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The Minimally Invasive 1-Hr Esophageal String Test (EST) Monitors Therapeutic Changes in Mucosal Inflammation in Eosinophilic Esophagitis

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Abbreviations used:

EST, Esophageal String Test®; ETK, EnteroTracker®; EoE, Eosinophilic Esophagitis; Eot-3, Eotaxin-3; MBP-1, Eosinophil Major Basic Protein-1; PPI, Proton Pump Inhibitor; PEC, Peak Eosinophil Count/HPF; FED, Food Elimination Diet; TCS, Topical Corticosteroid; PPV, positive predictive value; NPV, negative predictive value.

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Abstract

Background: Endoscopy, standard-of-care for monitoring Eosinophilic Esophagitis (EoE), assesses mucosal inflammation. The Esophageal String Test (EST®), a minimally invasive swallowed capsule and immunoassays, quantifies EoE inflammation. We determined whether the EST/EoEScore can monitor disease in patients undergoing treatment.

Methods: Thirty-three samples from 14 EoE patients (7 children, 7 adults) who underwent repeat endoscopies and ESTs were studied. Biopsies were analyzed for peak eosinophil counts; ESTs analyzed for EoEScores.

Results: Eosinophil counts and EoEScores significantly correlated during treatment, distinguishing patients with active EoE from treatment-associated remissions for 93.9% of ESTs performed.

Conclusion: The EST can be used to longitudinally monitor responses to treatment in EoE.

INTRODUCTION

Endoscopy remains standard-of-care for diagnosing and monitoring esophageal inflammation in EoE. To assess mucosal inflammation following initiation or changes in treatment, repeat endoscopies under anesthesia are used, but burdensome due to post-procedure recovery, costs and quality-of-life impacts (1, 2). The minimally invasive 1-hr Esophageal String Test (EST®) assesses esophageal mucosal inflammation in EoE (3). Its EoEScore®, based on eosinophil-associated biomarkers, represents the probability of mucosal inflammation equivalent to a peak eosinophil count (PEC) of ≥15eos/HPF (3), enabling identification of patients with active EoE. Our objective was to determine whether the 1-hr EST can longitudinally monitor disease activity during therapeutic changes.

METHODS

We analyzed ESTs from patients with multiple time points enrolled in our study validating the EST (3). Endoscopic biopsies and ESTs from patients before and during changes in treatment were analyzed to assess agreement between EoEScore and disease activity; follow-up EGDs were standard-of-care.

EnteroTracker® capsules (EnteroTrack, Aurora CO, USA) (4) replaced unavailable Enterotest® capsules (3, 5). ESTs were performed immediately before or within 2 days of endoscopy. One hour after swallowing the capsule, the string was recovered and processed for biomarkers (3-5). Subjects underwent endoscopy, diagnostic biopsies obtained, and eosinophil counts performed. Results are reported as peak eosinophils/high powered field (eos/HPF) (surface area = 0.26mm^2) (3).

EST biomarkers (MBP-1, Eotaxin-3) quantified by ELISA were used to calculate the EoEScore using a validated algorithm (3). The EoEScore (probability range 0-1) cutoff of ≥ 0.53 indicated disease activity corresponding to mucosal eosinophilia ≥15 eos/HPF, an EoEScore <0.53 to disease remission (<15 eos/HPF).

Statistical analyses are described in the on-line supplemental materials.

RESULTS

Thirty-three ESTs and biopsies were analyzed from 7 children and 7 adults with EoE (**Table 1)**. At initial endoscopy, 2 children were in remission, 5 active disease, while 3 adults were in remission, 4 active disease. Treatments included food elimination, topical corticosteroids or PPIs (**Table 1**). The EST was well-tolerated; one patient failed to swallow the capsule on first attempt, succeeding with a second capsule; none vomited during any of their ESTs.

Serial ESTs showed EoEScores distinguishing active disease (≥15eos/HPF) from treatment-associated remissions (<15 eos/HPF) **(Figure 1A, On-line supplemental Figure S1).** EoEScore subcomponents confirmed MBP-1 and Eotaxin-3 levels correlated with changes in mucosal eosinophilia (**On-line supplemental Figure S2A**) (3). Thirty-one of 33 biopsies and ESTs provided congruent data (93.9%); their EST accurately reflected mucosal eosinophilia (**Figure 1A**, **On-line supplemental Figure S2B)**. One adult's and one child's results were incongruent; EoEScore=0.467 and PEC=140 eos/HPF, and EoEScore=0.695 and PEC=12 eos/HPF, respectively. Re-review of EGD notes for the pediatric patient confirmed abnormal furrowing and edema

distally (**Table 1**), indicating active disease despite the PEC of 12eos/hpf, favoring interpretation of their EoEScore (0.695) as active disease.

EoEScores strongly correlated with EoE disease activity based on histologic assessment of PECs for all subjects (**Figure 2A**). Spearman analysis for EoEScore vs. PEC from 33 ESTs showed highly significant correlation (ρ = 0.755, p< 0.0001) (**Figure 2A**). Serial EoEScores for repeat ESTs correlated with mucosal eosinophilia during treatment changes, reflecting patients' clinical course in a variety of scenarios: (1) continued active disease, (2) remained in remission during four repeat ESTs, (3) showed food reintroduction-induced recurrence of eosinophilic inflammation, (4) full or partial treatment-induced remission, or (5) serially increased/decreased disease activity (**On-line supplemental Figure S1**).

Peak PECs and EoEScores from ESTs performed on visits 1 and 2 showed significant synchrony (**Figure 1**); where patient PECs increased or decreased above/below the 15eos/HPF cut-off for active disease, their EoEScores similarly increased or decreased above/below the 0.53 cut-off that distinguishes active disease from remission (**Figure 1A**). Change in PECs and EoEScores (visit 1 minus visit 2) were significantly correlated (Spearman ρ=0.63, p=0.016) (**Figure 1B**) demonstrating they co-vary.

Using data from the first two visits, we determined sensitivity, specificity, PPV and NPV of EoEScores for predicting patient remission status. An EoEScore<0.53 and PEC<15 eos/HPF served as cutoffs for assigning remission status (**Figure 2B**). Of the 14 patients, five (4 active, 1 remission) had no status change between visits, while 9 changed (5 active to remission, 4 remission to active). The EoEScore cutoff of 0.53 is

associated with an 80% sensitivity, 75% specificity (3); the sum of the sensitivity and specificity at each visit was >150%, supporting the EST EoEScore as useful for monitoring EoE disease status (6).

DISCUSSION

The EST EoEScore identified changes in eosinophilic inflammation during treatment, disease status correctly predicted in 31 of 33 patient visits (93.9%). Serial ESTs reflected longitudinal changes in mucosal inflammation, representing a clinically useful tool in lieu of repeat endoscopy for monitoring EoE.

Study limitations included repeat ESTs were not performed at regular intervals and relatively small sample size, potentially explaining variability in sensitivity and specificity, but this reflects real-life standard-of-care practices, as repeat ESTs were done at provider discretion. Some patients had EoEScores close to the active disease cutoff; depending on the clinical situation, a follow-up endoscopy could be recommended to confirm patient status. Real-life clinical utility studies are in progress; a 1-year experience showed the EST as safe, efficient, well-tolerated, lower cost, and guided changes in EoE management without endoscopy (7).

Other approaches for monitoring of EoE have been reported (8). The Cytosponge® collects epithelial scrapings from the entire esophagus, providing a biopsy-like sample (9, 10). Non-endoscopic devices, the EsoCheck® and EsophaCap®, being tested for Barrett's and esophageal adenocarcinoma (11, 12), may have promise for EoE.

EST applications across the spectrum of EoE include monitoring patients who: (1) are seen annually for surveillance, (2) have undergone treatment changes, (3) experience symptom changes uncertainly related to EoE, (4) are treatment non-adherent to assess inflammation and encourage adherence. Exclusions include patients with: (1) an inability to swallow pills or oral aversion, (2) problems keeping a string in the oropharynx, (3) endoscopic history of stenosis (<10mm esophageal diameter and/or stricturing) precluding capsule passage, (4) gelatin allergy (vegetarian capsules are available), and (5) risk of endoscopic complications (bleeding diatheses, connective tissue diseases). Finally, ESTs could be used to screen patients with a clinical history, signs and symptoms of EoE living in remote, low-resource rural and underserved communities with limited access to endoscopy, for referral for endoscopic diagnosis.

Tables

Table 1 - Patient characteristics[a]

Subject No.	Age (years)	Gender	Race/ Ethnicity	Treatment[b]	Disease Activity[c]	Peak Eos/HPF V1 / V2[d]	# ESTs performed
Pediatric							
1	20	M	W/C[d]	PPI	Active EoE	15 / 0	2
2	9	F	W/C	PPI, TCS	Remission[e]	12[f] / 40	2
3	18	F	W/C	PPI, FED	Active EoE	66 / 33	2
4	15	M	W/C	PPI, TCS	Active EoE	83 / 56	2
5	9	F	Other	PPI, TCS	Active EoE	57 / 0	2
6	19	F	W/C	TCS	Remission	0 / 102	2
7	17	M	W/C	PPI, TCS	Active EoE	31 / 0	2
Adult							
1	38	F	Other	PPI, TCS	Remission	0 / 108	2
2	20	F	Hispanic	PPI	Active EoE	100 / 55	2
3	51	F	W/C	TCS	Remission	0 / 24	3
4	34	F	W/C	FED	Active EoE	16 / 0	2
5	33	M	W/C	FED	Active EoE	52 / 45	4
6	58	F	W/C	PPI	Remission	3 / 0	2
7	59	M	W/C	PPI, FED	Active EoE	140 / 0	4

[a] Pediatric patients were enrolled from 3 sites (Children's Hospital Colorado CO; Lurie Children's Hospital IL; OSF Saint Francis Medical Center IL.); adult patients were enrolled from 2 sites (University of Colorado Hospital, CO; Northwestern Medical Center, IL).
[b] Treatment(s) at time of or following initial (first) visit; PPI, proton pump inhibitor; FED, Food elimination diet; TCS-topical corticosteroid.
[c] Disease activity (based on PEC) at initial (first) visit at which the EST was performed.
[d] PEC at V1=1st visit / V2=2nd visit at which the EST was performed.
[e] W/C, White/Caucasian.
[f] PEC <15eos/hpf suggested remission, but re-review of the EGD notes indicated evidence of active disease distally with abnormal presence of furrows and edema.

Figure Legends

Figure 1 – The peak eosinophil count and EoEScore® demonstrate longitudinal synchrony in repeat ESTs. A. Plots of individual PECs and EoEScores® for the 7 pediatric and 7 adult patients with active disease or treated disease in remission demonstrate how these independent measures show synchrony (covary) during EST sampling at the initial visit (#1) and repeat visit (#2). The peak eosinophil counts (left y-axis in blue) and EoEScore® (right y-axis in red) are plotted for visits 1 and 2 (x-axis). Dashed blue lines show a PEC of 15 eos/HPF and dashed red lines an EoEScore® of 0.53, the cutoffs for distinguishing between active and inactive EoE. The EoEScore® cutoff of 0.53 is associated with an 80% sensitivity, 75% specificity (3). **(B)** The correlation between changes in PEC and EoEScore® is plotted for ESTs performed at the 1st and 2nd visits; the change is plotted as Visit 1 minus Visit 2. Spearman (ρ) and Pearson (r) correlation coefficients and their significance are shown, indicating longitudinal synchrony between PEC and EoEScore® for repeat ESTs.

Figure 1

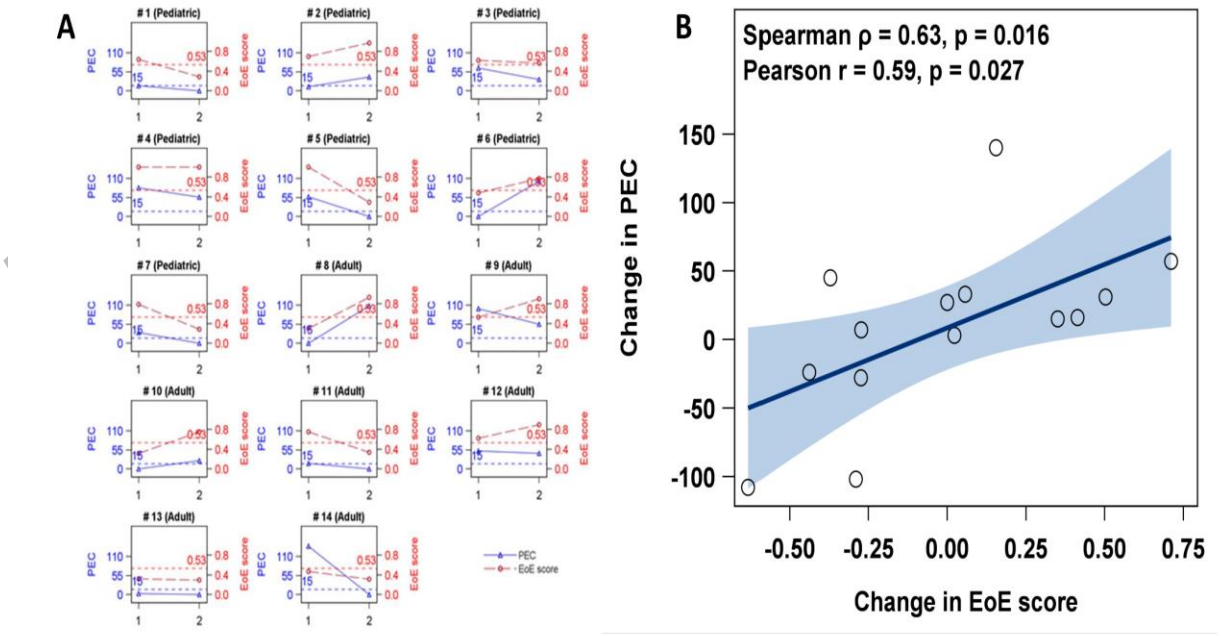
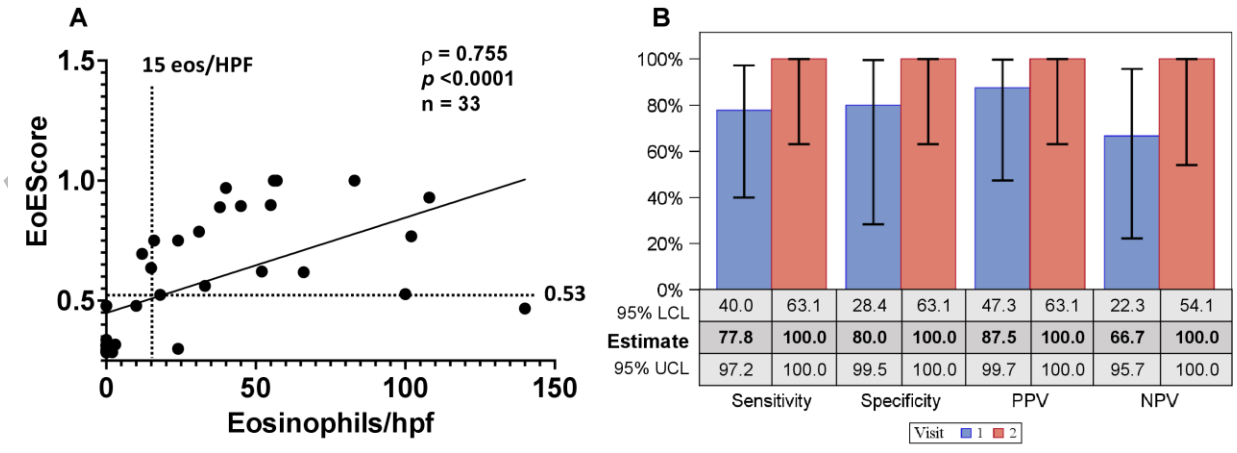


Figure 2 – Correlations and predictive features of the EST EoEScore® with esophageal inflammation during treatment. A. The EoEScore correlates with EoE inflammation during treatment. Spearman analysis is shown for a comparison of the EoEScore and histologic PEC (eos/HPF) in biopsies from 33 ESTs (including initial and all repeat ESTs) as performed by the 14 study subjects (ρ = 0.755, p<0.0001). The horizontal dashed line at 0.53 represents the cutoff for which an EoEScore indicates a high probability of subjects having active EoE, comparable to eosinophil counts of ≥15 eos/HPF, as represented by the vertical dashed line. **B. Predictive features of the ESTs related to treatment.** Using the EST EoEScore cutoff of 0.53 for distinguishing active from inactive EoE disease, the sensitivity, specificity, positive and negative predictive values (PPV, NPV) of the EoEScore for determining EoE remission status in the 14 subjects was calculated. The PEC of <15 eos/HPF served as the gold standard for EoE in remission. Only EST EoEScore and PEC data assessed at visits 1 and 2 were analyzed. The percent (%) values for sensitivity, specificity, PPV and NPV and associated 95% confidence intervals are indicated in the table below the graph; values for PPV and NPV are dependent upon the EoE case prevalence in the study.

Figure 2



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Supplemental Materials--http://links.lww.com/AJG/D460

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