Sentara Health Plans

**Esophageal Pathology Testing**

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| Policy Number: AHS – M2171 – Esophageal Pathology Testing | Prior Policy Name and Number, as applicable: |
| Initial Policy Effective Date: 12/01/2024 | |

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## I. Policy Description

The esophagus is a long tube that serves to connect the mouth to the stomach. Although the esophagus is primarily a connecting organ, it experiences significant chemical and mechanical trauma. The esophagus has mechanisms and structures to withstand this damage, but molecular injury is common (Zhang et al., 2020). Both serological and genetic markers have been suggested to identify, diagnose, or assess risk in the esophagus.

Eosinophilic esophagitis (EoE) is one such condition, as its nonspecific symptoms (pain, issues swallowing, vomiting, and so on) may be accompanied by inflammatory markers in the esophagus (Bonis & Gupta, 2021, 2023). Similarly, esophageal cancer is characterized by several nonspecific symptoms, while a predecessor condition, Barrett's esophagus (BE), may have no clinical symptoms at all (Saltzman & Gibson, 2021; Spechler, 2023).

For guidance concerning Tumor Mutational Burden Testing (TMB) and/or Microsatellite instability (MSI) analysis please refer to the AHS-M2178-Microsatellite Instability and Tumor Mutational Burden Testing policy.

## II. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in Applicable State and Federal Regulations of this policy document.

1) For consideration of therapy with PD-1 inhibitors for individuals with locally advanced, recurrent, or metastatic esophageal, gastric, or esophagogastric junction cancer, **any** of the following testing **MEETS COVERAGE CRITERIA**:
   a) Tumor analysis of PD-L1 expression by immunohistochemistry.
   b) Mismatch repair (MMR) analysis.
2) When trastuzumab is being considered for therapy for individuals with esophageal, gastric, or esophagogastric junction cancer, genetic testing of *HER2* **MEETS COVERAGE CRITERIA**.

3) When larotrectinib or entrectinib is being considered as a first-line or subsequent therapy for individuals with esophageal, gastric, or esophagogastric junction cancer, genetic testing for *NTRK* gene fusion **MEETS COVERAGE CRITERIA**.

4) The use of genetic testing (e.g., molecular panel tests and gene expression profiling) to assess the risk of eosinophilic esophagitis (EoE) **DOES NOT MEET COVERAGE CRITERIA**.

5) The use of genetic testing (e.g., molecular panel tests and gene expression profiling) to diagnose or monitor eosinophilic esophagitis (EoE) **DOES NOT MEET COVERAGE CRITERIA**.

6) For the diagnosis and evaluation of Barrett's esophagus, low-grade esophageal dysplasia, or high-grade esophageal dysplasia, wide-area transepithelial sampling (WATS) **MEETS COVERAGE CRITERIA**.

*The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.*

7) Assessing for risk of Barrett's esophagus and/or esophageal, including esophagogastric junction, cancer using a molecular classifier (e.g., BarreGEN test) **DOES NOT MEET COVERAGE CRITERIA**.

8) Epigenetic analysis for the likelihood for Barrett's esophagus, esophageal, or esophagogastric junction cancer (e.g., methylation analysis, EsoGuard) **DOES NOT MEET COVERAGE CRITERIA**.

9) To diagnose, assess, or monitor eosinophilic esophagitis (EoE), the Esophageal String Test **DOES NOT MEET COVERAGE CRITERIA**.

10) For esophageal and esophagogastric junction cancers, cell-free DNA/circulating tumor DNA (cfDNA/ctDNA) testing **DOES NOT MEET COVERAGE CRITERIA**.

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**NOTES:**

**Note:** For 5 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

## III. Table of Terminology

| Term | Definition |
|---|---|
| ACG | American College of Gastroenterology |
| AFS | American Foregut Society |
| AMACR | Alpha-methylacyl-CoA racemase |
| APC | *Adenomatous polyposis coli* |
| *ARID1A* | *AT-rich interactive domain-containing protein 1A* |
| *ARID2* | *AT-rich interactive domain 2* |

| Term | Definition |
|---|---|
| ASGE | American Society for Gastrointestinal Endoscopy |
| BAT | Bethesda marker |
| BE | Barrett's esophagus |
| BLM | Bloom syndrome protein |
| BMJ | British Medical Journal |
| BS | Bloom syndrome |
| *CAPN14* | *Calpain 14* |
| *CCL26* | *C-C motif chemokine ligand 26* |
| *CCNA1* | *Cyclin A1* |
| cfDNA | Cell-free tumor DNA |
| CLIA '88 | Clinical Laboratory Improvement Amendments Of 1988 |
| CMM1 | Familial cutaneous malignant melanoma-1 |
| CMS | Centers For Medicare and Medicaid Services |
| *COX2* | *Cyclooxygenase 2* |
| CPS | Combined Positive Score |
| CSCO | Chinese Society of Clinical Oncology |
| CTCs | Circulating tumor cells |
| ctDNA | Circulating tumor DNA |
| *DCC* | *Deleted in colorectal carcinoma* |
| DNA | Deoxyribonucleic acid |
| *DOCK2* | *Dedicator of cytokinesis 2* |
| EAACI | European Academy of Allergy and Clinical Immunology |
| EAC | Esophageal adenocarcinoma |
| ED | Esophageal dysplasia |
| EDP | Eosinophilic esophagitis diagnostic panel |
| *EGFR* | *Epidermal growth factor receptor* |
| EGJ | Esophagogastric junction |
| ELISA | Enzyme-linked immunoassay |
| *ELMO1* | *Engulfment and cell motility protein 1* |
| EoE | Eosinophilic esophagitis |
| ESMO | European Society for Medical Oncology |
| ESPGHAN | European Society of Pediatric Gastroenterology, Hepatology And Nutrition |
| EST | Esophageal string test |
| EUREOS | European Society of Eosinophilic Oesophagitis |
| FA | Fanconi anemia |
| FANC | FA complementation group A |
| FB | Forceps biopsy |
| FBE | Familial Barrett's esophagus |
| FDA | Food and Drug Administration |
| FISH | Fluorescence in situ hybridization |
| GERD | Gastroesophageal reflux disease |
| *HER2* | *Human epidermal growth factor receptor 2* |
| HGD | High-grade dysplasia |

| Term | Definition |
|---|---|
| HGD/EAC | High-grade dysplasia/esophageal adenocarcinoma |
| HIF1-ALPHA | Hypoxia-inducible factor 1-alpha |
| *HoGG1* | *8-oxoguanine DNA glycosylase* |
| ICER | Incremental cost-effectiveness ratio |
| IgE | Immunoglobulin E |
| IHC | Immunohistochemistry |
| IND | Indefinite for dysplasia |
| JSMO | Japanese Society of Medical Oncology |
| K20 | Potassium oxide |
| KSMO | Korean Society of Medical Oncology |
| LDTs | Laboratory developed tests |
| LGD | Low-grade dysplasia |
| MBP-1 | Major basic protein 1 |
| *MCC* | *Colorectal mutant cancer protein* |
| ML | Mutational load |
| MMR | Mismatch repair |
| MSI | Microsatellite instability |
| *MXI1* | *Max-interacting protein 1* |
| NBDE | Non-dysplastic intestinal metaplasia |
| NCCN | National Comprehensive Cancer Network |
| NDBE | Baseline nondysplastic BE |
| NF2 | Neurofibromatosis type 2 |
| NME1 | Nucleoside Diphosphate Kinase 1 |
| NNT | Number needed to test |
| *NOTCH3* | *Notch receptor 3* |
| NTRK | Neurotrophic tyrosine receptor kinase |
| PCR | Polymerase chain reaction |
| PD-1 | Programmed death-1 |
| PD-L1 | Programmed death-ligand 1 |
| PPK | Palmoplantar keratoderma |
| *PRG2* | *Proteoglycan 2, pro eosinophil major basic protein* |
| *PSEN2* | *Presenilin 2* |
| *PTEN* | *Phosphatase and TENsin homolog* |
| QALY | Quality-adjusted life-year |
| *RB* | *Retinoblastoma protein* |
| *RHBDF2* | *Rhomboid 5 homolog 2* |
| *RNF43* | *Ring finger protein 43* |
| SAGES | Society of American Gastrointestinal and Endoscopic Surgeons |
| SCCs | Squamous cell carcinomas |
| *SMAD4* | *SMA- and MAD-related protein 4* |
| *SMARCA4* | *Matrix associated, actin dependent regulator of chromatin, subfamily a* |
| SOC | Standard of care |
| *SPG20* | *Spastic paraplegia 20* |

| Term | Definition |
|---|---|
| SSO | Sequence-specific oligonucleotide |
| STMN1 | Stathmin 1 |
| TAVAC | Technology And Value Assessment Committee |
| *TFF1* | *Trefoil factor 1* |
| TML | Tumor mutational load |
| *TNFAIP8* | *TNF alpha induced protein 8* |
| TOS | Thoracic outlet syndromes |
| *TP53* | *Tumor protein 53* |
| TRK | Tropomyosin receptor kinase |
| *TSLP* | *Thymic stromal lymphopoietin* |
| TVAC | Technology And Value Assessment Committee |
| UEG | United European Gastroenterology |
| VHL | Von hippel-lindau syndrome |
| VIM | Vimentin |
| WATS | Wide-Area Transepithelial Sampling |
| WATS3D | Wide-Area Transepithelial Sampling with Computer-Assisted 3-Dimensional Analysis |

## IV. Scientific Background

The esophagus is a long tube that connects the mouth to the stomach. Its primary function is to transport food from the mouth to the stomach. However, this organ is often exposed to difficult conditions, from abrasive food to the acidic conditions of the stomach. Although mechanisms are in place to protect against injury (namely the tough squamous cells), it is common to see injury or disease in the esophagus (Zhang et al., 2020).

Many serological and genetic markers have been proposed as tools to assist in evaluation of esophageal pathology. Eosinophilic esophagitis (EoE), Barrett's esophagus (BE), and esophageal cancer are typically diagnosed with histological analysis from endoscopic biopsy (Bonis & Gupta, 2021; Saltzman & Gibson, 2021, 2023; Spechler, 2023), but biopsies frequently require careful consideration and resources to perform properly (NCCN, 2020, 2022b). For these reasons, serum and genetic markers have been suggested as noninvasive markers for esophageal pathologies.

*Eosinophilic Esophagitis (EoE)*

Eosinophilic esophagitis (EoE) marked by the presence of eosinophils in the esophagus. Eosinophils are typically associated with mitigating inflammation but are not normally found in the esophagus. EoE is represented by a broad set of clinical symptoms, such as difficulty swallowing, chest, or abdominal pain, and feeding dysfunction. Diagnosis is established through endoscopy with biopsies to confirm eosinophilia. The current diagnostic criteria set the cutoff for eosinophilia at ≥15 eosinophils per high power field, (60 eosinophils per $mm^2$) although this figure has been heavily discussed (Bonis & Gupta, 2021; Dellon et al., 2018).

***Proprietary Testing- EoE***

Laboratory tests have been suggested as a noninvasive adjunct for EoE. Serum IgE will be elevated in up to 60% of EoE patients, as allergy has a strong association with EoE. Many other markers, such as eotaxin-3, major basic protein-1, tryptase, chemokines, and serum eosinophil count, have all been suggested to assist in evaluation of EoE (Bonis & Gupta, 2021; Dellon et al., 2018). Immune system factors may also contribute to pathology. Since eosinophils are not normally found in the esophagus, their presence in the esophagus may suggest an underlying issue with the immune system. Various interleukins, mast cells, and T cells have all been proposed as contributing to pathogenesis, but the exact pathway and mechanisms are not completely understood (Rothenberg, 2023). Genetic features have also been used for EoE evaluation. Twin studies and family histories have indicated a role for genetics in EoE. Several genes have also been identified as potential risk factors, such as *CAPN14* (an interleukin-13 regulator), *TSLP* (a basophil regulator), and *CCL26* (promotes eosinophil movement into esophagus) (Sherrill & Rothenberg, 2014).

Wen et al. (2013) developed a diagnostic gene expression panel ("EDP") for EoE. The authors identified candidate genes using two cohorts of EoE and control patients, then validated these genes with a separate cohort of 194 patients (91 active EoE, 57 control, 34 ambiguous, 12 reflux). The panel was found to identify EoE patients at 96% sensitivity and 98% specificity. The authors also noted that the panel could separate patients in remission from unaffected patients (Wen et al., 2013).

Shoda et al. (2018) used an "EoE Diagnostic Panel" (EDP) to further classify EoE cases by histologic, endoscopic, and molecular features. The EDP consisted of 95 esophageal transcripts purported to identify EoE among both unaffected patients and patients with other conditions. 185 biopsies were studied. The authors identified three clear subtypes of EoE; subtype 1 with a normal-appearing esophagus and mild molecular changes, subtype 2 with an inflammatory and steroid-responsive phenotype, and subtype 3 with a "narrow-caliber" esophagus and severe molecular alterations. These findings were replicated in a 100-biopsy sample (Shoda et al., 2018).

Tests are commercially available for EoE. Noninvasive tests (as an alternative to endoscopy) have been recently popular. The Esophageal String Test (Testa et al.) is one such alternative. The patient swallows a gelatin-coated capsule with a string wrapped inside. Once the capsule is in the patient's stomach, the gelatin dissolves, allowing the capsule to pass through. The string itself is used to collect samples from the patient's esophagus and is easily removed from the patient. From there, the sample is analyzed for several biomarkers (major basic protein-1, eotaxins 2 and 3, and so on) to provide a probability% (a trademarked "EoEscore") of esophageal inflammation (Ackerman et al., 2019; EnteroTrack, 2019).

*Barrett's Esophagus (BE)*

Barrett's esophagus (BE) is a condition in which the normal squamous tissue lining the esophagus is replaced by metaplastic columnar epithelium. This new epithelium contains gastric features and is typically caused by chronic gastroesophageal reflux disease (GERD). This condition predisposes to esophageal cancer. When noxious substances (gastric acid, bile, et al) are exposed to the squamous esophageal tissue, the damage is usually repaired through regeneration of these squamous cells. In BE cases, this damage is repaired not through creation of new squamous cells, but through metaplastic columnar cells. The exact reason for this is unknown. Although these

metaplastic cells are more resistant to reflux-based damage than the normal squamous cells, these cells frequently show the oxidative DNA damage that is typical of cancer. Mutations in the p53 tumor suppressor gene appear to be the catalyst for cancers, as acquisition of this mutation in conjunction with the replication of the genome is conducive to carcinogenesis (Spechler, 2023).

Vollmer (2019) performed a review assessing incidence of adenocarcinoma detected during surveillance of BE. The author identified 55 studies encompassing 61371 total patients. Of the 61371 total patients, 1106 developed adenocarcinoma. Overall, the author found that the model created from the studies "predicted the per-person probability of developing cancer in 5 years of complete follow-up is approximately 0.0012". Variables affecting this probability included mean time of follow-up, definition of Barrett metaplasia, and fraction of patients followed up for at least 5 years (Vollmer, 2019).

***Proprietary Testing- BE***

Proprietary tests are commercially available for assessment of BE, usually to evaluate risk (BE progression to cancer, risk of BE itself, and such). For example, BarreGen, offered by Interpace Diagnostics, uses tumor mutational load (a measure intended to capture total genomic instability of a sample) to calculate risk of progression. Although many ways can estimate mutational load, BarreGen tests 10 key genomic loci which are as follows: "1p (*CMM1*, L-myc), 3p (*VHL*, *HoGG1*), 5q (*MCC*, *APC*), 9p (*CDKN2A*), 10q (*PTEN*, *MXI1*), 17p (*TP53*), 17q (*RNF43*, *NME1*), 18q (*SMAD4*, *DCC*), 21q (*TFF1*, *PSEN2*) and 22q (*NF2*)". These loci encompass integral tumor suppressors and are proposed to provide an accurate picture of genomic instability (Interpace, 2019; Trindade et al., 2019).

Another test, TissueCypher, also proposes to predict likelihood of progression from BE to esophageal cancer. The test measures 9 protein biomarkers that represent morphological and cellular changes (p53, p16, AMACR, CD68, COX2, HER2, K20, HIF1-alpha, CD45RO). These biomarkers are quantified and converted to a risk score (1-10) and probability of progression (Cernostics, 2021).

Esoguard, by Lucid Diagnostics, is an esophageal DNA test which analyzes 31 methylated biomarkers in the diagnosis of non-dysplastic Barrett's esophagus and adenocarcinoma. The assay uses next generation sequencing to examine individual DNA molecules for the presence or absence of cytosine methylation with a 90% specificity and 90% sensitivity (Lucid_Diagnostics, 2022).

Finally, a proprietary imaging system, WATS3D, is commercially available. This imaging system samples from a wider area, as opposed to only taking focal samples in a traditional biopsy. This technology also provides a 3-dimensional image of the sampled area. This technology purports to provide more precise sampling than the traditional 4-quadrant biopsies, claiming an increased detection rate of BE and other dysplasias (Diagnostics, 2023).

*Esophageal Cancer*

Esophageal cancers are largely divided into two groups: squamous cell carcinomas (SCCs) and adenocarcinomas (EAC). SCCs usually begin in the middle of the esophagus, whereas EACs

often originate near the gastroesophageal junction. Both share several risk factors, such as smoking. Due to the numerous environmental risk factors for both types of cancer, it is difficult to ascertain the true impact of genetic factors (Gibson, 2023). These cancers are primarily diagnosed through histologic examination, usually obtained through endoscopy (Saltzman & Gibson, 2021, 2023).

Advancements have been in the molecular characterization of both types of cancer. *TP53* mutations are the most common mutation seen in both types of cancer. Other frequently mutated genes in adenocarcinoma include *ELMO1* and *DOCK2* (enhance cell motility), *ARID1A*, *SMARCA4* and *ARID2* (chromatin remodelers), and *SPG20* (traffics growth factor receptors). BE, as the precursor to adenocarcinomas, includes certain similarities in genetic mutations but at a less severe rate. Further, the rate of overlap tended to increase with higher degree of dysplasia (Testa et al., 2017).

SCC mutations tend to be in genes associated with specific cellular pathways. Genes in ubiquitous pathways, such as *EGFR*, *NOTCH3*, and *RB*, are frequently mutated in SCC. The molecular profile of esophageal SCC tends to align more with other squamous cell cancers (such as head and neck cancers) rather than EAC (Testa et al., 2017). Numerous gene expression studies have been performed to further classify molecular subtypes of esophageal cancer (Gonzaga et al., 2017; McLaren et al., 2017; Visser et al., 2017). Gene expression profiles may have utility in assessing response to treatment, prognosis, or risk assessment.

Historically, Carcinoembryonic Antigen (CEA) has been used as the serum cancer marker in the diagnosis of esophageal cancer, as CEA levels have been shown to be significantly higher in these patients. The sensitivity (8-70%), specificity (57-100%), and positive likelihood ratio (5.94) of CEA means that patients with EC have a 6-fold higher chance of having higher CEA levels. Other markers include squamous cell cancer antigen (SCC-Ag) and cytokeratin 21-1 fragment (CYFRA21-1). The sensitivity and specificity Cyfra21–1 ranged from 36% to 63% and from 89% to 100%, respectively, with patients having a 12-fold higher chance of having EC. The sensitivity and specificity of SCC-Ag ranged from 13% to 64% and from 91% to 100%, respectively, whereas its PLR was 7.66 (Visaggi et al., 2021).

Li et al. (2019) investigated potential biomarkers for lymph node metastasis for esophageal squamous cell carcinoma. 6 studies encompassing 70 patients were included. The authors identified 9 biomarkers and 4 cellular mechanisms that influence lymph node metastasis. From there, they identified three biomarkers with broader influence on prognosis of disease, *PTEN*, *STMN1*, and *TNFAIP8*. The authors suggested that those three biomarkers should be researched further (Li et al., 2019).

Plum et al. (2019) evaluated *HER2* overexpression's impact on prognosis of esophageal adenocarcinoma (EAC). 428 EAC patients that underwent a "transthoracic thoraco-abdominal esophagectomy" were included. The authors identified 44 patients with *HER2* positivity (IHC score 3+ or 2+ with gene amplification). This cohort was found to have a better overall survival (OS, 70.1 months vs 24.6 months), along with better histology, absence of lymphatic metastases, and lower tumor stages. The authors also noted a similarity in results to a large 2012 study (Plum et al., 2019).

Frankell et al. (2019) examined the molecular landscape of esophageal adenocarcinoma (EAC). The authors assessed 551 genomically characterized EACs. A total of 77 driver genes and "21 non-coding driver elements" were identified. The authors also found an average of 4.4 driver events per tumor. A three-way association was found, between hyper-mutation, *Wnt* signaling, and loss of immune signaling genes. Finally, the authors also identified "sensitizing events" (events causing a tumor to be more susceptible to a therapy) to CD4/6 inhibitors in over half of the EAC cases studied (Frankell et al., 2019).

***Clinical Validity and Utility***

Ackerman et al. (2019) evaluated the ability of the 1-hour Esophageal String Test (Testa et al.) to distinguish between active eosinophilic esophagitis (EoE), inactive eosinophilic esophagitis, and normal esophagi. 134 patients (62 active EoE, 37 inactive EoE, 35 normal) were included. The authors found that eotaxin 3 measured from both EST samples and the control biopsy extracts to be the best marker for distinguishing active EoE from inactive EoE (by both sensitivity and specificity). Addition of major basic protein 1 (MBP-1) improved sensitivity by 0.039 (0.652 to 0.693) and specificity by 0.014 (0.261 to 0.275) across all patients (Ackerman et al., 2019).

Hao et al. (2019) performed a cost-effectiveness analysis of an "adenocarcinoma risk prediction multi-biomarker assay" (TissueCypher's Barrett's Esophagus Assay). A hypothetical cohort of 10000 patients with BE diagnoses (including non-dysplastic intestinal metaplasia [NBDE], indefinite for dysplasia [IND], and low-grade dysplasia [LGD]) was created. A Markov decision model was used to compare BE management costs between assay use and the standard of care (SOC). A surveillance interval of 5 years was used. Low-risk patients were found to have a 16.6% reduction in endoscopies. High-risk patients were found to have a 58.4% increase in endoscopic treatments (compared to the SOC arm), leading to a death total of 111 for the assay arm compared to 204 in the SOC arm (a 45.6% reduction). Overall, the authors calculated the incremental cost-effectiveness ratio (ICER) to be $52,483/quality-adjusted life-year (QALY), and they found that "the probability of the Assay being cost-effective compared to the SOC was 57.3% at the $100,000/QALY acceptability threshold" (Hao et al., 2019).

Eluri et al. (2018) aimed to validate a genomic panel intended to represent tumor mutational load (TML). Previously, the authors evaluated a panel of 10 genomic loci from which a TML score was calculated. This mean TML was found to be significantly higher in 23 BE patients that had progressed to high-grade dysplasia (HGD) or esophageal adenocarcinoma (EAC) as compared to 46 that had not progressed. The area under the curve in this prior study was found to be 0.95 at a mutational load (ML) cutoff of 1 (on a scale of 1-10). In the present study, 159 subjects were included. Cases had "baseline nondysplastic BE (NDBE) and developed HGD/EAC ≥ 2 years later." 58 subjects were progressors and 101 were nonprogressors. The authors identified no difference in mean ML in pre-progression tissue in both cohorts ("ML = 0.73 ± 0.69 vs. ML = 0.74 ± 0.61"). The area under the curve at the cutoff of ML 1 was only 0.50, and the authors concluded that the "utility of the ML to stratify BE patients for risk of progression was not confirmed in this study" (Eluri et al., 2018).

Trindade et al. (2019) evaluated tumor mutational load's (ML) ability to "risk-stratify those that may progress from non-dysplastic BE to dysplastic disease". 28 patients were included, and ML levels were compared between those that progressed to dysplasia and those who had not. 8 total

patients progressed to dysplasia (6 low-grade, 2 high-grade), and 7 of these patients had "some level" of genomic stability detected (ML ≥.5 on a scale of 1 to 10). 10 of the 20 patients that did not progress to dysplasia had "no" ML level. The authors also noted that at an ML of ≥1.5, the risk of progression to high-grade dysplasia was 33%, with a sensitivity of 100% and specificity of 85%. The authors concluded "that ML may be able to risk-stratify progression to high-grade dysplasia in BE-IND. Larger studies are needed to confirm these findings" (Trindade et al., 2019).

Moinova et al. (2018) evaluated the ability of two DNA methylation signatures to detect BE. Methylation signatures of the *VIM* and *CCNA1* loci were evaluated in 173 patients with or without BE. *CCNA1* methylation was found to have an area under the curve of 0.95 for distinguishing BE-related dysplasia compared to normal esophagi. When the data for *VIM* methylation was added, the resulting sensitivity was 95%, and the resulting specificity was 91%. These findings were replicated in a validation cohort of 86 patients, with the combination of methylation markers detecting BE metaplasia at 90.3% sensitivity and 91.7% specificity (Moinova et al., 2018).

Critchley-Thorne et al. (2016) validated a pathology panel to predict progression of BE to esophageal cancer. The authors identified 15 potential biomarkers, which were evaluated in both training and validation sets. This "classifier" separated patients into three different risk classes: low, intermediate, and high in the training set of 183. The authors calculated the hazard ratio of intermediate to low risk at 4.19 and high to low at 14.73. In the validation set (n = 183), the concordance index (an estimation of area under the curve) of the 15-factor classifier was 0.772, the best of the amounts tested (3, 6, 9, 12, 15, 17). The authors also noted that this classifier provided independent prognostic information that were outperformed predictions based on other clinicopathological factors, such as segment length, age, and p53 overexpression (Critchley-Thorne et al., 2016).

Another multicenter study investigated the use of WATS$^{3D}$ with either random or targeted FB in the detection of esophageal dysplasia (ED). 12,899 patients were enrolled in the study, and WATS$^{3D}$ detected an additional 213 cases of ED beyond the initial 88 cases identified by FB, representing an increase of 242%. Regarding screening for BE, WATS increased the overall detection by 153% (from 13.1% to 33% of the individuals enrolled). The authors noted that the order of testing (e.g., FB or WATS) did not impact the results. The authors conclude, "In this study, comprised of the largest series of patients evaluated with WATS, adjunctive use of the technique with targeted and random FB markedly improved the detection of both ED and BE. These results underscore the shortcomings of FB in detecting BE-associated neoplasia, which can potentially impact the management and clinical outcomes of these patients" (Smith et al., 2019).

A study into the cost-effectiveness of WATS$^{3D}$ testing as an adjunct to the standard-of-care forceps biopsy (FB) used a reference case of a 60-year-old white male with gastroesophageal reflux disease (GERD) to see the number of screens needed to avert one cancer and one cancer-related death as well as to calculate the quality-adjusted life years (QALYs) as measured in 2019 U.S. dollars. With this as a reference case, 320 – 337 individuals would need to be screened using WATS$^{3D}$ to avert one cancer, and 328 – 367 individuals would be required to avert one death. The additional cost associated with WATS$^{3D}$ was $1219, but an additional 0.017 QALYs

were produced, resulting in an ICER of $71395/QALY. The authors conclude, "Screening for BE in 60-year-old white male GERD patients is more cost-effective when WATS$^{3D}$ is used adjunctively to the Seattle protocol than with the Seattle protocol alone" (Singer & Smith, 2020).

One study compared the use of the WATS$^{3D}$ technology to standard forceps biopsy. 117 individuals with a history of Barrett's esophagus with dysplasia had both techniques performed. For the biopsy, a four-quadrant biopsy quadrant protocol was performed every 1 – 2 cm. Evaluation of the biopsy and the WATS$^{3D}$ technique was performed by separate pathologists, blinded to each other's results. "Brush biopsy [WATS$^{3D}$] added an additional 16 position 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)." The authors of the study noted that no statistical difference was evident between medical centers, the type of forceps used, or between sampling every 1 cm versus every 2 cm. They conclude, "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" (Anandasabapathy et al., 2011).

Another multicenter prospective trial of 4203 patients studied the use of WATS$^{3D}$ as an adjunct to four-quadrant random forceps biopsy (FB) in detecting Barrett's esophagus (BE) and esophageal dysplasia (ED). FB alone detected 594 cases of BE, and the addition of WATS$^{3D}$ detected an additional 493 cases, an increase of 83%. Likewise, WATS$^{3D}$ detected an increase of 88.5% of low-grade dysplasia (LGD). The authors conclude, "Adjunctive use of WATS to FB significantly improves the detection of both BE and ED. Sampling effort, an inherent limitation associated with screening and surveillance, can be improved with WATS allowing better informed decisions to be made about the management and subsequent treatment of these patients (Gross et al., 2018)." These findings support the earlier study by Johanson and colleagues. In their study of 1266 patients being screened for BE and ED, they noted an overall increase of 39.8% in the detection of BE when WATS$^{3D}$ (brush biopsy or BB) was used as an adjunct to FB. They also report that the number of patients needed to test (NNT) to obtain a positive BE result was 8.7. Interestingly, specifically for patients with gastroesophageal reflux disease (GERD), the addition of WATS$^{3D}$ resulted in an even higher increase in the detection of BE (by 70.5%) (Johanson et al., 2011).

Another study published in 2018 of a randomized trial at 16 different medical centers (n = 160 patients) compared the order of testing (WATS$^{3D}$ followed by biopsy sampling versus biopsy sampling followed by WATS$^{3D}$) to detect high-grade dysplasia/esophageal adenocarcinoma (HGD/EAC). The authors also stated secondary aims of determining the amount of additional time required for WATS$^{3D}$ and the ability of each procedure to separately detect neoplasia. The order of the procedures was not statistically relevant. The use of WATS$^{3D}$ as an adjunct to biopsy did result in a 14.4% absolute increase in the number of HGD/EAC cases detected. The authors noted that WATS$^{3D}$, on average, adds 4.5 minutes to the total procedure time. They conclude, "Results of this multicenter, prospective, randomized trial demonstrate that the use of WATS in a referral BE population increases the detection of HGD/EAC" (Vennalaganti et al., 2018).

Diehl studied the impact of TissueCypher Barrett's esophagus (BE) assay on clinical decisions in the management of BE patients. TissueCypher was ordered for 60 patients with BE and the impact of the test was assessed. TissueCypher results impacted 55.0% of management decisions,

resulting in either upstaging or downstaging of treatment. "In 21.7% of patients, the test upstaged the management approach, resulting in endoscopic eradication therapy (Wechsler et al.) or shorter surveillance interval. The test downstaged the management approach in 33.4% of patients, leading to surveillance rather than EET. In the subset of patients whose management plan was changed, upstaging was associated with a high-risk TissueCypher result, and downstaging was associated with a low-risk result" (Diehl et al., 2021). The authors conclude that TissueCypher will help target EET for high risk patients and reduce unneeded procedures in low risk patients (Diehl et al., 2021).

Wechsler studied the clinical utility of noninvasive biomarkers to identify EoE in children and predict esophageal eosinophilia. Blood/urine was collected from 183 children and several biomarkers were measured including Absolute eosinophil count (AEC), plasma eosinophil-derived neurotoxin (EDN), eosinophil cationic protein (ECP), major basic protein-1 (MBP-1), galectin-10 (CLC/GAL-10), Eotaxin-2 and Eotaxin-3, and urine osteopontin (OPN) and matrix metalloproteinase-9 (MMP-9). According to the results, all plasma and urine biomarkers were in increased in EoE. A panel that included all the other biomarkers was superior to measuring only AEC alone. AEC, CLC/GAL-10, ECP, and MBP-1 were significantly decreased in patients with esophageal eosinophil counts <15/hpf in response to treatment. AEC combined with MBP-1 best predicted the esophageal eosinophil counts. The authors conclude that eosinophil-associated proteins along with AEC are superior to AEC alone in distinguishing EoE and predicting eosinophil counts (Wechsler et al., 2021).

## V. Guidelines and Recommendations

**United European Gastroenterology (UEG), The European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), the European Academy of Allergy and Clinical Immunology (EAACI), and the European Society of Eosinophilic Oesophagitis (EUREOS)**

These joint guidelines were published by a task force of 21 physicians and researchers for eosinophilic esophagitis (EoE). In it, they note that noninvasive biomarkers (inflammatory factors, total IgE, chemokines, tryptase, et al) are “not accurate” to diagnose or monitor EoE. They remark that absolute serum eosinophil count fared best in correlating with severity of disease but had a diagnostic accuracy of 0.754. The guidelines state that histology is necessary for monitoring. The String Test was also mentioned as having good preliminary results but required further corroboration (Lucendo et al., 2017).

**Updated International Consensus Diagnostic Criteria for Eosinophilic Esophagitis: Proceedings of the AGREE Conference**

These newly published international diagnostic criteria primarily include endoscopic findings. Although the guidelines emphasize ruling out other diagnoses (in which biomarkers may be useful), it does not mention any serum or genetic factors for EoE itself (Dellon et al., 2018).

**National Comprehensive Cancer Network (NCCN)**

The NCCN notes four syndromes that predispose to an increased risk for esophageal and esophagogastric junction (EGJ) cancers; tylosis with non-epidermolytic palmoplantar keratoderma (PPK) with esophageal cancer (including Howel-Evans syndrome), familial Barrett esophagus (FBE), Bloom Syndrome (BS, *BLM* gene*)*, and Fanconi Anemia (FA, *FANC A-E genes*). The *RHBDF2* gene has been associated with tylosis (with non-epidermolytic palmoplantar keratosis) for genetic risk assessment. Though FBE may be associated with "one or more autosomally inherited dominant susceptibility alleles," no gene has been validated. With regards to next-generation sequencing, the NCCN concludes that "when limited tissue is available for testing, sequential testing of single biomarkers or use of limited molecular diagnostic panels may quickly exhaust the sample. In these scenarios, comprehensive genomic profiling via a validated NGS assay performed in a CLIA-approved laboratory may be used for the identification of *HER2* amplification, MSI [microsatellite instability], and *NTRK* gene fusions. It should be noted that NGS has several inherent limitations and thus whenever possible, the use of gold-standard assays (IHC [immunohistochemistry]/FISH [fluorescence *in situ* hybridization]/targeted PCR [polymerase chain reaction]) should be performed"(NCCN, 2022a).

Liquid biopsy aids in identifying genetic mutations in solid cancers by looking at circulating tumor DNA (ctDNA) in blood and can be used in those with advanced disease and cannot undergo clinical biopsies for disease surveillance and management. Detecting mutations in DNA from esophageal and EGJ carcinomas "can identify targetable alterations or the evolution of clones with altered treatment response profiles." The NCCN has also stated that "a negative result should be interpreted with caution, as this does not exclude the presence of tumor mutations or amplifications" (NCCN, 2022a).

The NCCN notes that "testing for MSI by polymerase chain reaction (PCR) or *MMR* [mismatch repair] by IHC should be considered on locally advanced, recurrent, or metastatic esophageal and EGJ cancers in patients who are candidates for treatment with PD-1 inhibitors." The NCCN also identifies several targeted therapeutic agents currently approved by the FDA; trastuzumab, pembrolizumab/nivolumab, and entrectinib/larotrectinib. Trastuzumab is based on HER2 overexpression and pembrolizumab is based on "testing for MSI by PCR or NGS/MMR by IHC or PD-LA immunohistochemical expression by CPS or high mutational burden (TMB)." Select TRK inhibitors have also been FDA-approved for *NTRK* gene fusion-positive tumors (NCCN, 2022a).

Genetic biomarkers such as aneuploidy and loss of p53 heterozygosity have been proposed as useful for identifying increased risk of progression in BE patients, but the NCCN remarks that these biomarkers require "further prospective evaluation as predictors of risk for the development of HGD [high-grade dysplasia] and adenocarcinoma of the esophagus in patients with Barrett esophagus" (NCCN, 2022a).

The NCCN notes that wide-area transepithelial sampling (WATS) has been used to detect esophageal carcinomas in BE patients. They state, "The use of wide-area transepithelial sampling with computer-assisted 3-dimensional analysis (WATS3D), a relatively new sampling technique combining an abrasive brush biopsy of the Barrett esophagus mucosa with computer-assisted pathology analysis to highlight abnormal cells, may help increase the detection of esophageal dysplasia in patients with Barrett esophagus." They go on to cite the 2017 study by Vennalaganti

and colleagues that shows a 14.4% increase in the number of additional cases of HGD/esophageal adenocarcinoma captured by using WATS. However, the NCCN remarks that the "utility and accuracy of WATS for detecting HGD/adenocarcinoma in patients with Barrett esophagus needs to be evaluated in larger phase III randomized trials" (NCCN, 2022a).

For squamous cell carcinoma, the NCCN recommends performing microsatellite and PD-L1 testing (if not done previously) if metastatic cancer is suspected. NGS may be considered via validated assay (NCCN, 2022a).

**American Society for Gastrointestinal Endoscopy**

The ASGE recommends the use of WATS3D as an adjunct to "Seattle protocol biopsy sampling" in patients with known or suspected BE (conditional recommendation, low quality of evidence). The society stated that they had downrated the certainty of the recommendation due to possible risk bios, insistency, and indirectness of the studies that were available at the time of publication since some of the studies had included LGD (whereas others had not) and many of the studies had been sponsored by the test's manufacturer. The society also had noted that, as of the date of publication, no studies addressing the cost-effectiveness of WATS-3D had been published. (Qumseya et al., 2019) It should be noted that since the publication of these guidelines the 2020 cost-effectiveness study by Singer and Smith (2020) has been published.

**Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) Technology and Value Assessment Committee (TAVAC)**

The TAVAC of SAGES evaluated WATS$^{3D}$ and published their findings and recommendations within the journal *Surgical Endoscopy* in 2020. They note that WATS$^{3D}$ is not recommended "as a stand-alone substitute for cold forcep biopsies." Within their expert panel recommendation section:

- They state that no significant morbidity or mortality is associated with the testing.
- They also state that "WATS$^{3D}$ increases diagnostic yield by 38 – 150% for Barrett's Esophagus, by 40 – 150% for Low Grade Dysplasia; and by 420% for High Grade Dysplasia; when compared to forceps biopsy alone."
- WATS$^{3D}$ testing also "has very high inter-observer agreement for the pathological diagnosis of non-dysplastic and dysplastic Barrett's Esophagus."

Regarding value, "Increased detection of pre-malignant diseases of the esophagus by the adjunctive use of WATS$^{3D}$ supports screening and surveillance by the adjunctive use of WATS$^{3D}$ during upper endoscopy in appropriate patients" (Docimo et al., 2020).

**American Foregut Society**

The AFS published a white paper reviewing WATS$^{3D}$ in 2020. After reviewing the literature, they state, "The American Foregut Society (AFS) Board has concluded that there are sufficient data to support the routine use of WATS$^{3D}$ technology in the diagnosis and ongoing evaluation of Barrett's esophagus" (AFS, 2021).

**American College of Gastroenterology**

The ACG published guidelines on the diagnosis and management of Barrett's Esophagus. In it, they state that no single biomarker (including genetic abnormalities) is "adequate" as a risk stratification tool. Further, they remark that an entire panel of biomarkers may be required, but no panels were ready for clinical practice (Shaheen et al., 2016).

**European Society for Medical Oncology**

ESMO does not mention any molecular testing for diagnosis or risk assessment of esophageal cancer. Testing for HER2 is mentioned for targeted therapy with trastuzumab. The guidelines recommend following the 2016 ACG guidelines regarding Barrett's Esophagus screening (Lordick et al., 2016).

**Pan-Asian adapted ESMO Clinical Practice Guidelines: a JSMO-ESMO initiative endorsed by CSCO, KSMO, MOS, SSO and TOS**

The only biomarker mentioned in these guidelines is HER2; intended "to select patients with metastatic esophageal adenocarcinoma for treatment with…trastuzumab". The guidelines go on to state that evidence for the role of other biomarkers or agents is "limited" (Muro et al., 2019).

**The Brazilian Group of Gastrointestinal Tumours' (GTG)**

The Brazilian Group of Gastrointestinal Tumours' (GTG) published guidelines and discussed the use of biomarkers in gastric, esophageal and esophagogastric junction (OGJ) cancer. The following recommendations were made:

- "In biopsies of localised OGJ or oesophageal adenocarcinomas, the assessment of HER2 status is optional
- In biopsies of metastatic adenocarcinomas of the OGJ or oesophagus, with the intention of palliative treatment, HER2 status should be investigated
- The assessment of HER2 status in metastatic adenocarcinomas of the OGJ or oesophagus can be made in surgical specimens, biopsies or cell blocks of primary or metastatic tumours, by using immunohistochemistry and interpreted according to the recommended scoring system. Borderline cases (++) in immunohistochemistry must be confirmed by FISH test.
- For oesophageal, OGJ and gastric tumours, PD-L1 expression should be determined by using the combined positive score (CPS) instead of tumour proportion score (TPS). When compared to TPS, CPS is a more sensitive prognostic biomarker in these tumour types" (Rocha-Filho et al., 2021).

## VI. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website https://www.cms.gov/medicare-

coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

**Food and Drug Administration (FDA)**

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

## VII. Applicable CPT/HCPCS Procedure Codes

| CPT | Code Description |
|---|---|
| 81301 | Microsatellite instability analysis (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) of markers for mismatch repair deficiency (eg, BAT25, BAT26), includes comparison of neoplastic and normal tissue, if performed |
| 81479 | Unlisted molecular pathology procedure |
| 88104 | Cytopathology, fluids, washings or brushings, except cervical or vaginal; smears with interpretation |
| 88271 | Molecular cytogenetics; DNA probe, each (eg, FISH) |
| 88272 | Molecular cytogenetics; chromosomal in situ hybridization, analyze 3-5 cells (eg, for derivatives and markers) |
| 88273 | Molecular cytogenetics; chromosomal in situ hybridization, analyze 10-30 cells (eg, for microdeletions) |
| 88274 | Molecular cytogenetics; interphase in situ hybridization, analyze 25-99 cells |
| 88275 | Molecular cytogenetics; interphase in situ hybridization, analyze 100-300 cells |
| 88341 | Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure) |
| 88342 | Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure |
| 88344 | Immunohistochemistry or immunocytochemistry, per specimen; each multiplex antibody stain procedure |
| 88360 | Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; manual |
| 88361 | Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; using computer-assisted technology |
| 88367 | Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), using computer-assisted technology, per specimen; initial single probe stain procedure |
| 88368 | Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), manual, per specimen; initial single probe stain procedure |

| CPT | Code Description |
|---|---|
| 88369 | Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), manual, per specimen; each additional single probe stain procedure (List separately in addition to code for primary procedure) |
| 88373 | Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), using computer-assisted technology, per specimen; each additional single probe stain procedure (List separately in addition to code for primary procedure) |
| 88374 | Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), using computer-assisted technology, per specimen; each multiplex probe stain procedure |
| 88377 | Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), manual, per specimen; each multiplex probe stain procedure |
| 0095U | Inflammation (eosinophilic esophagitis), ELISA analysis of eotaxin-3 (CCL26 [C-C motif chemokine ligand 26]) and major basic protein (PRG2 [proteoglycan 2, pro eosinophil major basic protein]), specimen obtained by swallowed nylon string, algorithm reported as predictive probability index for active eosinophilic esophagitis<br>Proprietary test: Esophageal String Test™ (EST)<br>Lab/Manufacturer: Cambridge Biomedical, Inc. |
| 0108U | Gastroenterology (Barrett's esophagus), whole slide–digital imaging, including morphometric analysis, computer-assisted quantitative immunolabeling of 9 protein biomarkers (p16, AMACR, p53, CD68, COX-2, CD45RO, HIF1a, HER-2, K20) and morphology, formalin-fixed paraffin-embedded tissue, algorithm reported as risk of progression to high-grade dysplasia or cancer<br>Proprietary test: TissueCypher® Barrett's Esophagus Assay<br>Lab/Manufacturer: Cernostics |
| 0114U | Gastroenterology (Barrett's esophagus), VIM and CCNA1 methylation analysis, esophageal cells, algorithm reported as likelihood for Barrett's esophagus<br>Proprietary test: EsoGuard™<br>Lab/Manufacturer: Lucid Diagnostics |
| 0386U | Gastroenterology (Barrett's esophagus), P16, RUNX3, HPP1, and FBN1 methylation analysis, prognostic and predictive algorithm reported as a risk score for progression to high-grade dysplasia or esophageal cancer<br>Proprietary test: Envisage<br>Lab/Manufacturer: Capsulomics, Inc |



*Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.*

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## IX. Review/Revision History

| Effective Date | Summary |
|---|---|
| 12/01/2024 | Initial Policy Implementation |