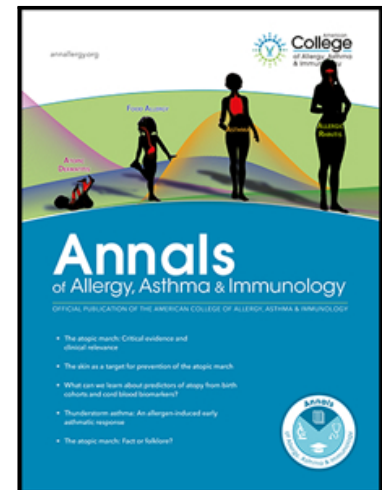


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Non-Invasive Tests for Eosinophilic Esophagitis: Ready for Use?

Emily C. McGowan MD, PhD Associate Professor ,
Seema S. Aceves MD PhD Professor

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Non-Invasive Tests for Eosinophilic Esophagitis: Ready for Use?

Emily C. McGowan MD, PhD, Associate Professor, University of Virginia School of Medicine, Division of Allergy and Immunology, Charlottesville, VA; Adjunct Assistant Professor, Johns Hopkins University School of Medicine, Division of Allergy and Clinical Immunology, Baltimore, MD (ekc5v@virginia.edu)

Seema S. Aceves MD PhD, Professor University of California San Diego, Division of Allergy and Immunology, Departments of Pediatrics and Medicine, San Diego, California, Rady Children's Hospital, San Diego (saceves@health.ucsd.edu)

Corresponding Author Information:

Emily C. McGowan, MD, PhD
University of Virginia School of Medicine
PO Box 801355
Charlottesville, VA 22908
Ph: (434) 924-8648
Fax: (434) 924-5779
Email: ekc5v@virginia.edu

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Keywords: eosinophilic esophagitis, non-invasive tests, esophageal string test, cytosponge, biomarkers, transnasal endoscopy

Abbreviations/Acronyms: eos- eosinophils, hpf- high power field, MBP- major basic protein, EST- esophageal string test, EoE- eosinophilic esophagitis, SPECT/CT- single-photon emission computed tomography, EGD- esophagogastroduodenoscopy, TNE- transnasal endoscopy, AEC- absolute eosinophil count

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Objective: To summarize the existing literature for several promising minimallyinvasive tests to measure disease activity in EoE.

Data Sources: Literature searches were performed using PubMed. Keyword combinations included eosinophilic esophagitis and minimally-invasive techniques, including the esophageal string test, Cytosponge, transnasal endoscopy, technetiumlabeled heparin, and non-invasive biomarkers.

Study Selections: Retrospective and prospective observational studies, peer-reviewed reviews, and systematic reviews were selected. Data were reviewed and summarized.

Results: Various techniques have been developed in recent years to measure disease activity in EoE without the need for conventional endoscopy. Our review summarizes the data on these techniques, the benefits and limitations, and future directions for implementation in both research and clinical care.

Conclusion: Tremendous progress has been made towards developing minimallyinvasive techniques to measure disease activity in EoE. Each of the techniques mentioned in this review has advantages and disadvantages, and some are closer to widespread use than others.

Introduction

Over the last 20 years, eosinophilic esophagitis (EoE) has transformed from a case-reportable disease to a major cause of upper gastrointestinal morbidity.¹ EoE is characterized by symptoms of esophageal dysfunction — such as nausea, vomiting, abdominal/chest pain, dysphagia, and food impactions — and esophageal eosinophilia (≥ 15 eosinophils per high-powered field [eos/hpf]).² Unfortunately, symptoms of EoE do not always correlate with histologic findings;^{3,4} therefore, the assessment of esophageal eosinophilia is critical for both diagnosing and monitoring individuals with this disease.

Currently, in order to identify esophageal eosinophilia, patients presenting for diagnosis or monitoring of EoE must undergo an esophagogastroduodenoscopy (EGD). This procedure is expensive, necessitates time off work, poses a risk for esophageal perforation,⁵ and requires the use of anesthesia which may impact neurodevelopment in young children.⁶ These risks are particularly concerning for individuals with EoE pursuing dietary therapy who require multiple

EGDs in order to identify food triggers. There is also emerging data suggesting disparities in the diagnosis of pediatric EoE in rural communities that do not have access to highly trained pediatric gastroenterologists to perform these procedures in children.⁷ Therefore, the development of alternative methodologies to measure disease activity, which would minimize the limitations arising from our reliance on EGDs and enable more providers to diagnose and monitor this disease, is a critical need in our field.

In this review, we will summarize the existing literature for several promising minimally-invasive tests to measure disease activity in EoE, including the Esophageal String Test, the Cytosponge Cell Collective Device, transnasal endoscopy, radioactively-labeled heparin, and non-invasive, non-esophageal biomarkers. We will additionally comment on the benefits and limitations of these methodologies, as well as future directions to implement these surrogate markers in both research and clinical care.

Esophageal String Test

Overview of the Test

The Esophageal String Test (EST, EnteroTrack, LLC) is derived from the enterotest string device, which was initially designed to assess for gastric and small intestinal pathogens.⁸ Over the years, this test has been adapted to sample bile,⁹ identify *H. pylori* in the stomach,¹⁰ assess gastroesophageal reflux disease (GERD),¹¹ and most recently detect disease activity in EoE.^{12,13} The enterotest string device consists of a weighted gelatin capsule that contains 90 cm of nylon string. To administer the EST, one end of the string is first pulled from the capsule and taped to the patient's cheek. The patient then swallows the capsule which travels to the duodenum. For the EST, this string remains in place for one hour, after which time it is removed

and the esophageal portion of the string is assayed for eosinophil-associated proteins, as shown in **Figure 1**.

Review of the Literature

The use of the EST in EoE has been assessed in two studies to date.^{12,13} In 2013, Furuta *et al* published the first study examining whether the EST could identify markers of eosinophilic mucosal inflammation in EoE.¹² In this study, ESTs were performed in 41 patients, ages 7-20 years, who were undergoing an endoscopy with biopsy. The EST was swallowed the night prior to the EGD and remained in place until the time of the endoscopy (~16 hours). Of the 41 patients enrolled, 14 were found to have active EoE, 8 had inactive EoE, 4 had GERD, and 15 were healthy controls. The authors found that the levels of eosinophil-associated proteins in the EST samples correlated significantly with the peak and mean number of tissue eos/hpf, as well as the levels of eosinophil-associated proteins. This study provided the proof of concept that an esophageal string could be utilized to measure the degree of eosinophilic inflammation in EoE.

Based on these encouraging findings, the authors extended this study and assessed whether similar results could be obtained when decreasing the dwell time of the EST to 1 hour.¹³ In this study, Ackerman *et al* recruited 134 individuals, ages 7-55 years, who were undergoing a clinically-indicated endoscopy for evaluation of confirmed or suspected EoE at 5 centers. The EST was performed within 4 hours of the endoscopy and eosinophil-associated proteins were assessed in the EST eluate and esophageal tissue. In addition, the authors recorded the EoE Endoscopic Reference Score (EREFS) for each patient and assessed subject satisfaction with the procedure. The authors confirmed their previous findings that eosinophil-associated proteins obtained via the EST correlated with peak eos/hpf and that eosinophil products could be

measured close to equivalently in esophageal biopsies as on EST. In addition, they found that these proteins obtained via the EST correlated with EREFS scores, and they developed an “EoE Score” using eotaxin-3 and major basic protein-1 concentrations that could be used clinically to differentiate patients with active EoE from inactive EoE and controls using the EST. Finally, they found that there was high patient and parent satisfaction with the procedure.

Commentary

The EST is a promising non-invasive technique which has several strengths for assessing esophageal eosinophilia. First, the EST has been studied in a large number of pediatric and adult EoE patients and has been shown to correlate with endoscopic findings. Second, the associated EoE Score will facilitate the clinical classification of patients with active EoE. In addition, no specialized equipment is necessary to collect the string sample, which could potentially be used in areas where there is limited access to pediatric gastroenterologists.

The EST, however, does also have some notable limitations. First, 14% of consented patients in the most recent study¹³ were unable to complete the procedure due to gagging, which suggests that the EST may not be a viable option in all patients. Second, the EST has not yet been validated for longitudinal use, so it remains unclear whether this test could be used to guide management in individual patients. Despite these limitations, this novel tool will likely be important for both clinical care and research in this field, and in some hospitals, the EST is already being used as a billable procedure during clinical care.

Cytosponge Cell Collection Device

Overview of the Test

The Cytosponge is a novel, minimally-invasive device that was originally designed as a screening tool to assess for Barrett's esophagus¹⁴⁻¹⁷ and esophageal cancer.^{18,19} This device consists of a 30 mm spherical polyurethane sponge that is compressed into a capsule and attached to a string (**Figure 2**). Upon swallowing, the capsule dissolves in the stomach, and after 7.5 minutes, the sponge is withdrawn through the esophagus via the string. The cells retrieved from the sponge are then collected and embedded to produce a cell block for histologic analysis, enabling enumeration of eosinophils in the esophageal tissue. In comparison to the EST that dwells for 1 hour, the Cytosponge procedure is completed in approximately 10 minutes.

Review of the Literature

The use of the Cytosponge in patients with EoE has been assessed in three studies to date.²⁰⁻²² In 2015, Katzka *et al* published the results of a feasibility study, in which the diagnostic accuracy of the Cytosponge was compared to that of endoscopy. The authors enrolled 20 adults (ages 22 – 51) with confirmed EoE, and the Cytosponge was performed two hours prior to endoscopy. All enrolled patient had symptoms of dysphagia, 15 were found to have a stricture, and 13 were found to have active disease based on conventional histology. When using a cut-off of 15 eos/hpf, the sensitivity of the Cytosponge was 84.6%, and the specificity was 56%. Interestingly, of the 7 patients in histologic remission (< 15 eos/hpf) on biopsy, 3 were found to have active disease via the Cytosponge. The correlation between the average number of eosinophils on biopsy and Cytosponge was 0.50 (p=0.025). In addition to eosinophils, the authors were able to detect other histologic features of EoE via the Cytosponge, including eosinophilic abscesses, spongiosis, and basal cell hyperplasia. Finally, there were no adverse

events when using the Cytosponge in this population, and 100% of the patients stated that they would choose the Cytosponge over endoscopy for their next procedure.

Given these promising findings, the authors conducted a two-center cross-sectional study in patients with EoE to further examine the diagnostic accuracy, safety, and patient preference of the Cytosponge in comparison to conventional endoscopy. In this study, the authors modified the method for counting eosinophils in the Cytosponge sample — the count was adjusted according to the percentage of the total field occupied by the esophageal tissue fragment. For example, if the tissue fragment occupied 50% of the high-powered field, then the eosinophil count was doubled (**Figure 3**). The authors enrolled 86 patients (ages 18 – 69) who underwent a total of 105 procedures. Six patients (7%) were unable to swallow the Cytosponge. The authors found good correlation between eosinophil counts obtained via the Cytosponge and histology ($r=0.78$; $p<0.0001$). When using a cut-off of 15 eos/hpf, the sensitivity and specificity of the Cytosponge were 75% and 86%, respectively. There were no adverse events, and the Cytosponge received higher patient rating scores than endoscopy ($p=0.002$).

In 2017, Paterson *et al* used the Cytosponge to evaluate for esophageal pathology in patients presenting with reflux symptoms.²² A total of 820 patients were enrolled, all of whom had an endoscopy following the Cytosponge procedure. The authors found 6 cases of active EoE detected by endoscopy, 4 of which were identified by the Cytosponge. In one patient, EoE was detected by the Cytosponge but not endoscopy. These results were pooled with the previous two studies, for an overall sensitivity of 76.3% and a specificity of 98.8% for detecting EoE using the Cytosponge.²³

The safety of the Cytosponge was also assessed in a recent pooled analysis of 2,418 individuals — including 102 patients with EoE. The Cytosponge was found to be safe and well-

tolerated, with a complication rate of <1:2000.²⁴ The two complications noted in this pooled analysis included one minor pharyngeal bleed and one case of sponge detachment.²⁴ neither of which occurred in the patients with EoE.

Commentary

The Cytosponge is another very promising, minimally-invasive diagnostic tool for identifying esophageal inflammation in patients with EoE that has multiple advantages. First, the entire Cytosponge procedure can be performed by a clinical nurse in approximately 10 minutes in an outpatient setting and does not require the use of anesthesia. Second, the Cytosponge sample can be processed by standard laboratories, does not require special stains, and can be used to directly visualize eosinophils and other histologic features of EoE. Third, the Cytosponge has been shown to identify esophageal eosinophilia in cases where the endoscopic biopsies were negative, suggesting it may provide broader sampling of the esophagus. Fourth, this tool has also been shown to be safe and well-tolerated, with the important caveat that the studies in EoE to date have been small. Finally, the Cytosponge cell pellet can also be used for DNA/RNA extraction,^{25,26} immunohistochemistry,^{20,21} and microbiome analyses,²⁷ which could be leveraged for research purposes. Given these advantages, the Cytosponge is currently being used for clinical care in patients with EoE at select institutions.

The Cytosponge, however, does have limitations for use in EoE. First, some patients are unable to swallow the Cytosponge (7% in the most recent study²¹) or have persistent gagging after swallowing the capsule, limiting its use in select patients. Second, while the Cytosponge enables histologic assessment of eosinophils, it does not provide direct visualization of the esophagus which is important to identify other clinical features of EoE such as strictures. It

further primarily samples the superficial epithelium and does not enable histologic assessment of deeper tissue layers. Third, because the Cytosponge samples the entire esophagus, inflammation localized to one specific region (for example, the distal esophagus), cannot be identified. Fourth, while the Cytosponge is FDA-approved to collect and retrieve surface cells in the esophagus for cytologic and histologic analyses, the use of this device in patients with dysphagia is still considered off-label because these patients were not included in the initial studies to detect Barrett's esophagus and esophageal cancer.¹⁴⁻¹⁹ In addition, with an overall sensitivity of 76.3%, it is possible that some patients with active disease will be missed. Finally, the Cytosponge has only been studied in adults, and its use in children would likely require modification of the sponge diameter.

Transnasal endoscopy

Overview of the Test

Transnasal endoscopy (TNE) was first designed as a cost-effective tool to screen for Barrett's esophagus and esophageal cancer in adults,²⁸⁻³³ and it has recently been studied to detect esophageal pathology in patients with EoE. This procedure is performed in an outpatient procedure room and does not require the use of sedation. Patients are first given topical lidocaine spray in the nares, and then an ultrathin endoscope (2.8 to 6.0 mm outer diameter) is inserted through the nose and down the esophagus to the stomach. The stomach and esophagus are visualized, and biopsies are taken of the esophageal tissue. In children, virtual reality goggles have been used to facilitate patient distraction during the TNE, and the procedure time is approximately 5-15 minutes.³⁴⁻³⁶

Review of the Literature

In 2015, Philpott *et al* first assessed the use of TNE in patients with EoE.³⁷ In this two-center prospective study, the authors offered 96 consecutive adult patients with EoE — who were scheduled for outpatient endoscopy — the choice of TNE or conventional endoscopy. The authors found that while only 24 patients (25%) chose to proceed with TNE, the procedure was safe, well-tolerated, and resulted in adequate histologic specimens.

The use of TNE in pediatric patients with EoE was first assessed by Friedlander *et al*, in a pilot study published in 2016.³⁴ In that study, the team enrolled 22 patients (ages 8-17) with a confirmed diagnosis of EoE who were referred for a follow-up endoscopy. Of the 22 patients, 21 underwent the TNE, and the most common self-reported symptoms included gagging (57%) and a sore throat (47%). There was no significant difference in the total epithelial surface area when comparing biopsies obtained via the TNE to those obtained via the patient's previous endoscopy, and the visual findings correlated with histologic findings in 85.7% of patients. The TNE was preferred over endoscopy by 85.7% of parents and 52.4% of patients, and the charges associated with TNE were 60.1% lower than that of their previous endoscopies.

In 2019, the same group published a retrospective study of 190 children and young adults (ages 3-22) with EoE who underwent TNE for clinical care.³⁶ Of 300 TNE attempts, 294 procedures (98%) were successfully completed. Fifty-four patients underwent multiple TNEs to assess response to therapy changes. The visual and histologic findings were found to be adequate for diagnosis. There were no significant adverse events noted, and the cost of TNE was 53.4% less than the patients' previous endoscopies.

Interviews were conducted with 21 sets of these patients and parents to qualitatively examine their experience with the TNE procedure.³⁸ Parents and patients noted that positive

aspects of the TNE included the lack of exposure to intravenous anesthesia and the shortened duration of the procedure compared to conventional endoscopy, the ability of the parent to stay in the room with the child, clear communication prior to the procedure, and the use of the virtual reality goggles as distraction. Negative aspects included the taste of the topical anesthesia, patient discomfort during the procedure, and patient stress prior to the TNE.

Most recently, TNE was used as a research tool to examine the kinetics of eosinophil recruitment after single-food reintroduction in patients with EoE (n=5).³⁵ While this study ended prematurely due to research restrictions during the covid-19 pandemic, the authors made a number of interesting observations. First, they found that the TNE was well-tolerated in all subjects, and when given a choice, these patients chose to proceed with TNE over conventional endoscopy. Second, esophageal eosinophilia was present at 2 and 4 weeks after dietary reintroduction in 50% (2/4 patients) and 75% (3/4 patients) of those who developed inflammation. This study further demonstrates that the TNE could be a promising research tool to further examine the kinetics of eosinophil recruitment and potentially monitor for the development of EoE in individuals at high-risk for developing this disease.

Commentary

TNE is another very promising emerging tool. The advantages of TNE include the fact that it can be used in pediatric patients as young as age 3, provides direct visualization of the esophagus, enables the acquisition of biopsy samples that are comparable to conventional endoscopy, and could be used by providers in Allergy/Immunology and ENT. TNE also does not require the use of anesthesia and enables parents to be in the room with their child, which are additional advantages for pediatric EoE patients.

Widespread use of TNE is currently limited by the need for virtual reality to distract patients during the procedure and the need for specialized training in TNE by pediatric gastroenterologists. In addition, the use of this technique may be limited in adults, as the one study conducted to date in this population demonstrated reluctance to choose this procedure over conventional endoscopy. In addition, while the cost of TNE is 50-60% lower than endoscopy, the average cost for this procedure was still high (\$4393.00),³⁶ which may limit its widespread use compared to other emerging tests.

^{99m}Tc-Technetium-Labeled Heparin

Overview of the Test

The use of radiolabeled heparin to identify eosinophil granule proteins in the esophagus has recently become a topic of interest. This approach is based on the concept that heparin — an anionic molecule that is not absorbed from the GI tract — will bind cationic molecules released from eosinophils, including major basic protein (MBP-1), in the esophagus.³⁹⁻⁴² By labeling heparin with ^{99m}Tc-Technetium (Tc), these complexes in the esophagus can be identified radiographically. With this approach, an oral preparation of ^{99m}Tc-labeled heparin is swallowed, and radioactivity is thereafter measured by single-photon emission computed tomography (SPECT/CT).

Review of the Literature

The use of radiolabeled heparin in EoE was first proposed by Saffari *et al* in 2013.⁴¹ In this study, heparin was labeled with ^{99m}Tc in the presence of stannous chloride. Esophageal tissue samples, which were previously collected from patients with active EoE (n=5), inactive EoE

(n=3), and controls (n=2), were incubated with ^{99m}Tc -labeled heparin. These samples were then imaged using SPECT. The authors found that SPECT counts correlated with eosinophil density ($r=0.67$; $p<0.05$), and their data suggested that this technique could be used to distinguish patients with active EoE from those without eosinophilic inflammation.

^{99m}Tc -labeled heparin was then used *in vivo* in 5 research subjects (4 with EoE and 1 control) in order to assess the feasibility, biodistribution, and radiation exposure of this technique.⁴² ^{99m}Tc -labeled heparin was prepared and diluted in sterile saline to a final volume of 15 mL. The solution was then ingested, and the patients underwent dynamic imaging using SPECT/CT during ingestion. Imaging was additionally performed at 1, 2, 4, 6, 8, and 24 hours after ingestion, and the subjects had an endoscopy the day after imaging. The authors found that all subjects tolerated the oral solution, ^{99m}Tc -labeled heparin was not appreciably absorbed throughout the GI tract, and the dose of radiation was comparable to nuclear imaging techniques using other ^{99m}Tc agents. In addition, visual binding of ^{99m}Tc -heparin was strongly associated with EREFS scores ($r=0.91$; $p<0.001$), peak eosinophil counts ($r=0.84$; $p<0.001$), and MBP-1 immunostaining ($r=0.87$; $p=0.001$).

Commentary

The use of ^{99m}Tc -labeled heparin to identify eosinophilic inflammation in EoE is an exciting technique that warrants further study. If additional studies demonstrate that ^{99m}Tc -labeled heparin can accurately identify eosinophilic inflammation without direct sampling of the esophagus, then there could be a substantive advantage for this technique compared to the others mentioned in this review. Furthermore, there is a potential that ^{99m}Tc -labeled heparin could identify eosinophilic inflammation in other organs, or other areas of the GI tract, which could be

an added advantage.⁴² Disadvantages of this technique include the risk of radiation exposure, especially as patients with EoE often require repeated procedures to assess response to therapy. In addition, the use of ^{99m}Tc-labeled heparin in the pediatric population could pose additional safety concerns and technique modifications. Further study is clearly warranted to answer these questions, as well as provide additional data on safety, before this technique is ready for widespread use.

Non-invasive biomarkers

A significant amount of research in recent years has been dedicated to identifying non-invasive biomarkers that could be used to diagnose and monitor EoE. These have included biomarkers assessed in peripheral blood,⁴³⁻⁴⁶ oral or throat swabs,^{47,48} breath condensate,⁴⁹ urine,^{50,51} and stool.^{52,53} A systematic review on this topic was published in 2018, at which time 49 studies were assessed.⁵⁴ The authors found that while some promising markers have been identified, very few have been shown to discriminate patients with EoE from allergic controls. The authors also noted that these studies were limited by the timing of specimen collection, retrospective study design, and the lack of community-based samples.

Since this time, additional studies have assessed novel biomarkers in EoE with some promising results. Henderson *et al* demonstrated that circulating levels of eosinophil progenitors identified via flow cytometry can differentiate children with active EoE from those with inactive disease.⁵⁵ Flow cytometry has further been used to measure expression of eosinophil surface markers, including α_{IIB} integrin, which correlate with eosinophilic inflammation in longitudinal samples of adult patients with EoE.⁵⁶ Weschler *et al* examined plasma levels of eosinophil-associated proteins in pediatric EoE patients and controls and found that the measurement of

absolute eosinophil count (AEC) combined with MBP-1 best predicted esophageal eosinophil counts.⁵⁷ One small study demonstrated the use of urinary bromotyrosine for assessing the presence of esophageal eosinophilia in patients with EoE as compared to non-atopic and atopic controls.⁵⁰ Further research is needed to prospectively validate these findings in large, diverse, cohorts.

Conclusion

In recent years, tremendous progress has been made towards developing minimally-invasive techniques to measure disease activity in EoE. The use of a safe and effective minimally-invasive tool would decrease our reliance on repeated endoscopies, an invasive and costly procedure. In addition, given the increasing prevalence of EoE and the chronic nature of this disease, it would provide non-GI practitioners — such as allergists — a means to assess disease activity in the esophagus. Finally, it would improve access to care in areas where GI and pediatric GI specialists are limited.

Each of the techniques mentioned in this review has advantages and disadvantages (**Table 1**), and some are closer to widespread use than others. While some may depend on close collaborations with other specialties, such as radiology and pathology, others could be performed in community practice. And, while some are applicable to all populations, others may be more suitable only for adults. Ultimately, the right choice for an individual patient will likely involve careful consideration of these factors.

While these tests will not negate the need for endoscopy in many situations — such as when initially diagnosing EoE, when there is a concern for esophageal stricture, or when ruling out other causes of secondary esophageal eosinophilia — they could be a paradigm shift in our

management of and potentially screening for this disease. For example, these technologies could be used to replace endoscopies in patients with confirmed EoE who are pursuing dietary therapy with serial food reintroductions. They could also be used in patients undergoing oral or sublingual immunotherapy to monitor for the development of EoE. These could also be invaluable tools for confirmed EoE patients with seasonal flares, and they could be used to screen for EoE in high-risk populations. Further research into whether these tools could be used in these clinical situations is clearly needed and will help guide how they can be implemented in clinical practice, especially for the Allergist-Immunologist.

Learning Objectives

- To become familiar with the performance of emerging minimally-invasive techniques to measure disease activity in eosinophilic esophagitis (EoE).
- To review the literature evaluating the use of these minimally-invasive techniques, in comparison to conventional endoscopy, in patients with EoE.

CME Questions

1. Which of the following eosinophil-associated proteins were used to create an “EoE Score,” which could be used to differentiate patients with active EoE from inactive EoE and non-EoE controls using the esophageal string test?
 - a. MBP-1 and eotaxin-2
 - b. MBP-1 and eotaxin-3**
 - c. ECP and MBP-1
 - d. ECP and eotaxin-2
 - e. EPX and ECP

Rationale: Eosinophil-associated proteins measured via the esophageal string test correlate significantly with peak eosinophils/hpf and concentrations of the same proteins in the esophageal tissue. Using statistical modeling, the combination of MBP-1 and eotaxin-3 concentrations demonstrated the greatest ability to discriminate patients with active EoE from inactive EoE, and active EoE from controls. These concentrations were used to create an EoE score, which provides a probability for a patient with EoE to have active or inactive disease.

Reference: Ackerman SJ, Kagalwalla AF, Hirano I, et al. One-Hour Esophageal String Test: A Nonendoscopic Minimally Invasive Test That Accurately Detects Disease Activity in Eosinophilic Esophagitis. *Am J Gastroenterol* 2019;114:1614-25.

2. What is the reported complication rate for the Cytosponge?
- a. 1 : 20
 - b. 1 : 100
 - c. 1 : 200
 - d. 1 : 1000
 - e. **1 : 2000**

Rationale: In a pooled analysis of 2,418 individuals, the Cytosponge was found to be safe and well-tolerated, with a complication rate of <1:2000. The two complications noted in this pooled analysis included one minor pharyngeal bleed and one case of sponge detachment, neither of which occurred in the patients with EoE.

Reference: Januszewicz W, Tan WK, Lehovsky K, et al. Safety and Acceptability of Esophageal Cytosponge Cell Collection Device in a Pooled Analysis of Data From Individual Patients. *Clin Gastroenterol Hepatol* 2019;17:647-56.e1.

3. In a pooled analysis of three studies examining the use of the Cytosponge, what was the overall sensitivity and specificity for detecting EoE when compared to conventional endoscopy?
- a. 65% and 85%
 - b. 65% and 99%
 - c. 76% and 85%
 - d. **76% and 99%**
 - e. 85% and 85%

Rationale: In a recent systematic review, the pooled sensitivity and specificity for detecting EoE via the Cytosponge, when compared to the gold standard of endoscopy, was 76% and 99%, respectively.

Reference: Iqbal U, Siddique O, Ovalle A, Anwar H, Moss SF. Safety and efficacy of a minimally invasive cell sampling device ('Cytosponge') in the diagnosis of esophageal pathology: a systematic review. *Eur J Gastroenterol Hepatol* 2018;30:1261-9.

4. In a retrospective study of 190 children undergoing transnasal endoscopy (TNE), what was the reduction in cost with this procedure compared to conventional endoscopy?
- a. 25%
 - b. 32%
 - c. 47%
 - d. **53%**
 - e. 76%

Rationale: In a retrospective study of 190 children and young adults with EoE who underwent a TNE at Children's Hospital Colorado, the authors compared the cost for this procedure to that of previous endoscopies in the same patients. They found that the cost of TNE was 53.4% less than the patients' previous endoscopies.

Reference: Nguyen N, Lavery WJ, Capocelli KE, et al. Transnasal Endoscopy in Unsedated Children With Eosinophilic Esophagitis Using Virtual Reality Video Goggles. *Clin Gastroenterol Hepatol* 2019;17:2455-62.

5. Which of the following describes ^{99m}technetium-labeled heparin?
- A radiolabeled cationic molecule that is absorbed from the GI tract
 - A radiolabeled anionic molecule that is absorbed from the GI tract
 - A radiolabeled cationic molecule that is not absorbed from the GI tract
 - A radiolabeled anionic molecule that is not absorbed from the GI tract**
 - A non-radiolabeled cationic molecule that is absorbed from the GI tract

Rationale: Heparin is an anionic molecule that is not absorbed from the GI tract, which has been shown to bind cationic molecules released from eosinophils — including major basic protein-1 — in the esophagus. By labeling heparin with ^{99m}Technetium, these complexes in the esophagus can be identified radiographically.

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Figure 1: The esophageal string test (EST) is adapted from the enterotest string device. The weighted capsule with nylon string is swallowed and the weight carries the string to the duodenum. The string remains in place for 1 hour, at which time the esophageal portion is assayed for eosinophil-associated proteins. This figure was created with Biorender.com.

Figure 2: The Cytosponge Cell Collection device consists of a 30 mm spherical polyurethane sponge that is compressed into a 20 mm capsule and attached to a string. After swallowing, the capsule dissolves, and the sponge is withdrawn through the esophagus by the string.

Figure 3: Histology obtained via esophageal biopsy (A) and cytology obtained via the Cytosponge (B) from the same individual with active EoE (≥ 15 eos/hpf). Arrows represent eosinophils.

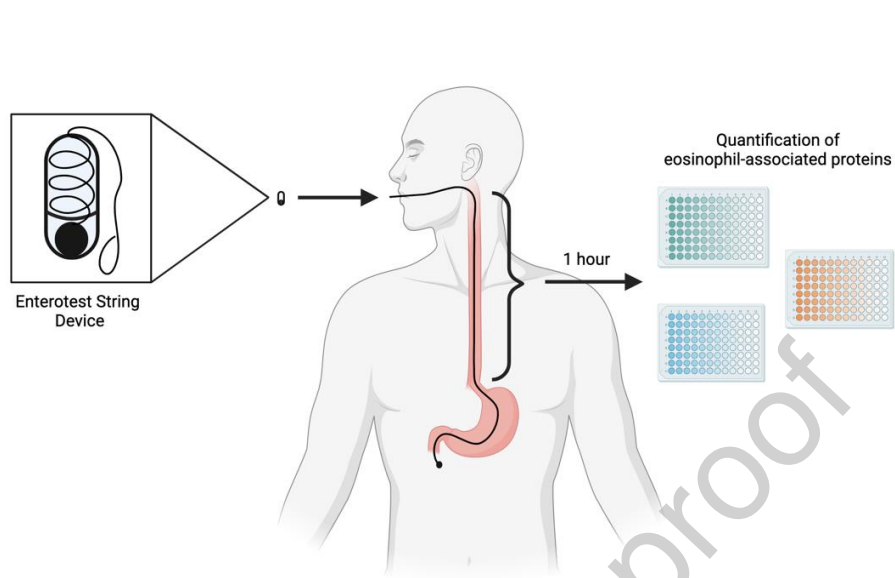


Figure 1: Overview of the Esophageal String Test

Figure 2: The Cytosponge in capsule (left) and expanded (right)

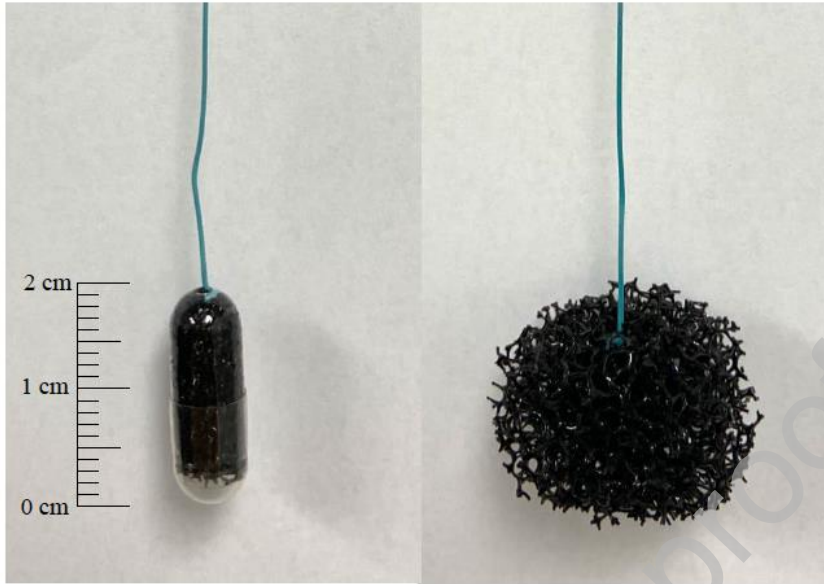
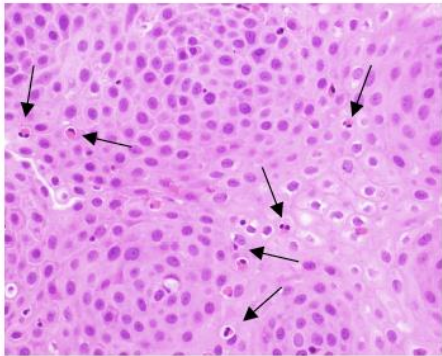


Figure 3: Eosinophils identified in esophageal tissue via the Cytosponge

A. Biopsy histology



B. Cytosponge cytology

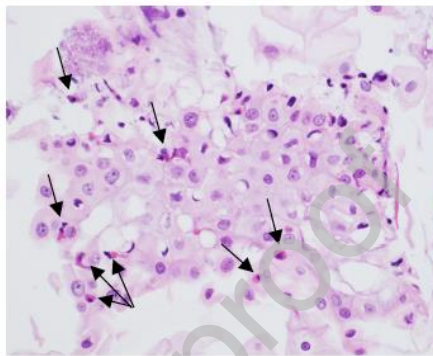


Table 1: Comparison of Emerging Non-Invasive Tests for EoE

	EST^{12,13}	Cytosponge²⁰⁻²⁴	TNE³⁴⁻³⁸
Time to perform	1 hour	10 minutes	5-15 minutes
Age[§]	≥ 7 years	≥ 18 years	≥ 3 years
Potential adverse effects	Gagging Nausea Sore throat	Gagging Sore throat Pharyngeal bleed* Sponge detachment*	Vomiting Epistaxis Nausea
Tolerance of Procedure	86 – 100%	93 – 100%	98 – 100%
Specialty Required	None	Pathology	GI or ENT
Cost	\$	\$	\$\$\$

EST: Esophageal string test; TNE: Transnasal endoscopy

[§]Based on referenced studies

*Risk of occurrence is <1:2000 and did not occur in patients with EoE.