its ability to detect subepithelial disease in BE,[26-28] although the clinical relevance of these findings remains uncertain.[29]

Multiple case reports and case series have demonstrated the potential of VLE to identify dysplasia in BE not detected by high-definition white-light endoscopy or electronic chromoendoscopy.[24-26,30] A large, single-center, retrospective series found an incremental yield of dysplasia detection by using VLE with laser marking compared with VLE without laser marking or random biopsies.[31] A study looked at the interobserver agreement between users at high-volume academic centers based on still images, and the study found strong agreement for non-neoplastic and neoplastic BE (kappa 0.66 and 0.79).[32] Although the learning curve for interpretation of VLE images appears to be favorable,[33] a large amount of complex data are interpreted by the endoscopist in real time. Thus, computer-aided detection of BE neoplasia is under development, which will allow a software program to pinpoint areas concerning for neoplasia on a VLE image.[22,23,34] A study evaluated a prototype computer-aided detection program that uses 60 VLE images of ex vivo endoscopic resection specimens.[35] The study found that computer-aided detection was able to detect neoplasia with 90% sensitivity and 93% specificity. Computer-aided detection of neoplasia is not yet commercially available.

MOLECULAR AND BIOMARKER TECHNOLOGIES FOR DIAGNOSIS AND RISK STRATIFICATION

Cytosponge

The Cytosponge-TFF3 (Trefoil Factor 3; BD Diagnostics, Durham, NC, USA) is a cell collection device that has been evaluated as a non-endoscopic form of screening for BE.[6] The device contains polyester sponge material compressed within a capsule 8.5 mm in diameter and 25 mm in length. The sponge is attached to a 70-cm polyester string (Fig. 3). The capsule is composed of a gelatin vegetable derivative that disintegrates in the stomach within 3 to 5 minutes of swallowing,[36] releasing the sponge to its full 30-mm diameter. The capsule is swallowed, leaving a portion of the string exiting the patient's mouth. The string is then retracted to retrieve the sponge, which collects cells from the gastroesophageal junction and the esophagus during its return passage. The collected cells are then processed by immunohistochemistry testing for TFF3, a secretory protein from mucin-producing cells that specifically differentiates BE from gastric cardia cells and squamous esophageal cells.[37] A commercial version (Cytosponge; Medtronic, Dublin, Ireland) is currently available outside the United States. The Cytosponge is FDA-cleared but is currently being used in the United States only in a research capacity; a limited commercial release in the United States is underway as of early 2019.

A multicenter prospective study evaluated a prototype version of the Cytosponge in 504 patients aged 50 to 70 years undergoing endoscopy for GERD and compared the results with those of endoscopy.[38] Ninety-nine percent of the patients were able to swallow the capsule, and there were no serious adverse events. The sensitivity and specificity of the Cytosponge were 73.3% (95% CI, 44.9%-92.2%) and 93.8% (95% CI, 91.3%-95.8%) for ≥1 cm circumferential BE and 90.0% (95% CI, 55.5%-99.7%) and 93.5% (95% CI, 90.9% - 95.5%) for BE segments of ≥2 cm. In the Barrett's Esophagus Screening Trial 2, a multicenter case-control study from the United Kingdom, 1100 patients (647 patients with known BE and 463 controls) underwent the Cytosponge procedure followed by endoscopy at tertiary-care centers.[36] The overall sensitivity of the Cytosponge for diagnosing BE was 79.9% (95% CI, 76.4%-83.0%), and the specificity was 92.4% (95% CI, 89.5%-94.7%). In patients with circumferential BE >3 cm the sensitivity increased to 87.2% (95% CI, 83.0%-90.6%). No adverse events related to the Cytosponge occurred. The Cytosponge received higher acceptability scores than endoscopy with sedation or local anesthetic spray when rated by the patients ($P < .001$). A prospective study evaluated the commercial Cytosponge-TFF3 test (The Cytosponge**)** in 73 patients with known BE undergoing endoscopy.[39] It was found to be safe, acceptable, and had a sensitivity of over 90% for the detection of BE. There are no published studies from the United States on the Cytosponge as yet.

Mutational load

Mutational load (ML) is a measure of genetic aberration and represents a potential biomarker to risk-stratify disease progression in BE.[40] BE tissue with a higher proportion of genetic aberrations (ie, loss of heterozygosity of tumor suppressor genes) more frequently progresses to advanced disease.[41] ML can be assessed by using a commercially available kit (BarreGEN; Interpace Diagnostics, Pittsburgh, Pa, USA). This technology is used currently in clinical practice to provide additional data in patients with BE regarding their risk for progression to esophageal cancer. The test quantifies the degree of derangement of 10 genetic loci for tumor suppressor genes relevant in Barrett's neoplasia, specifically assessing the presence of loss of heterozygosity mutations and new alleles consistent with microsatellite instability. ML is analyzed by using formalin-fixed, paraffin-embedded tissue from biopsy specimens taken at the time of endoscopy. Hematoxylin and eosin (H&E)-stained slides are examined

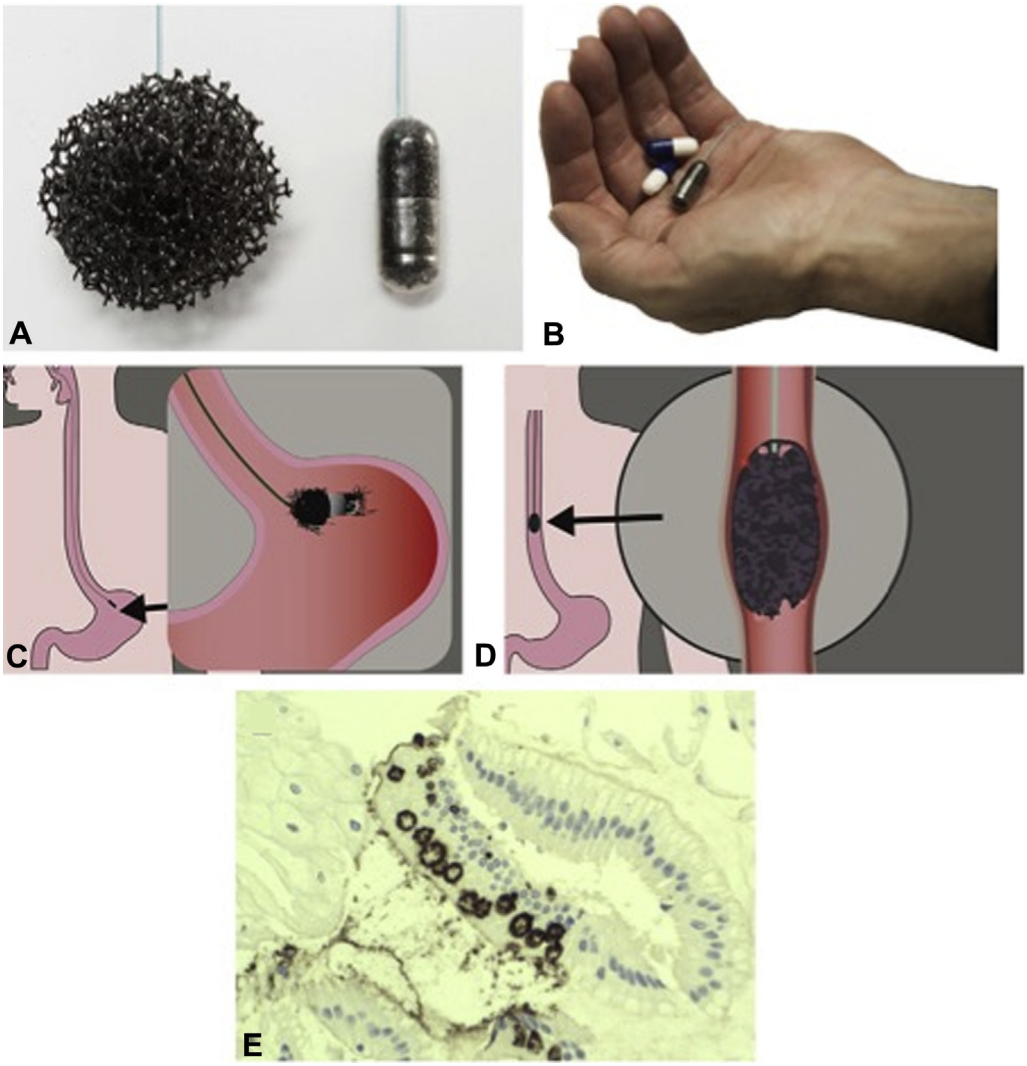

Figure 3. Use of the Cytosponge test: **A,** Expanded Cytosponge (*left*) and Cytosponge embedded in a gelatin capsule (*right*). **B,** The Cytosponge compared with paracetamol capsules in the palm of a hand. **C,** The Cytosponge is swallowed, and the gelatin capsule dissolves in the stomach within 5 minutes. **D,** The Cytosponge is retrieved by a nurse, collecting cells as it is pulled up. The arrows indicate the enlarged area containing the Cytosponge. **E,** Immunohistochemical images (orig. mag. ×20), illustrating Trefoil Factor 3 (TFF3)–positive staining in cells collected with the Cytosponge (immunostaining for TFF3 with proprietary monoclonal antibody; BD Diagnostics). Image and caption used with permission by Elsevier.

microscopically to identify representative patient histology and targets of interest. The H&E slides are then used as a guide for microdissection of recut, unstained, 4 micron-thick, formalin-fixed, paraffin-embedded slides from the region of interest. DNA from microdissection of the targets is then analyzed by using polymerase chain reaction and quantitative capillary electrophoresis. For each tissue target, it is determined whether each loss of heterozygosity mutation is of low (50%-75% of DNA affected) or high (>75% of DNA affected) clonality. The sum of the clonality of each genetic locus is the mutational load.[42]

A case-control study (n = 69) evaluated the utility of ML in predicting progression to HGD or EAC based on samples of nondysplastic BE or BE with LGD at baseline.[40] Cases that progressed to HGD and/or EAC during follow-up (n = 23) were compared with 46 controls who did not progress during a 4-year follow-up period. The baseline mean ML was higher in cases than in controls (2.21 vs 0.42; $P < .001$). ML may serve as an adjunctive test in cases of equivocal histology. A retrospective study evaluated ML in 271 patients with varying degrees of dysplasia (indefinite, LGD, and HGD)[42] and reported that ML correlated to the degree of dysplasia (1.1 vs 2.2 vs 3.3, respectively; correlation coefficient = 0.60; $P < .0001$). Another retrospective study of 877 targets from BE biopsy specimens that underwent microdissection described a similar correlation of ML with increasingly worse degrees of dysplasia (correlation coefficient = 0.68; $P < .0001$).[43]

Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH)–based biomarker assays that use esophageal brushing specimens

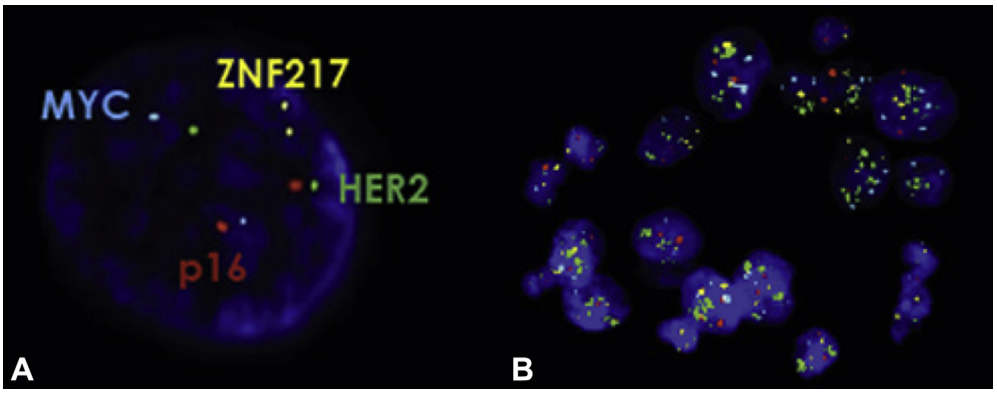

Figure 4. Use of fluorescence in situ hybridization (FISH) in the evaluation of Barrett's epithelium. **A,** A normal esophageal cell nucleus with the expected 2 signals from each probe. **B,** Esophageal brushing with the majority of nuclei demonstrating FISH anomalies. Used with permission from NeoGenomics.

may offer another tool for prediction of BE progression and treatment response. FISH uses fluorescently labeled DNA probes to detect gain or loss of specific chromosomal regions (Fig. 4). Prior studies have evaluated FISH probes directed against tumor suppressor and proto-oncogenes, such as 8q24 (c-MYC), 9p21 (p16), 17q11.2 (HER2/*neu*), 17q13.1 (p53), and 20q13.2 (ZNF217) in various BE populations.[44]

FISH assays can be performed by using commercially available kits available through multiple companies and tertiary-care centers. These assays are being used in practice to aid in risk stratification of patients with BE for progression to esophageal cancer. Brushings are collected during endoscopy by using a standard cytology brush before biopsies. Brushings should be performed initially on any visible abnormalities, such as nodularity. A separate brushing is then obtained from the remaining BE segment. Many available assays use a 4-probe panel (Vysis; Abbott Molecular, Des Plaines, Ill, USA) to detect gains and losses of MYC (8q24), p16 (CDKN2A at 9p21), HER2 (ERBB2 at 17q12), and ZNF217 (20q13), which have been associated with neoplastic progression.[45-48]

A recent multicenter study reported the utility of the Vysis 4-probe panel to discriminate degrees of dysplasia in BE.[45] The authors studied a total of 46 non-BE, 42 nondysplastic BE, 23 indefinite for dysplasia, 10 LGD, 29 HGD, and 42 EAC specimens. The presence of polysomy on FISH was able to identify HGD and/or EAC with a sensitivity of 80% and a specificity of 88%.[45] Other studies that use different probes also have reported high sensitivity and specificity of FISH for differentiating LGD from HGD and/or EAC.[46-48]

The utility of FISH to risk-stratify patients with HGD who will progress to EAC also has been evaluated.[44] In a single-center retrospective study, 245 patients with prior biopsy-proven HGD without EAC underwent brush cytology during surveillance endoscopy. The authors evaluated the brushing specimens by using the previously described Vysis 4-probe panel for the presence of polysomy; 93 patients (38%) had polysomy detected in at least 1 target gene, and 152 patients (62%) did not. Patients with a polysomic FISH result had a significantly higher risk of developing EAC within 2 years (14.2 vs 1.4%; $P < .001$).[44]

FISH also has been studied to predict the response to treatment of BE neoplasia. A retrospective cohort study evaluated 181 patients who underwent endoscopic therapy for HGD or early EAC.[49] Cytology specimens were obtained from all participants within 3 months before ablation therapy, which was performed by using radiofrequency ablation and multipolar coagulation, among other techniques. FISH analysis was performed by using the 4-probe panel described previously. A total of 130 patients (72%) achieved complete remission of dysplasia, defined as the absence of dysplasia or neoplasia during 2 consecutive surveillance endoscopies performed at least 3 months apart. Normal FISH results before ablation were associated with achievement of complete remission of dysplasia (hazard ratio 1.53; 95% CI, 1.06-2.21), whereas multiple gains of target loci found in the pretreatment cytology samples were associated with a decreased chance of complete remission of dysplasia (hazard ratio 0.57; 95% CI, 0.40-0.82).[49] Similar findings were observed in 2 studies of patients with BE and HGD and/or EAC who were treated with photodynamic therapy.[50,51]

Immunohistochemistry

In addition to routine cytology analysis, collected cells can be analyzed with molecular markers for diagnosis and risk stratification. Dutch investigators have prospectively evaluated immunohistochemical analysis of p53, AMACR, cyclin A, and SOX2 in several studies of patients with BE to characterize the predictive value of these markers for neoplastic progression.[50-52] In a large, prospective, multicenter study, 625 patients with BE were followed in an endoscopic surveillance program for a median duration of 6.7 years. Fifty patients (8%) developed neoplastic progression (HGD, n = 37; EAC, n = 13) during surveillance after a median follow-up of 3.2 years. Cyclin A immunopositivity was seen in 10% of nondysplastic biopsy

specimens, in 33% of LGD biopsy specimens, and in 69% of HGD and/or EAC biopsy specimens, and was associated with an increased risk of neoplastic progression (adjusted relative risk, 2.4; 95% CI, 1.7-3.4). The same authors evaluated p53 and SOX2 expression in earlier studies, and these markers demonstrated similar prognostic value.[50] In another cross-sectional prospective study of 175 patients with BE,[53] aneuploidy and p53 or cyclin A immunopositivity had the strongest associations with dysplasia in a per-biopsy analysis and, as a panel, had an area under the receiver operating characteristic curve of 0.97 (95% CI, 0.95-0.99) for diagnosing HGD and/or EAC. These findings were reproduced in a validation cohort of 46 patients.[53]

A commercial TissueCypher (Cernostics, Inc, Pittsburgh, Pa, USA) BE assay has been developed for use with histologic biopsy specimens. TissueCypher is currently being used in clinical practice to provide additional data in patients with BE regarding their risk for progression to esophageal cancer. This assay uses algorithms to interpret quantitative biomarker (immunohistochemistry and FISH), cellular, and subcellular morphology feature data. A total of 1184 features and/or biopsy specimens are extracted from the biomarkers and morphology by the software and then summarized as multiple measures. Based on these data, a 5-year risk score for progression to HGD and/or EAC is generated.

A nested case-control study evaluated TissueCypher in 79 patients with BE with no or indefinite dysplasia or LGD who progressed to HGD and/or EAC at least 1 year later and 287 samples from matched control patients who did not show progression.[54] The assay incorporates 3-tier stratification into low, intermediate, or high risk for progression. The predicted high-risk group developed HGD and/or EAC at a 9.4-fold greater rate (95% CI, 4.6-19.2; $P < .0001$) than the low-risk group. The performance characteristics of TissueCypher in a prospective study or in BE cohorts beyond the institutions that developed the assay's risk score have not yet been reported.

AREAS FOR FUTURE RESEARCH

The technologies reviewed in this document are commercially available in the United States, with the exception of the Cytosponge (a limited U.S. commercial release of Cytosponge is underway as of early 2019. The endoscopy-based technologies, WATS 3D and VLE, are both FDA-cleared and have associated category I CPT codes. Further evaluation of these established technologies will enhance the understanding of their utility in diverse patient populations, including those with a low prevalence of BE and dysplasia.

Several of the molecular and biomarker technologies discussed in this document have the potential to help risk stratify patients with BE. Although mutational load, FISH analysis, and immunohistochemistry are available commercially and have data supporting their use, current utilization is per physician discretion, and adoption has not been widespread. The competing and potentially complementary roles of these technologies in risk stratification and their role in best clinical practice has yet to be defined. In addition, further clarification is needed in how to manage or survey positive results in molecular studies in the setting of a negative EGD result. Finally, many of these technologies are associated with significant expense; cost effectiveness analyses will guide best practices with regard to adoption of these technologies in routine care.

Summary

The incidence of EAC is increasing despite awareness of BE as the precursor lesion. Improved, less invasive screening approaches that better identify undiagnosed BE may be beneficial. The use of dysplasia as a biomarker and random biopsy protocols for its detection has limitations. Several technologies discussed in this document may improve dysplasia detection in BE in a wide-field manner. Moreover, the addition of different biomarkers may aid in enhanced risk stratification to optimize approaches to surveillance or treatment for patients with BE.

DISCLOSURE

H. Aslanian is a consultant for Boston Scientific and Olympus. M. Bhutani is on the advisory board for MediGlobe. D. Lichtensteinis a consultant for Olympus. J. Melson received investigator-initiated funds from Boston Scientific and is on the medical advisory board for Clinical Genomics. U. Navaneethan is a consultant for Takeda, AbbVie, and Janssen. R. Pannala is a consultant for Boston Scientific and received research support from Apollo Endosurgery. M. Parsi received honoraria from Boston Scientific. A. Schulman is a consultant for Boston Scientific and MicroTech. A. Sethi is a consultant for Boston Scientific and Olympus. G. Trikudanathan is on the advisory board for AbbVie. All other authors disclosed no financial relationships relevant to this publication.

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Abbreviations: ASGE, American Society for Gastrointestinal Endoscopy; BE, Barrett's esophagus; CPT, Current Procedural Terminology; 3-D, 3-dimensional; EAC, esophageal adenocarcinoma; FDA, U.S. Food and Drug Administration; FISH, fluorescence in situ hybridization; H&E, hematoxylin and eosin; HGD, high-grade dysplasia; LGD, low-grade dysplasia; ML, mutational load; VLE, volumetric laser endomicroscopy; WATS, wide-area transepithelial sampling.

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