Editorial

Implementation of the esophageal string test in clinical practice and research

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Eosinophilic esophagitis (EoE) is a chronic, progressive inflammatory disease that requires indefinite monitoring to assess disease status and tailor treatments. Esophagogastroduodenoscopy (EGD) with biopsies is the standard of care for monitoring, exposing patients to potential risks of anesthesia and complications. EGDs also require time away from work or school and represent a significant component of the $1.36 billion estimated annual health care costs for EoE in the United States.[1]

The esophageal string test (EST) is a minimally invasive diagnostic tool used to measure mucosal inflammation without sedation. The test involves swallowing a weighted, cellulose capsule device containing a stainless steel ball and a highly absorbent nylon string. The end of the string is taped to the cheek, and the patient swallows the capsule (Fig 1, *A*). As the capsule is swallowed, the remainder of the string deploys in the upper gastrointestinal tract to collect luminal secretions. The capsule dissolves in the stomach and the stainless steel ball is eventually passed through the stool. After an hour, the string is removed and segments are cut according to the predicted esophageal length and location (based on height or pH) (Fig 1, *B*). Specifically, the location of the gastroesophageal junction is identified by using a height formula or pH stick. The distal end of the esophageal segment is cut 2 cm proximal to the gastroesophageal junction. The proximal end of the esophageal segment is estimated to begin 7 cm distal to the lip. The length of string from 0 to 7 cm is the oropharyngeal segment, which is removed. The esophageal segment is placed in elution buffer on dry ice for storage and shipping. In a reference laboratory, a protease inhibitor is added and the protein from the string is isolated and assessed by ELISA for the eosinophil-associated proteins major basic protein-1 (MBP-1) and eotaxin-3. Eosinophil-associated proteins are compared against a nomogram to generate a cumulative score (EoEScore). The EoEScore alone is reported with the reference range for inactive disease (<0.53) and is associated with a probability of the presence of eosinophilic inflammation.

Originally, the EST was called the Entero-Test and used to diagnose gastrointestinal infections, assess gastrointestinal hemorrhage, and sample bile acids among other applications. It was adapted for use in EoE as the EnteroTracker capsule (enterotrack, Aurora, Colo), a class I US Food and Drug Administration–registered device that is exempt from 510(k) requirements. The clinical assay for MBP-1 and eotaxin-3 used for EoE is a separate Clinical Laboratory Improvement Amendments–approved laboratory-developed test performed by EnteroTrack.[2]

The initial trial of the EST tested diagnostic performance of the device in children aged 7 to 20 years with active EoE (n = 14), inactive EoE (n = 8), and gastroesophageal reflux disease (n = 4), as well as in a group of controls (n = 15) after the EST was placed overnight.[3] The study was carried out in a single pediatric center in Colorado. An EGD was performed the day after the patient had swallowed the EST; peak number of eosinophils per hpf (eos/hpf) in esophageal biopsy samples were used as the criterion standard for comparison. Multiple eosinophil granule proteins were also compared with peak the eos/hpf value. Spearman correlations were moderate to strong for each of the eosinophil granule proteins assessed when compared with the peak eos/hpf value on tissue biopsy samples ($r > 0.60$). Sensitivity and specificity were not directly reported, but the area under the curve (AUC) values suggested strong diagnostic sensitivity for MBP-1 (AUC = 0.97). In this study, eotaxin-3 was not assessed. A larger, multisite (in Denver, Chicago, and Indianapolis) follow-up study compared eosinophil-associated protein measurements with the peak eos/hpf values in adult (n = 60) and pediatric patients (n = 74) aged 7 to 55 years with active EoE (n = 62), inactive EoE (n = 37), and no EoE (the controls) (n = 35) (N = 134).[4] The correlations between eosinophil-associated proteins and peak eos/hpf values in biopsy samples were more modest ($r \geq 0.4$). The EST had moderate diagnostic accuracy (AUC = 0.83; sensitivity = 0.80; specificity = 0.75) in patients with known EoE.

Our center has performed more than 100 ESTs in patients aged 6 to 22 years with a known diagnosis of EoE in our outpatient allergy and gastroenterology clinics. Our preferred criteria for EST candidates are outlined in Table I. Typically, we use the EST in asymptomatic or minimally symptomatic patients who are undergoing stable treatment and modifying their diet or refining or assessing their medication regimens. We avoid using the EST in patients with significant dysphagia or suspected esophageal stricture, who may require esophageal dilation. Patients are scheduled in a dedicated EST outpatient clinic block, which can be administered and billed as a physician or nurse visit. They are counseled to fast for 2 hours before the visit to prevent regurgitation or aspiration. First, height is obtained by using a stadiometer. After the device has been placed and secured to the patient's cheek with transparent medical dressing (Tegaderm, Maplewood, Minn), he or she is taken to the waiting room. Books or electronic devices may be useful distractions and prevent manipulation of the device. After 1 hour, the string is marked at the lip, removed, and processed with caution to prevent contamination. We lay parafilm over a dedicated polyethylene cutting board (48 × 12 × 1/2 inches) with a measuring tape adhered to its surface. We prefer using a height formula to determine the location of the

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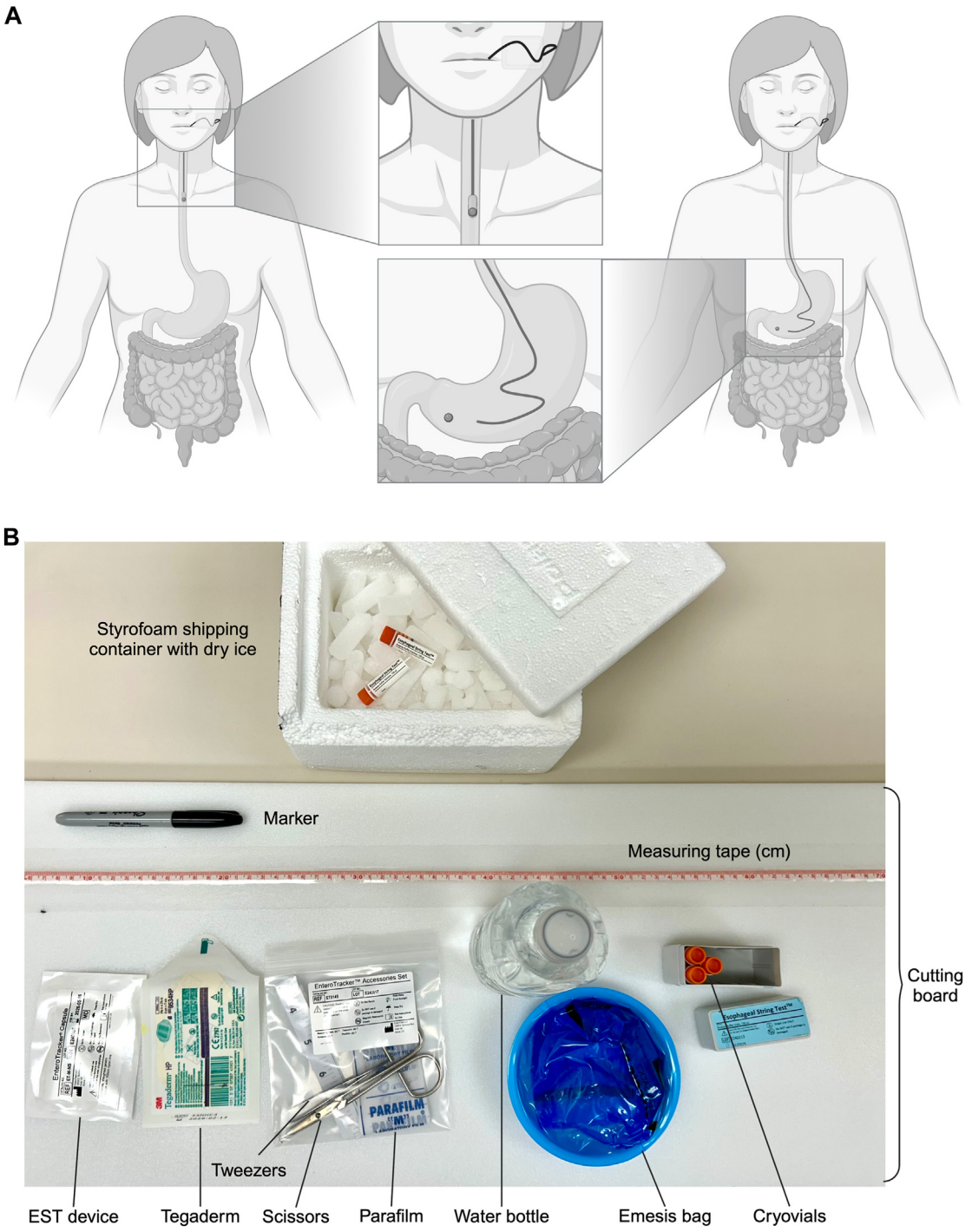

FIG 1. EST administration and necessary supplies. **A,** Diagram demonstrating how the first portion of the string is secured to the cheek and the weighted capsule is swallowed. The string deploys throughout the upper gastrointestinal tract. The capsule dissolves in the stomach and the stainless steel ball eventually passes through the stool. The EST is removed and processed after 1 hour. **B**, Supplies for administering, collecting, and processing the EST are shown. Patients may drink up to 8 ounces of water to facilitate swallowing the EST. An emesis bag should be available in case of vomiting. The EST is removed from its packaging, and the end of the string is secured to the cheek with transparent medical dressing before it is swallowed. Before removal, the EST is marked at the lip with a marker. Tweezers are used to catch the distal end of the string, which is subsequently laid flat on parafilm to prevent contamination. A measuring tape on a cutting board (optional) is used to identify locations to cut the various EST segments (ie, oropharyngeal, esophageal, gastric, and duodenal) with scissors. The esophageal segment is placed in a cryovial and placed on dry ice in an extruded polystyrene foam (Styrofoam) container for shipping. **A,** Created with BioRender.

TABLE I. Preferred EST candidate characteristics

Required	Relative exclusions
• Has a known diagnosis of EoE • Is able to swallow pills	• Has severe dysphagia • Has overlap gastroesophageal reflux disease • Has suspected steroid-induced esophageal candidiasis • Has persistent dysphagia requiring endoscopic evaluation (eg, using EndoFLIP) or intervention (eg, dilation)

gastroesophageal junction as gagging/vomiting, proton pump inhibitor use, or reflux may obscure or change the anatomic location of pH transition. After the sample has been shipped to the EnteroTrack reference laboratory on dry ice, the results are sent back within 10 to 14 days.

The EST is generally well tolerated. The direct risks of the EST capsule include sore throat, gagging, and (rarely) vomiting.[4] In our center, gagging is the most common complication (32%), and inability to swallow the capsule is the most common reason for EST failure. The EST contains a small stainless steel metal ball to facilitate transit. Magnetic resonance imaging should not be performed until the stainless steel ball has passed through the stool, and if this is uncertain, an abdominal x-ray should be obtained. The expected colonic transit time is 24 to 72 hours. In the validation study, approximately 14% of individuals were unable to swallow the capsule. Slightly higher failure rates have been seen in real-world settings.[5] Our EST failure rate (11%) is comparable to that in the validation study. The capsule is approved for use in patients as young as 7 years.[4] Occasionally, the string will not deploy because it will knot, but recent adaptations were made to the EST to shorten the length of the string and alter packing of the capsule to improve unraveling. In addition, the capsule was made smaller and composed of vegetarian cellulose, negating the prior contraindication for use with individuals having gelatin allergy.

In terms of diagnostic accuracy, the test performance characteristics of the EST based on the reported sensitivity and specificity suggest a positive likelihood ratio of 3.2 and a negative likelihood ratio of 0.27. This means that the test is useful for detecting eosinophilic inflammation when the pretest probability is low or high. Because symptoms are often discordant with biopsy findings[6] and the efficacy of dietary therapy usually ranges from 40% to 60%,[7] the utility of the EST in some clinical scenarios is uncertain and a positive or negative result may not provide sufficient information to justify instituting or withdrawing treatment.

Another aspect that may affect test performance is variability in the way the EST is swallowed. The EST is designed so that the initial oropharyngeal segment of the string is thinner than the collection portion, which dwells in the esophagus and stomach. This makes the string more tolerable to maintain in place, because the thick portion begins below the vocal cords. Unfortunately, some patients may swallow variable lengths of the thin portion, reducing collection of luminal secretion in the proximal esophagus. The EST is not normalized for esophageal length or protein content, and the validation studies did not address differences in esophageal length. Additional limitations of the EST include a delay in processing time, reliance on a single reference laboratory, and shipping logistics. We do not ship EST specimens on Fridays or over long holiday weekends. We consider shipment distance, anticipated delays, and seasonal temperatures when determining the amount dry ice to include with the shipment.

The primary obstacle to clinical implementation of the EST has been approval for insurance reimbursement. The Current Procedural Terminology code for the EST (0095U) is a Proprietary Laboratory Analysis code. In 2021, the Centers for Medicare and Medicaid Services agreed to increase reimbursement for this code. The Current Procedural Terminology codes for returns visits and facility fees can also defray cost; however, institutions, practices, or patients must shoulder any residual expense. Our institution has identified the EST as an area of strategic importance to enhance the patient experience and has supplemented the initial cost of implementation. Efforts are under way by EnteroTrack to reduce laboratory expenses, minimize patient costs, and decrease the financial burden on clinics and hospitals offering the EST.

We have used the EST for both clinical and research purposes. In some instances, we have collected residual samples from clinical EST specimens for use in research after obtaining appropriate consent. A key advantage of the EST is that it can be segmented according to length, providing spatial resolution of markers across the upper gastrointestinal tract. We generally snap freeze string segments for research purposes in liquid nitrogen to minimize protein degradation. This allows us to use the specimens for applications that may not be compatible with the EST extraction buffer.

The EST has already been used in research settings to assess other biomarkers, including the microbiome and periostin.[8,9] Unbiased approaches such as proteomics may identify additional markers. The EST may also be useful for screening for disease activity in asymptomatic patients or those at risk for developing EoE. We are actively pursuing this approach by monitoring for the development of esophageal eosinophilia in clinical trials of oral immunotherapy for IgE-mediated food allergy. Consistent with its original applications, the EnteroTracker capsule can sample the stomach and duodenal mucosa, which may prove useful in patients with nonesophageal eosinophilic gastrointestinal disease or other gastrointestinal diseases. Because it can collect bile, the Entero-Test has previously been used to evaluate the biliary disposition of drugs enabling studies of pharmacokinetics, metabolism, and excretion.[10] Finally, ELISAs may be adapted for point-of-care testing, which could enable home collection and processing.

In summary, the EST is an exciting tool that enables minimally invasive sampling of the esophageal mucosa. Allergists and gastroenterologists can use the EST to monitor children, adolescents, and adults with known EoE in an outpatient setting. Patient selection is important, as some patients may have clinical indications that favor traditional EGD. The EST is usually well tolerated, although some patients cannot swallow the capsule and

device failures may occur. In the future, the EST may be useful for sampling additional biomarkers in clinical scenarios beyond EoE.

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