Use of Esophageal String Test to Understand symptoms, inflammation and function in Eosinophilic Esophagitis

Sponsor: End Allergies Together (501(c)(3))

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Version: 3.0
Date: 4/25/18
COMIRB: 17-0796

STATEMENT OF COMPLIANCE

The study will be conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6), the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and the NIDCR Clinical Terms of Award. All personnel involved in the conduct of this study have completed human subjects protection training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator or Clinical Site Investigator:

Signature		Date	
Print Name		Title	

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LIST OF ABBREVIATIONS

{Please add all disease or study-specific abbreviations/acronyms in this section. Modify this list as needed for your particular study and remove abbreviations that are not used in the document.}

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CHCO	Children's Hospital Colorado
CHOP	Children's Hospital of Philadelphia
CRF	Case Report Form
DCC	Data Coordinating Center
DSMO	Data and Safety Monitoring Officer
EOE	Eosinophilic Esophagitis
EREFS	Endoscopic Reference System
EST	Esophageal String Test
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
ISM	Independent Safety Monitor
MOP	Manual of Procedures
N	Number (typically refers to participants)
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OHSR	Office of Human Subjects Research
PEESS	Pediatric Eosinophilic Esophagitis Symptom Scores
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
US	United States

Title:	Use of Esophageal String Test to understand symptoms, inflammation and function in eosinophilic esophagitis
Overview:	Presently, the only method available to monitor disease activity in Eosinophilic Esophagitis is endoscopy with pathological review of biopsies. The overall goal of this study is to determine the ability of the Esophageal String Test (EST), a minimally invasive capsule based technology, to measure disease activity in children with EoE.
Objectives:	**Primary Aim:** Do eosinophil proteins captured by the EST correlate with symptoms related to pediatric EoE? **Secondary Aim 1:** Do eosinophil proteins captured by the EST correlate with the markers of EoE pathogenesis? **Secondary Aim 2:** Do eosinophil proteins captured by the EST correlate with a measure of esophageal function?
Population:	40 subjects: males and females, ages 7-18 years old undergoing upper endoscopy for monitoring of EoE or for symptoms of esophageal dysfunction concerning for EoE
Study Sites:	**Lead Site:** Children's Hospital of Colorado **Recruitment Site:** Children's Hospital of Philadelphia **Clinical Laboratory:** University of Illinois, Chicago
Site Principal Investigators	Stephen Ackerman, PhD; University of Illinois, Chicago Amanda Muir, MD; Children's Hospital of Philadelphia
Study Duration:	2 years
Subject Participation Duration:	1 month
Estimated Time to Complete Enrollment:	18 months

1 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Background Information

Eosinophilic Esophagitis (EoE) is a chronic disease characterized by symptoms related to esophageal dysfunction and large numbers of eosinophils in the lining of the esophagus (1). An endoscopy is required to make the diagnosis, because a biopsy of the esophageal tissue needs to be examined under the microscope. Eosinophils contain a number of proteins that likely participate in their function. Eosinophil peroxidase, major basic protein, eosinophil cationic protein and eosinophil derived neurotoxin are four of the major proteins that are associated with the eosinophil's function.

The EST is a capsule filled with a 90cm nylon string. A trailing end of the string protrudes from one end of the capsule. This end is taped to the cheek and the capsule is swallowed. As the capsule travels to the small intestine, the string inside the capsule is dislodged, leaving a string that goes from the cheek to the small intestine. The capsule dislodges from the string and the string is left in place, in the mouth, esophagus, stomach and small intestine for an hour. During this time, the string rubs against the inside of the esophagus and collects eosinophil proteins. After one hour, the string is removed through the mouth and placed in preservative to save the eosinophil proteins. The fluid extracted from the string is analyzed in the laboratory for eosinophil proteins. In a previous study, we showed that the eosinophil proteins captured by the string correlated with the number of eosinophils and amount of eosinophil proteins in the tissue biopsy (2). In this proposal, we will measure eosinophil proteins contained in the EST and correlate that with esophageal symptoms, inflammatory proteins and function.

1.2 Rationale

Procurement of mucosal biopsies during sedated endoscopy is the only method to determine whether a child may have eosinophilic esophagitis (EoE). Counting esophageal eosinophils in the squamous epithelium remains key to establishing the diagnosis. To date, no blood, stool, breath or other peripheral sampling of eosinophil related biomarkers has been shown to correlate with mucosal eosinophilia in esophageal biopsies, thus leaving patients and families with the only option of undergoing this invasive, costly and time consuming procedure. Sedated upper endoscopy with biopsy is a safe procedure, but alternative methods that limit or eliminate exposure to anesthetic agents and address other problematic features are needed to obtain diagnostic samples. Although eosinophils are easy to count, variability exists with respect to reporting, and better biomarkers must be determined. In this regard, we, and others showed that effluents obtained from the esophageal lumen are reflective of esophageal eosinophilia. Katzka et al determined that the esophageal sponge is able to harvest mucosal scrapings to measure eosinophils (10). This approach is not suitable for children because of its size and tolerability. Our work documents that the Esophageal String Test (EST) can detect luminal biomarkers that reflect esophageal eosinophilic infiltration (2). This capsule based technology is well tolerated by children ages 7 years and up, and fluid adherent to the string has been shown to contain eosinophil granule proteins that correlate with esophageal eosinophilia and amounts of these proteins in biopsies.

The overall goal of this study is to discover a less invasive fashion to make the EoE diagnosis and assess disease activity in children. Here, we will determine whether eosinophil granule proteins captured by the EST correlate with: a) EoE symptoms, b) EoE histopathological scores and inflammatory proteins and c) a new functional biometric, distensibility. In this proposal, we hypothesize that luminal eosinophilic granule proteins will correlate with symptoms, inflammatory proteins and distensibility.

1.2.1 Potential Risks

The study involves *greater than minimal risk with no prospect of direct benefit.*

Since the patient will be undergoing endoscopy/colonoscopy for diagnostic purposes regardless of this study, the benefits to the subject and society outweigh the minimal risks associated with the additional biopsies. The procedure will present the subject with an experience that is the same as that expected in the standard of care. Risks associated with endoscopy and colonoscopy with biopsy include perforation, hemorrhage and the risks of conscious sedation.

While upper endoscopy with esophageal biopsy is greater than minimal risk, only those patients consenting for and receiving upper endoscopy with biopsy for evaluation of GI symptoms as recommended by their primary clinician as part of routine care will be approached for study. The additional biopsies for research purposes are therefore of minimal risk beyond routine clinical care.

The safety of multiple biopsies is supported by studies on adult patients with Barrett's esophagus that have shown that multiple esophageal biopsies (as many as 35 to 120 esophageal biopsies in an individual patient) do not produce esophageal perforation or bleeding when performed by an experienced team of physicians, nurses, and technicians (48). In addition, a recent NIH study demonstrated that obtaining multiple mucosal biopsies for research purposes during elective endoscopy is well tolerated and appears to have no more than minimal risk without appreciably increasing the risk of otherwise routine endoscopy (49). Importantly, there was no statistically significant association between the number of biopsies, type of procedure, anatomic location of research biopsies, endoscopist, or the use of nonsteroidal anti-inflammatory drugs and the risk of complications.

A recent review of the incidence of perforation and other severe complications associated with upper endoscopy was performed at Children's Hospital Colorado. In a 42 month period of study there were 8 documented episodes of perforation out of 8130 endoscopic procedures. In all 8 cases the patient had undergone an interventional endoscopic procedure (4 esophageal dilations, 2 gastrojejunostomy placements, 1 foreign body removal and 1 polypectomy). None of the perforations was the result of esophageal mucosal biopsies performed during diagnostic EGD. The incidence of perforation associated with pediatric gastrointestinal endoscopy performed by pediatric gastroenterologists is low. In the 3.5 year period of study, 5683 diagnostic EGD's were performed here at CHCO. There were no documented complications resulting from esophageal mucosal biopsies, including bleeding requiring blood transfusion, tamponade, or cauterization. There were 5 diagnostic EGD's (0.09%) which presented after endoscopy with symptoms concerning for upper GI bleeding (hematemesis/coffee ground emesis), 4 of which were referred to the ED, but none of whom required blood transfusion or additional intervention.

EndoFLIP has been shown to be safe in adults and children without increased risk of bleeding or perforation. Parameters can be set to limit the degree of inflation permitted. EndoFLIP will add approximately an additional 10 minutes of sedation time to overall procedure.

Allergic reaction to the gelatin in the capsule is the only associated risk with the EST. The EnteroTracker device contains a small metal ball to weigh down the string. Subjects should tell their providers they have swallowed the ball and xray should be ordered prior to undergoing an MRI after completed the study. The small ball should pass within 24-48 hours in healthy individuals.

There are no differences in the risks to the experimental group (patients with eosinophilic GI diseases) and the control groups (other inflammatory diseases and those with normal

mucosae). No treatments will be administered. Specific procedures are in place to protect the patient including using the smallest biopsy forceps and smallest capsule indicated as well as automatic stoppage of EndoFLIP insufflation at set intrabag pressures and volumes. Risks are minimized by qualified investigators closely monitoring subjects, and appropriate use of inclusion/exclusion criteria.

Patient confidentiality will be maintained. Violation of privacy and loss of confidentiality are both risks to which research participants are exposed. The possibility of these risks increases when protected health information is collected

Describe plan to minimize risk. We will minimize risk by excluding those patients at potential or theoretical increased risk of bleeding or perforation from esophageal mucosal biopsies (See exclusion criteria) and with the below mentioned stopping rules. EndoFLIP will only be performed by endoscopists trained in these tools to avoid unnecessary prolongation of procedure and anesthesia time.

Stopping Rules. A subject will be able to discontinue the study any time after giving consent up until time of procedure. Subjects will need to be re-consented if ever undergoing repeat procedure. If, during the procedure, there is real or anticipated complication on the part of the physician(s) related to anesthesia or procedure, the research procedure will be stopped. Protocol stopping criteria include any grade 3 complication.

1.2.2 Potential Benefits

No immediate benefits are available to the subject.

Potential benefits to the subject are related to the long-term outcome of these studies. If the pathogenic mechanisms for GI inflammatory diseases can be further dissected, more specific diagnostic and appropriate therapeutic measures can be developed. Specifically, this study will expand the knowledge as to the role of eosinophils and eosinophil-derived granule proteins in gastrointestinal inflammation. In addition, these studies will provide the basis for future diagnostic criteria and treatments for certain GI inflammatory diseases.

Treatment of EoE is in large part administered to prevent the development of esophageal stricture. However, we are extremely limited in the available clinical parameters to monitor disease progression in this regard. The overall aim of this proposal is to evaluate the association between patient reported symptoms, esophageal function and eosinophil activity using standard and investigative assessment tools. This data will advance our understanding of the relationship between patient reported symptoms, esophageal inflammation and esophageal function and provide novel information regarding the utility of potentially less invasive disease assessment tools. Second, there is the potential to gain further understanding of the pathogenesis of disease. Future studies will use tools identified here to define the natural history and measure response to treatment.

Taken together, results from these studies will provide a rich assessment of the esophageal mucosa using a minimally invasive device, the EST. We anticipate that EST protein measurements will correlate with symptoms, inflammatory proteins and a functional biometric. We will take advantage of the robust resources and rich collaboration of three academic institutions to address these aims.

2 OBJECTIVES

2.1 Study Objectives

Specific Aim 1: Do eosinophil proteins captured by the EST correlate with symptoms related to pediatric EoE?

Prior to children undergoing a standard of care endoscopy and biopsy, we will assess their symptoms with a validated score sheet (PEESS)(3). Then, they will swallow an EST and leave it in place for one hour. At the end of the hour, we will remove the EST and freeze it so that we can measure eosinophil proteins that stick to the EST at a later time. We will compare the symptoms score with the EST protein levels. Previous studies show variability in the correlation of symptoms with inflammation, but we anticipate that because the EST samples all of the esophagus, and not just a small piece, like the esophageal biopsy, the EST protein levels will correlate with symptoms (2).

Specific Aim 2: Do eosinophil proteins captured by the EST correlate with the markers of EoE pathogenesis?

In the same pediatric subjects described in Aim 1, we will obtain esophageal biopsies and measure the amount of inflammation in that tissue using 3 different specialized scoring systems (4-6). We will correlate these inflammation scores in the biopsy with the eosinophil protein levels from the EST. We anticipate that the inflammation scores from the tissue will correlate with the eosinophil protein levels on the ESTs.

Specific Aim 3: Do eosinophil proteins captured by the EST correlate with a measure of esophageal function?

In the same pediatric subjects described in Aim 1, we will perform EndoFLIP measurments during the endoscopy. The EndoFLIP is a slender probe covered by a smooth long balloon that is passed into the esophagus and slowly expanded with a salt solution (7). As the EndoFLIP is expanded, sensors inside the balloon identify pressure values that reflect how well the esophagus can stretch. Our early study shows that these readings can identify reduced distensibility in children with EoE (8). We will correlate distensibility measurements obtained by the EndoFLIP with the eosinophil protein levels from the EST.

3 STUDY DESIGN

When children are scheduled to undergo an endoscopy, research assistants will contact the family to arrange for the EST to be performed within one week of the endoscopy as previously described (REF). Prior to swallowing the EST, the patient will complete the PEESS form to assess symptoms. The string will be left in place for 1 hour and then removed. The endoscopy and EREFs and EndoFLIP will be performed as previously described (2). Endoscopic severity: Endoscopic esophageal appearances will be scored using a validated EoE scoring system (EREFS). Five features of EoE will be scored: Edema (0-1), Rings (0-3) Exudates (0-2), Furrows (0-1), Stricture (0-1). Results will be reported as an inflammatory score (edema, exudates, furrows) and fibrostenotic score (rings, stricture). Functional Luminal Imaging Probe (FLIP): After endoscopic visualization, the FLIP, a 16 cm probe (Crospon) will be placed transorally and positioned 3 cm distal to the lower esophageal sphincter. Esophageal cross sectional areas and intrabag pressure will be measured during stepwise distensions beginning with 5 mL and increasing to a maximum of 70mL or intrabag pressure of 50 mmHg is achieved, whichever comes first. FLIP is FDA-approved without age restriction and its use has been approved by our IRBs previously. Primary results will be reported as distensibility plateau (mm).

EST and biopsy samples: The EST will be placed in a labeled tube and frozen until analysis as below. Four mucosal biopsies will be obtained from the distal esophagus for protein (3) and histological (1 biopsy) analysis. Samples for protein analysis will be snap frozen for batch processing and assessment. Samples for histological analysis will be paraffin embedded and cut into 5μM sections for batch staining.

4 STUDY ENROLLMENT AND WITHDRAWAL

Children with EoE or symptoms of esophageal dysfunction concerning for EoE, who are 7 Years of age and older, will be enrolled in this study at Children's Hospital Colorado (CHCO) and Children's Hospital of Philadelphia (CHOP). Most children 7 years and older can swallow capsules. Subjects will be identified by screening appointment schedules, a well-tested method used at both institutions. Informed consent and assent will be obtained. After endoscopy, subjects will be identified as: 1) EoE active or 2) EoE inactive or 3.) other-symptoms but normal histology or vice versa. EoE will be defined based on current diagnostic criteria as having clinical symptoms of esophageal dysfunction, >15 eos/HPF, and other causes of eosinophilia ruled out either by negative pH/impedance study or greater than 6 weeks of treatment with proton pump inhibitor prior to endoscopy (1). EoE active will be defined as having symptoms and abnormal histology. EoE inactive will be defined as being asymptomatic and <15 eos/HPF. "Other" patients will be analyzed separately based on clinical diagnoses. Subjects will be age and sex matched for data analysis.

Participation of Minorities: Subjects will be recruited/enrolled without regard to racial/ethnic group.

Participation of Children: Children aged 7 years and older will be recruited/enrolled.

4.1 Subject Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

- Provide signed and dated informed consent form.
- Willing to comply with all study procedures and be available for the duration of the study.
- Male or female, aged 7 to 18 years old, inclusive
- Undergoing upper Endoscopy with biopsy for clinical care at CHCO or CHOP
- Current or historical diagnosis of EoE, or suspected of having EoE

4.2 Subject Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

- Known connective tissue disease, other eosinophilic disorders
- Past history of caustic ingestion, esophageal surgery or other esophageal injury
- Known inflammatory bowel disease or esophageal motility disease (achalasia)
- Unwilling or unable to swallow the EST
- Oral or intravenous steroids in the preceding 60 days (not including swallowed topical fluticasone, budesonide, etc)
- Pregnancy
- Participation in a clinical study that may interfere with participation in this study

4.3 Rationale for Study Population

Based on prior experience with the EST in a similar population, one out of three subjects is unable to swallow the EST. In order to achieve the enrollment goal of 40 subjects completing all study procedures, 70 subjects would need to be consented at both CHCO and CHOP. It is anticipated that 35 subjects would sign consent at CHCO in order to reach 20 subjects completing the study.

4.4 Strategies for Recruitment and Retention

Potential participants will be screened from the clinical schedules at CHCO and CHOP. Should this method of recruitment yield poor accrual, the study teams will pursue other methods of recruitment including, but not limited to: print advertisement, social media and email communications. Research coordinators will contact the parents of the subjects and explain the study at least one week prior to the scheduled endoscopy. Interested potential participants will be scheduled for consenting and completion of the EST procedure. If the EST procedure cannot be completed prior to the Endoscopy with EndoFLIP, then the consent will be completed prior to the upper endoscopy and the EST procedure will be scheduled within one week of the Endoscopy. Subjects will receive reminder telephone calls prior to all scheduled procedures.

4.5 Subject Withdrawal

Subjects may withdraw voluntarily from the study or the investigator may terminate a subject's participation.

4.5.1 Reasons for Withdrawal

Subjects are free to withdraw from participation in the study at any time upon request.

An investigator may terminate a study subject’s participation in the study if:

- Any medical condition, event or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- The subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

4.5.2 Handling of Subject Withdrawals

Participation in research is always voluntary. A subject can decide to withdrawal from the study at any time. If the subject choses to withdrawal prior to completing any procedures, it will be recorded as such in the study Case Report Forms. If a subject withdraws after completing some of the procedures, the data collected for those procedures will be entered in the CRFs and the uncompleted procedures will be noted as not completed prior to withdrawal.

4.6 Premature Termination or Suspension of Study

It is understood that the study could be terminated prematurely based on poor accrual or prior to reaching recruitment goals, but at the end of the grant cycle.

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party. If the study is prematurely terminated or

suspended, the principal investigator will promptly inform the IRB and will provide the reason(s) for suspension or termination.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects.
- Insufficient adherence to protocol requirements.
- Data that are not sufficiently complete and/or evaluable

5 STUDY SCHEDULE

Screening/ Consenting (Day -7 to 0) When children are scheduled to undergo an endoscopy, research assistants will review the child's information for basic inclusion/exclusion criteria. They will then contact the family to discuss the study. The CRC will email or fax the consent form to families in order to provide ample opportunity to review the consent form. Families who agree to the study will be scheduled to consent for the study up to one week prior to the scheduled EGD or on the day of the EGD before the EGD scheduled time.

Baseline Visit (Day 0)***:*** All procedures can be completed in one day if the subject agrees. If so, the baseline visit will be the only visit for the subject. The baseline visit should be completed as follows:

- Obtain Informed Consent
- Obtain Subject Medical History and complete Study Inclusion/Exclusion criteria
- Obtain subject's height
- Administer the PEESS
- Administer EST device (subjects are allowed up to 8 ounces of water to assist in the swallowing of the EST)
- After 1 hour, remove the EST and assess for adverse events
- Accompany subject to procedure center for check in
- Obtain biopsies during EGD
- Perform EndoFLIP during EGD

Follow Up Visit (Day 1-3): Research Coordinators should contact subjects between 1-3 days after procedures are completed and assess for any adverse events.

6 STUDY PROCEDURES/ EVALUATIONS

6.1 Study Procedures

When children are scheduled to undergo an endoscopy, research assistants will contact the family to arrange for the EST to be performed within one week of the endoscopy. Prior to swallowing the EST, the patient will complete the PEESS form to assess symptoms. The string will be left in place for 1 hour, removed. The EST will be removed from the subject at least 2 hours before the scheduled upper endoscopy time to align with NPO guidelines for upper endoscopy. The endoscopy and EREFs and EndoFLIP will be performed per clinical guidelines and procedures for EndoFLIP (described below).

Pediatric EoE Symptom Score (PEESS): PEESSv2.0 is a validated pediatric PRO that contains patient and parent proxy instruments that can be easily understood. Scoring is based on two domains that evaluate frequency and severity of the symptoms reported by the patient (see Appendix 1). PEESS Score v2.0 will be used to assess EoE symptoms. The PEESSv2.0 Children and Teens Report (8-18) is intended for children ages 8-18 years old to complete independently. The PEESSv2.0 Parent Report for Children and Teens (2-18) is completed for all subjects by the parent.

Endoscopic severity**:** Endoscopic esophageal appearances will be scored using a validated EoE scoring system (EREFS). Five features of EoE will be scored: Edema (0-1), Rings (0-3) Exudates (0-2), Furrows (0-1), Stricture (0-1). Results will be reported as an inflammatory score (edema, exudates, furrows) and fibrostenotic score (rings, stricture) (see Appendx 2).

EST Satisfaction Survey**:** Following the string procedure, the subject and parent will be asked to complete a survey within 2-5 days of the endoscopy. The survey will assess symptoms/side effects after the string and endoscopy procedures, and collect information on patient preference with string versus the endoscopy.

Functional Luminal Imaging Probe (FLIP): After endoscopic visualization, the FLIP, a 16 cm probe (Crospon) will be placed transorally and positioned 3 cm distal to the lower esophageal sphincter. Esophageal cross sectional areas and intrabag pressure will be measured during stepwise distensions beginning with 5 mL and increasing to a maximum of 70 mL or intrabag pressure of 50mmHg is achieved, whichever comes first. To prevent unintended dilation of a stiff esophagus, infusion will stop and alarm message will sound if intrabag pressure exceeds 60 mm Hg. Raw data will be exported to EndoFLIP Analytics and will be filtered to remove measurements during esophageal contractions and vascular and respiratory artifacts. A polynomial regression method to identify pressure to cross sectional area relationships will be used to determine outcomes, narrowest cross sectional area, and distensibility plateau. FLIP is FDA-approved without age restriction and its use has been approved by our IRBs previously. Primary results will be reported as distensibility plateau (mm).

EST and biopsy samples: The EST will be placed in a labeled tube and frozen until analysis as below. Four mucosal biopsies will be obtained from the distal esophagus for protein (3 biopsies) and histological (1 biopsy) analysis. Samples for protein analysis will be snap frozen for batch processing and assessment. Samples for histological analysis will be paraffin embedded and cut into 5μM sections for batch staining.

6.2 Lab Procedures

EST samples and corresponding biopsies will be shipped in 3 month intervals to Dr. Ackerman at the University of Illinois, Chicago. EST sample processing and analysis- EST samples will be thawed (previously harvested and frozen in extraction buffer with protease inhibitors) on ice and vortexed for 1 min at maximum vortex speed. Biomashers will be used to separate the string from the EST sample following manufacturer instructions to homogenize the sample, centrifuge the filter column (1min/13,400 rpm/+4°C) and collect the flow through. The EST eluates are aliquoted and stored at -80°C until used in biomarker assays. ELISAs for selected eosinophil biomarkers including MBP1 and CLC/Gal-10 (in house ELISAs at UIC), and commercial ELISAs for EPX and EDN will be performed as per manufacturer instructions (2). Measurement of non-eosinophil protein biomarkers including CCL24, CCL26, TSLP, Lox5, EMSY, IL-13, periostin and others will be done by commercial ELISAs and nanostring methods (2,16,17). Results will be reported as mass amount of protein/ml after adjusting for sample dilution factors.

1. Histopathological scores- Cut paraffin embedded samples will be assessed and scored for each of EoEHSS(4), EPX (5) and EMT (6) as previously described.
2. Data capture- PEESS survey results, FLIP results, histopathology and EREFs scores and molecular readouts will be loaded into the REDCAP study database after obtained.
3. Data Analysis- In order to address our aims, we will perform the following correlation analyses.
   a. EST protein analyte concentration vs. PEESS scores
   b. EST protein analyte concentration vs. EREFS scores
   c. EST protein analyte concentration vs. EoEHSS scores
   d. EST protein analyte concentration vs. EPX scores
   e. EST protein analyte concentration vs. EMT scores
   f. EST protein analyte concentration vs. nano-string biomarkers
   g. EST protein analyte concentration vs. EndoFLIP numerical values

Correlation of biomarkers from 1 hr ESTs with PEESS symptom score or histology scores. Analysis of variance or its non-parametric counterpart will be used to compare the differences in biomarkers between patients with different endoscopic appearance (e.g. Rings+/- Furrows + Stricture vs. Rings+/- Furrows vs. None of Rings, Furrows or Stricture). The semi partial correlation of biomarker level from string test with cross sectional area at 40 mmHg pressure, a metric of extensibility from FLIP test, will be examined using multiple linear regression (MLR) while adjusting for age of patient. MLR will also be used to examine the association of the outcomes with multiple biomarkers. No adjustment for comparisons of multiple outcomes will be applied in order to preserve some power for this study.

6.3 BioSpecimens Procedures

1. Purpose (if known)
   - Immunohistochemical analysis, bacterial DNA and RNA extraction and analysis, protein measurement, barrier function analysis or microbial culture and staining will be performed. Samples may undergo short-term culture in the lab prior to processing as above.
2. Handling of specimens
   - Samples will be collected from endoscopy suite unfixed into a tube with physiological saline, will undergo short-term cultures in the lab and then either snap frozen or formalin fixed, stored in either a -80 freezer in a locked room until analysis is complete or in a slide box behind a locked office in a secure building until analyzed.

- All samples will be coded with indirect identifiers and records will be kept in a notebook locked in a cabinet behind locked door or in a password protected computer stored in a locked room.
- Patient will be able to request destruction of sample.
- Samples will be destroyed if subject withdraws consent for further participation.
- The PI will have ownership of the specimens.
- Portions of the sample will be sent to University of Illinois, Chicago for laboratory analysis.

7 ASSESSMENT OF SAFETY

7.1 Specification of Safety Parameters

Due to the nature of the EST, expected adverse events in this study include gagging, vomiting or sore throat up to 24 hours following the procedure. Subjects who vomit the EST will be given the option to attempt the procedure again if they would like. We will contact subjects 24 -72 hours after the EST procedure to assess for any unanticipated events.

Safety monitoring for this study will focus on unanticipated problems involving risks to participants, including unanticipated problems that meet the definition of a serious adverse event.

7.1.1 Adverse Events

An Adverse Event is any untoward medical occurrence in a patient or clinical investigation subject administered an investigational product and which does not necessarily have a causal relationship with this treatment.

Immediate gagging and/or vomiting and sore throats 24 hours after the EST will be documented but these AEs are expected. All other adverse events will be documented and reviewed using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Study coordinators will contact subjects 2-3 days after the procedures to assess for AEs. The PI will determine causality as well as severity and all AEs will be reviewed on a quarterly basis by a DSMO.

7.1.2 Unanticipated Problems

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.1.3 Serious Adverse Events

For the purposes of this study, serious adverse events will be tracked if they meet the below criteria and are determined to be related to the research procedures.

A serious adverse event (SAE) is one that meets one or more of the following criteria:

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect

An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical study protocol, Good Clinical Practice, or Manual of Procedures requirements. The noncompliance may be on the part of the subject, the investigator, or study staff. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly.

These practices are consistent with investigator and sponsor obligations in ICH E6:

- Compliance with Protocol, Sections 4.5.1, 4.5.2, 4.5.3, and 4.5.4.
- Quality Assurance and Quality Control, Section 5.1.1
- Noncompliance, Sections 5.20.1 and 5.20.2.

All deviations from the protocol must be addressed in study subject source documents and promptly reported to the local IRB, according to their requirements.

8.1 Major Protocol Deviation (Protocol Violation)

A Protocol Violation is a deviation from the IRB approved protocol that may affect the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data. In addition, protocol violations include willful or knowing breaches of human subject protection regulations, or policies, any action that is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles, and a serious or continuing noncompliance with federal, state, local or institutional human subject protection regulations, policies, or procedures.

8.2 Non-Major Protocol Deviation

A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that does not have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

8.3 Reporting and Managing Protocol Deviations

The study site principal investigator has the responsibility to identify, document and report protocol deviations as directed by the study Sponsor. However, protocol deviations may also be identified during study conduct review.

Upon determination that a protocol deviation (major or minor) has occurred, the study staff will a) notify the site Principal Investigator, b) will complete a Protocol Deviation form. The Protocol Deviation form will document at a minimum the date PD occurred, the date PD identified, a description of event, whether the deviation resulted in SAE/AE, the signature of PI, report to local IRB, and documentation of a corrective action plan. The DSMO may request discussion with the PI to determine the effect of the protocol deviation on the study participant and his/her further study participation, the effect of the protocol deviation on the overall study, and corrective actions. The PI will complete and sign the Protocol Deviation form and submit it

to the local IRB, per IRB regulations. Major protocol deviations will be reported to the DSMO. All deviations, major or minor will be collated submitted to local IRBs at annual review periods.

9 ETHICS/PROTECTION OF HUMAN SUBJECTS

9.1 The Belmont Report

In accordance with the National Institutes of Health's federal wide assurance (FWA00005897): "This institution assures that all of its activities related to human subject research, regardless of funding source, will be guided by the ethical principles of The Belmont Report." Additionally, the investigator assures that all activities of this protocol will be guided by the ethical principles of The Belmont Report, 45 CFR 46 and all of its subparts (A, B, C and D).

9.2 Institutional Review Board

A copy of the protocol, informed consent forms, assent form, any advertising/recruitment materials and other information to be completed by participants, such as survey instruments or questionnaires, will be submitted to the local IRBs for written approval.

The investigator must submit and obtain approval from the IRB for all subsequent amendments to the protocol, informed consent documents and other study documentation referenced above. The investigator will be responsible for obtaining IRB approval of the annual Continuing Review throughout the duration of the study.

The investigator will notify the IRB of serious adverse events and protocol violations.

9.3 Informed Consent Process

Written informed consent will be obtained from each participant before any study-specific procedures or assessments are done and after the aims, methods, anticipated benefits, and potential hazards are explained. The research study will be explained in lay terms to each potential research participant. The participant's willingness to participate in the study will be documented in writing in a consent form, which will be signed by the participant with the date of that signature indicated. The investigator will keep the original consent forms and signed copies will be given to the participants. It will also be explained to the participants that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. Written and/or oral information about the study in a language understandable by the participant will be given to all participants.

Once the informed consent has been signed, the participant is considered enrolled in the study.

9.3.1 Assent or Informed Consent Process (in Case of a Minor)

All participants will receive a full oral explanation of the study, and informed consent will be obtained prior to participation. The consent form that is used for this study will be approved by a central IRB. Informed consent will be obtained by one of the investigators or his/her authorized delegate. All study staff receive training in the informed consent process. Prospective subjects are given time to read over the entire consent form in order to make their decision and are also given an opportunity to ask any questions concerning the study. They may request what they would like done with their material and clinical information when the study is completed, or if they should happen to withdraw from the study. They are then asked to check boxes indicating

the components of the study to which they will consent; for example, some individuals can participate in database entry but not consent for tissue procurement. If the child is over the age of assent (varies by institution), they are also asked to sign an assent form. A copy of the consent/assent form is given to the family so that they have the information regarding the study. Another copy is placed in their medical chart, and the third copy is kept in the research office.

9.3.2 Consent of Non-English Speaking Subjects

As per, 45 CFR 46.117 (b)(2), we anticipate that no more than 3 subjects of Non-English speaking subjects of the same language will be enrolled. If a 4th subject is to be enrolled then a translation of the entire consent form will be provided. We will provide oral presentation of informed consent in conjunction with a short form stating that the elements of informed consent have been presented orally, for studies that will include non-English speaking subjects but not purposely recruit them. The translated consent form will be done according to the conditions stated above. A translator fluent in the subject's language and English will read the consent summary to the subject or their representative. A written summary of what is presented orally will be completed. A witness to the oral presentation will be provided and the translator may serve in this role. The subject or their representative and the witness (translator) will sign the Short Form. The person obtaining consent as authorized under the protocol and the witness (translator) will sign the Consent Summary. The subject will be given copies of the Short Form document and the Consent Summary. We will answer the subject's questions and assess comprehension in the subjects' native language. The subject or his/her representative will walk away with: (1) the short form written consent in the subject's language; (2) the long standard consent in English; (3) a copy of the summary of the oral presentation.

9.4 Participant Confidentiality

All of the practices will adhere to institutional and national policies regarding the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

Following Health Insurance Portability and Accountability Act guidelines, a participant's privacy and confidentiality will be respected throughout the observational study. Each participant will be assigned a sequential identification number, and this number rather than a name will be used to collect, store, and report participant information. Data reported in medical journals or scientific meetings will be presented in aggregate for participants as a whole. No individual participant will be identified in any way.

Participant confidentiality will be strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biologic samples in addition to the clinical information relating to participating subjects.

Research samples will be labeled with code numbers so that direct identifiers will not be visible. All information concerning patient identification will be kept in protected storage areas, including password-protected computer files and locked files and/or offices. The Principal Investigator, primary researchers, and clinical research coordinator(s) will have access to the patient identifiers

Some samples that are collected during an endoscopy and/or colonoscopy for research purposes may be frozen and shipped to other hospitals, institutions, and testing companies for analysis. Data may also be shared. The data and/or samples will be coded with indirect identifiers per HIPAA and have no PHI associated with them. The data and/or samples will be

used in a collaborative relationship between institutions, or testing company receiving the data and/or samples. All of these samples will be shared under an MTA, or other applicable agreement

9.5 Registration on ClinicalTrials.gov

A description of this study will be available on http://www.ClinicalTrials.gov.

9.6 Future Use of Stored Specimens and Other Identifiable Data

All specimens will include a research study identifier but otherwise will be de-identified. Specimens remaining after study analysis will be stored for analysis in future related protocols. The use of these samples will be described in those protocols and requests will be routed through proper institutional and IRB channel for approval.

10 STATISTICAL CONSIDERATIONS

No previous data exists for estimating the potential magnitude of correlation of biomarkers from the 1-hour EST with other outcome variables for this power analysis. We want to have sufficient power to detect a significant correlation if the true underlying correlation is 0.45 or greater, corresponding a medium to large effect size by Cohen's operational cutoff. In this case, a sample of 40 is required to ensure 80% chance at 5% significance to detect significant correlation between a biomarker and an outcome variable.

Correlation of each biomarker level from 1 hour string test with PEESS symptom score, EREFS histopathological score or luminal diameter measured by EndoFlip at 50 mmHg pressure will be analyzed using Spearman correlation analysis. Wilcoxon rank sum test will be used to compare the difference in biomarker level between patients with different endoscopic appearance (e.g. Rings+/- Furrows + Stricture vs. Rings+/- Furrows vs. None of Rings, Furrows or Stricture). Multiple linear regression (MLR) will be used to examine the above relationship while adjusting for other covariates such as age and gender of patients. Log transformation will be used for biomarker in MLR.

10.1 Sample Size Considerations

Preliminary studies- With respect to evidence for completion of aims in this proposal, we feel that we have either published on our ability to both establish technical feasibility and scientific rationale as summarized below

10.1.1 Technical feasibility

a. Tolerability and performance of EST- (2)
b. Ability of EST to capture luminal mediators - (2)
c. Ability to perform EREFs- (20)
d. Ability to assess pathological inflammation in tissue sections- (5)
e. Tolerability and performance of EndoFLIP - (8)

10.1.2 *Scientific rationale*

a. Luminal measurements correlate with mucosal inflammation - (2)

b. EndoFLIP reflects differences in pediatric esophageal distensibility in EoE - (8)

11 DATA HANDLING AND RECORD KEEPING

Data will be entered into a REDCap database developed and maintained in the University of Colorado REDCap system. REDCap is a secure web application designed to support data capture for research studies, providing user-friendly web-based case report forms, real-time data entry validation (e.g. for data types and range checks), audit trails and a de-identified data export mechanism to common statistical package

All study coordinators and PIs will be required to complete the REDCap training platforms provided by the University of Colorado in order to obtain a username and password. Passwords should remain unique and confidential to the assigned team members.

11.1 Data Entry

Data will be collected either electronically (i.e. via tablet/computer) or on paper CRFs at each site and entered into online electronic case report forms maintained by the DMCC. Participants will be given the option to complete questionnaires at home. The first recording of any information captured for the registry will be considered the source document, which may be, but is not limited to, a medical record, a laboratory or clinical report, a paper CRF, or an eCRF.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Source data are all information, original records of clinical findings, observations, or other activities in a clinical research study necessary for the reconstruction and evaluation of the study. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aid or evaluation checklists, recorded data from automated instruments, audio recordings of data collection events, copies or transcriptions certified after verification as being accurate and complete, photographs or digital photo files, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the study. If CRFs are used as source documents, it should be stated in this section what data will be collected on CRFs and what data will be collected from other sources.

All source documents should be created and maintained according to Standard Operating Procedures of the individual sites. Source documents should be accurate, legible, contemporaneous, original and attributable and documentation for each subject should be maintained on site for the duration of the study.

Study staff will maintain appropriate medical and research records for this study, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. Study staff may be asked to provide de-identified documents to the PI at CHCO for verification of data points and regulatory agencies to examine (and when required by applicable law, to copy) research records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress and data validity.

13 QUALITY CONTROL AND QUALITY ASSURANCE

This section will address the plans for local quality assurance and quality control.

Quality Management is the overall process of establishing and ensuring the quality of processes, data, and documentation associated with clinical research activities. It encompasses both quality control (QC), and quality assurance (QA) activities.

Each site should have standard operating procedures (SOPs) and/or a quality management plan that describe:

- How data will be evaluated for compliance with the protocol and for accuracy in relation to source documents.
- The documents to be reviewed (e.g., CRFs, clinic notes, product accountability records, specimen tracking logs, questionnaires, audio or video recordings), who is responsible, and the frequency for reviews.
- Who will be responsible for addressing quality assurance issues (correcting procedures that are not in compliance with protocol) and quality control issues (correcting errors in data entry).
- Staff training methods and how such training will be tracked.
- Training on the EST procedure will be provided by staff from CHCO via webinar and video training. Sites should maintain records of the training for all staff administering the EST.

14 PUBLICATION/DATA SHARING POLICY

We will comply with all NIH guidelines of February 26, 2003 on data sharing as well as all subsequent policies and best practices that have evolved since this initial policy was released. Data sharing will be customized according to the type of data and setting in which it was collected.

Human Subjects Trials
Our projects that include human subjects have datasets stored in on-site secure databases. Before release of any information, the raw data is stripped of identifiers to uphold compliance with HIPAA and other governing agencies' guidelines; and is published as subsets of the original dataset, rather than allowing access to the original database. All databases housing any personal health information (PHI) are constructed with appropriate security to prevent unauthorized access; and have defined user roles that restrict access to PHI to those authorized by the University of Colorado School of Medicine's HIPAA Office for that project, and to the database administrator, a quality assurance auditor, and authorized go-betweens. When appropriate CCTSI Translational Informatics Core or the Children's Clinical Research Organization will be responsible for the coordination of these databases.

Laboratory-based Data
Our laboratory will maintain the associated data within our laboratory. Requests for data are sent to the Technology Transfer Office of the University of Colorado. They will negotiate an appropriate data sharing agreement with the requesting investigator and institution.

Data Generated by Shared Resources
Each Shared Resource has established policies that have been reviewed and approved by GEDP Leadership that define the length of time service-generated data is stored and the security provisions in place to prevent unauthorized access to the data. Data is not released to researchers other than the originating investigator without prior written approval from the originating investigator.
Biosamples: Access to biosamples and related data collected using federal funding is overseen by the PI and when appropriate by the GEDP Leadership team.

Operational Procedures
The Technology Transfer Office works closely with us to help protect our intellectual property. The office provides guidance on and formally negotiates data use agreements with requesting parties when appropriate.

15 LITERATURE REFERENCES

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PEESS

Pediatric Eosinophilic Esophagitis (EoE) Symptom Severity Module

Version 2.0

PARENT REPORT FOR CHILDREN AND TEENS (Ages 2 - 18)

DIRECTIONS

Tell us about your child's problems with EoE in the past *MONTH*.

There are no right or wrong answers. Please circle the best number.

Please answer the question in the Frequency section and then the related question in the Severity section.

Frequency				
Never	Almost never (less than once a week)	Sometimes (1 or more times a week)	Often (1 time a day)	Almost always (2 or more times a day)
How often does your child get sick?				
0	1	2	3	4

Severity				
Not bad at all	A little bad	Kind of bad	Bad	Very bad
How bad is it when your child gets sick?				
0	1	2	3	4

Office Use Only

Study ID: ☐☐☐☐☐☐☐☐☐ Subject ID: ☐☐☐☐☐ Date Completed: ☐☐ / ☐☐ / ☐☐☐☐ (Month / Day / Year)

Tell us about your child's problems with EoE in the past *MONTH*.

Frequency				
Never	Almost never (less than once a week)	Sometimes (1 or more times a week)	Often (1 time a day)	Almost always (2 or more times a day)
1. How often does your child have chest pain, ache, or hurt?				
0	1	2	3	4
3. How often does your child have heartburn (burning in the chest, mouth, or throat)?				
0	1	2	3	4
5. How often does your child have stomach aches or belly aches?				
0	1	2	3	4
7. How often does your child have trouble swallowing?				
0	1	2	3	4
9. How often does your child feel like food gets stuck in his/her throat or chest?				
0	1	2	3	4
11. How often does your child need to drink a lot to help swallow food?				
0	1	2	3	4
13. How often does your child vomit (throw up)?				
0	1	2	3	4
15. How often does your child feel nauseous (feel like throwing up, but doesn't)?				
0	1	2	3	4

Severity				
Not bad at all	A little bad	Kind of bad	Bad	Very bad
2. How bad is your child's chest pain, ache, or hurt?				
0	1	2	3	4
4. How bad is your child's heartburn (burning in the chest, mouth, or throat)?				
0	1	2	3	4
6. How bad are your child's stomach aches or belly aches?				
0	1	2	3	4
8. How bad is your child's trouble swallowing?				
0	1	2	3	4
10. How bad is it when your child gets food stuck in his/her throat or chest?				
0	1	2	3	4
12. How bad is it when your child needs to drink a lot to help swallow food?				
0	1	2	3	4
14. How bad is your child's vomiting (throwing up)?				
0	1	2	3	4
16. How bad is your child's nausea (feeling like throwing up, but doesn't)?				
0	1	2	3	4

Please turn to the next page for the rest of the questions. Thank you! Next page

Tell us about your child's problems with EoE in the past ***MONTH***.

Frequency				
Never	Almost never (less than once a week)	Sometimes (1 or more times a week)	Often (1 time a day)	Almost always (2 or more times a day)

Severity				
Not bad at all	A little bad	Kind of bad	Bad	Very bad
				

17. How often does your child have food come back up in his/her throat when eating?

0	1	2	3	4

18. How bad is it when food comes back up in your child's throat?

0	1	2	3	4

19. How often does your child eat less than others?

0	1	2	3	4

20. How often does your child need more time to eat than others?

0	1	2	3	4

Thank you very much for participating!

PEESS

Pediatric Eosinophilic Esophagitis (EoE) Symptom Severity Module

Version 2.0

CHILDREN AND TEENS REPORT (Ages 8 - 18)

DIRECTIONS

Tell us about your problems with EoE in the past *MONTH*.

There are no right or wrong answers. Please circle the best number.

Please answer the question in the Frequency section and then the related question in the Severity section.

Frequency				
Never	Almost never (less than once a week)	Sometimes (1 or more times a week)	Often (1 time a day)	Almost always (2 or more times a day)
How often do you get sick?				
0	1	2	3	4

Severity				
Not bad at all	A little bad	Kind of bad	Bad	Very bad
How bad is it when you get sick?				
0	1	2	3	4

Office Use Only

Study ID: ☐☐☐☐☐☐☐☐☐☐ **Subject ID:** ☐☐☐☐☐ **Date Completed:** ☐☐ / ☐☐ / ☐☐☐☐ (Month / Day / Year)

Tell us about your problems with EoE in the past *MONTH*.

	Frequency					Severity				
	Never	Almost never (less than once a week)	Sometimes (1 or more times a week)	Often (1 time a day)	Almost always (2 or more times a day)	Not bad at all	A little bad	Kind of bad	Bad	Very bad
1.	How often do you have chest pain, ache, or hurt?	0	1	2	3	4				
2.	How bad is the chest pain, ache, or hurt?						0	1	2	3	4
3.	How often do you have heartburn (burning in your chest, mouth, or throat)?	0	1	2	3	4				
4.	How bad is your heartburn (burning in your chest, mouth, or throat)?						0	1	2	3	4
5.	How often do you have stomach aches or belly aches?	0	1	2	3	4				
6.	How bad are the stomach aches or belly aches?						0	1	2	3	4
7.	How often do you have trouble swallowing?	0	1	2	3	4				
8.	How bad is the trouble swallowing?						0	1	2	3	4
9.	How often do you feel like food gets stuck in your throat or chest?	0	1	2	3	4				
10.	How bad is it when food gets stuck in your throat or chest?						0	1	2	3	4
11.	How often do you need to drink a lot to help swallow your food?	0	1	2	3	4				
12.	How bad is it if you don't drink a lot to help swallow your food?						0	1	2	3	4
13.	How often do you vomit (throw up)?	0	1	2	3	4				
14.	How bad is the vomiting (throwing up)?						0	1	2	3	4
15.	How often do you feel nauseous (feel like you're going to throw up, but don't)?	0	1	2	3	4				
16.	How bad is the nausea (feeling like you're going to throw up, but don't)?						0	1	2	3	4

Please turn to the next page for the rest of the questions. Thank you! **Next page**

Tell us about your problems with EoE in the past *MONTH*.

Frequency				
Never	Almost never (less than once a week)	Sometimes (1 or more times a week)	Often (1 time a day)	Almost always (2 or more times a day)

Severity				
Not bad at all	A little bad	Kind of bad	Bad	Very bad
				

17. How often does food come back up your throat when eating?

0	1	2	3	4

18. How bad is the food coming back up your throat when eating?

0	1	2	3	4

19. How often do you eat less food than others?

0	1	2	3	4

20. How often do you need more time to eat than others?

0	1	2	3	4

Thank you very much for participating!

APPENDIX 2: EREFS

Protocol Number: _____ Participant ID: _____
Site: _____ Date of Visit: _____
Interviewer User ID: _____

Endoscope Type:
(Please select one)

Olympus ○ Pentax ○ Fujinon ○

Endoscope Size:

Adult ○ Pediatric ○

Feature/Location:
(Please select a grade for each feature/location)

Feature	Location			
	Proximal/Mid Esophagus (> 5cm above the squamocolumnar junction)		Distal Esophagus (within 5 cm above the squamocolumnar junction)	
Edema (mucosal "pallor", decreased clarity or absence of vascular markings)	○	Grade 0: absent	○	Grade 0: absent
	○	Grade 1: present	○	Grade 1: present
	○	Cannot Assess	○	Cannot Assess
Rings (Fixed esophageal rings, "Trachealization", Corrugations)	○	Grade 0: none (normal)	○	Grade 0: none (normal)
	○	Grade 1: mild (subtle circumferential ridges)	○	Grade 1: mild (subtle circumferential ridges)
	○	Grade 2: moderate (distinct rings that do not impair passage of a standard diagnostic adult upper endoscope (diameter 9-10 mm))	○	Grade 2: moderate (distinct rings that do not impair passage of a standard diagnostic adult upper endoscope (diameter 9-10 mm))
	○	Grade 3: severe (distinct rings that do not permit passage of a diagnostic adult upper endoscope (diameter 9-10 mm))	○	Grade 3: severe (distinct rings that do not permit passage of a diagnostic adult upper endoscope (diameter 9-10 mm))
	○	Cannot Assess	○	Cannot Assess
Exudates (also referred to as white spots, plaques)	○	Grade 0: none	○	Grade 0: none
	○	Grade 1: mild (lesions involving <10% of the esophageal surface area)	○	Grade 1: mild (lesions involving <10% of the esophageal surface area)

Protocol Number: _____ Participant ID: _____
Site: _____ Date of Visit: _____
Interviewer User ID: _____

	○	Grade 2: severe (lesions involving >10% of the esophageal surface area)	○	Grade 2: severe (lesions involving >10% of the esophageal surface area)
	○	Cannot Assess	○	Cannot Assess
Furrows (also referred to as vertical lines, longitudinal furrows)	○	Grade 0: absent	○	Grade 0: absent
	○	Grade 1: mild	○	Grade 1: mild
	○	Grade 2: severe (vertical lines with mucosal depth (indentation))	○	Grade 2: severe (vertical lines with mucosal depth (indentation))
	○	Cannot Assess	○	Cannot Assess
Stricture	○	Grade 0: absent	○	Grade 0: absent
	○	Grade 1: present	○	Grade 1: present
	○	Cannot Assess	○	Cannot Assess
	____ ____	Diameter (mm) [0-25] Length (cm) [0-25]	____ ____	Diameter (mm) Length (cm)

Miscellaneous Features

Crepe Paper Esophagus (mucosal tearing or laceration upon passage of endoscope)	○	Grade 0: None
	○	Grade 1: Present
	○	Cannot Assess
Narrow Caliber Esophagus (reduced luminal diameter involving greater than 50% of the esophageal length)	○	Grade 0: None
	○	Grade 1: Present
	○	Cannot Assess
Esophageal erosions (Los Angeles Classification)	○	No erosions (mucosal breaks)
	○	A: One (or more) mucosal break no longer than 5 mm, that does not extend between the tops of two mucosal folds
	○	B: One (or more) mucosal break more than 5 mm long,

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	that does not extend between the tops of two mucosal folds
○	C: One (or more) mucosal break that is continuous between the tops of two or more mucosal folds, but which involves less than 75% of the circumference
○	D: One (or more) mucosal break which involves at least 75% of the esophageal circumference
○	Cannot Assess