Cytosponge Protocol

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Title **Cytosponge Protocol**

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Eosinophilic esophagitis is a relatively new disease in which esophageal eosinophilia leading to inflammation and stricture formation results from exposure to food antigens. Although it would seem logical that identification and removal of food antigens would be the definitive treatment, this is more easily said than done.

There are several reasons for this:

- Current available allergen testing, mostly through blood and skin, do not reliably reflect specific allergen sensitivity in the esophagus.

- As there has not been a non-invasive test to determine the esophageal response to single food allergens, clinicians have had to choose between allergen avoidance therapy because it is too cumbersome or performing serial and at times numerous endoscopies and biopsies to fully evaluate a range of potential foods that may trigger the inflammatory response.

- Treatment of eosinophilic esophagitis is also further hampered by a distinct lack of symptoms in adults due to what is typically a silent inflammatory response until stricture formation and subsequent dysphagia and impaction occur.

- Furthermore, adult patients with this disease typically accommodate by careful chewing and slow eating such that an absence of symptoms commonly underestimates the severity

of disease. As a result, an invasive test that reliably determines esophageal eosinophilic would be extremely valuable for monitoring disease activity and the response to food allergen withdrawal or introduction.

Cytosponge is an ingestible gelatin capsule containing a compressed mesh attached to a string. The capsule is swallowed and once in the stomach, the gelatin dissolves and a spherical mesh of 3 cm diameter is released. The mesh is withdrawn through the mouth by the attached string and a cytologic specimen is collected.

Current cytosponge from University of Cambridge

Covidien Cytosponge

In a recent study evaluating the Cytosponge in patients with Barrett's esophagus, excellent results were achieved with a the sensitivity and specificity of the test was 73.3% (95% confidence interval 44.9% to 92.2%) and 93.8% (91.3% to 95.8%) for 1 cm or more circumferential length and 90.0% (55.5% to 99.7%) and 93.5% (90.9% to 95.5%) for clinically relevant segments of 2 cm or more.

There was also overall excellent tolerance by patients using the Cytosponge. As a result, one might wonder if the Cytosponge would similarly be a reliable relatively non-invasive tool for evaluating patients with eosinophilic esophagitis.

Specific Aim: To evaluate the sensitivity and specificity of Cytosponge when compared to upper endoscopy and biopsies/swab testing in patients undergoing clinically indicated endoscopy for evaluation of eosinophilic esophagitis and healthy controls

Methods:

- Patients with eosinophilic esophagitis and health controls, (patients without esophageal symptoms such as GERD or dysphagia), will be enrolled.
- Patients with EoE will be having a clinically indicated upper endoscopy and biopsies either for initial diagnosis or for monitoring the activity of their disease.
- All patients will be asked to be fast, no solid food 8 hours prior to swallowing the cytosponge, can drink clear liquids up to 2 hours prior to swallowing the cytosponge
- The EoE subjects two hours prior to endoscopy will be asked to swallow the Cytosponge. Briefly, the capsule and bunched up string are swallowed with water. Two hours is chosen as the time interval because water will likely be needed to swallow the capsule and this is the standard time allowed for to last safely consume a clear liquid prior to endoscopy.
- Controls will be will have the same fasting instructions but will not be required to be having and endoscopy.
- The string is held without any tension to allow the capsule to move into the stomach. The patient holds onto the string for five minutes after ingestion to allow the capsule to dissolve in the proximal stomach, where a spherical mesh of 3 cm diameter is released.
- The back of the throat is then sprayed with 1% lidocaine and the expanded mesh withdrawn by pulling on the string with the patient's head in an extended position.
- After retrieval the string is cut and the Cytosponge containing the cytological specimen placed in preservative fluid and kept at room temperature until transportation to the laboratory.
- The whole process of administering the Cytosponge, including instructing the patient, takes less than 10 minutes.
- Following this, the clinically indicated endoscopy will be performed. The cytology specimens will be reviewed by one pathologist who will read them blindly without knowledge of the results of the biopsy specimens. The cytology specimens will be

compared to the biopsy in terms of esophageal eosinophil presence and density. A scoring system for cytology will be devised.

- Samples from the University of North Carolina will be sent to the Mayo Clinic to be reviewed by the same pathologist.
- After the Mayo pathologist have completed their analysis, slides and part of the uncut blocks will be sent to Dr. Rebecca Fitzgerald's lab at the University of Cambridge looking at reproducibility of results, evaluation of the mucosal microbiome by sequencing and additional biomarker studies including RNA, methylation and protein analyses.
- We will send samples from a cell block made from the cytology fluid from the cytosponge and a portion of the primary cytosponge placed in RNAlater solutionFor extra-cellular RNA signature analysis with possible detection of EoE specific lumen protein products proposed to Dr. Rodenberg's lab at the University of Cincinnati.
- FFPE RNA form the concurrent biopsies at the time of the EGD if available as biopsy tissue represents the source of the dislodged cells/tissue pieces by the cytosponge. Parallel comparison will provide key clue to validate the new diagnostic approach.
- We will stain sections from esophageal biopsies and Cytosponge for the eosinophil secondary granule protein eosinophil peroxidase (EPX). This may increase the diagnostic sensitivity of the Cytosponge because EPX staining accounts for eosinophil degranulation products in addition to intact eosinophils. H+E stains and their matching EPX stains will be digitized using an Aperio slide scanner and Aperio ImageScope software will be used for quantification of EPX.
- Modification 11/24/2021 – we would also like to utilize slides from IRB15-004741 on patients that had a cytosponge and biopsy. These will undergo the EPX staining as described above. Possible samples used will be cross checked in Ptrax to ensure the subjects consented to allowing their samples to be utilized in future research and only the applicable samples will be used. Total Mayo participants to use from this study will be up to 30.

Recruitment: We will recruit 130 participants at Mayo there will be 180 total subjects in this trial

Inclusion Criteria:

- Adult patients between the ages of 18 and 65 with eosinophilic esophagitis or health controls including lactating or pregnant women undergoing clinically indicated endoscopy and esophageal biopsy.

Exclusion Criteria:

- Patients for whom clinically indicated endoscopy is not safe. Patients who have small caliber esophagus, an esophageal stricture that will not permit passage of the gelatin capsule or are unable to swallow the capsule. Patients who have taken any antithrombotic medication within 10 days of the procedure.

- Vulnerable populations, such as those with diminished mental acuity, will be excluded.

Patients with known Lidacaine/Acetylcysteine allergies

Study flow:

- The study coordinator will look for patients being seen in Esophageal clinic with the indication of EoE or healthy controls (patients without esophageal symptoms such as GERD or dysphagia)
- When the physician has an EoE patient scheduled for a clinically indicated upper endoscopy, to diagnose or monitor their disease, these patients will be approach by the study coordinator to see if they are interested in the cytosponge study?
- Subjects willing to swallow the cytosponge that do not have esophageal symtoms will be approached by the study coordinator.
- Patients with EoE will then be scheduled to come to Gonda 9s two hours prior to the endoscopy. Healthy controls will be scheduled to come to Gonda 9 at their convenience as long as they adhere to the fasting instructions.
- The physician will have the patient swallow the Cytosponge.
- The patient holds onto the string for five minutes after ingestion to allow the capsule to dissolve in the proximal stomach, where a spherical mesh of 3 cm diameter is released.
- The back of the throat is then sprayed with 1% lidocaine and the expanded mesh withdrawn by pulling on the string with the patient's head in an extended position.
- After retrieval the string is cut and the Cytosponge containing the cytological specimen placed in preservative fluid and kept at room temperature until transportation to the laboratory.
- The whole process of will take appoximately 10 minutes.
- A throat swab for collection will also be performed at this time to be processed for bacterial population of the esophageal mucosa.
- Following this, the clinically indicated endoscopy will be performed. Prior to biopsies we will do a brushing of the esophagus.
- The cytology specimens will be reviewed by one Mayo pathologist who will read them blindly without knowledge of the results of the biopsy specimens.
- The cytology specimens will be compared to the biopsy in terms of esophageal eosinophil presence and density. A scoring system for cytology will be devised.
- We will do further EoE/allergy analysis with FFPE slides including EDN and tryptase staining.
- When the Mayo pathologist have completed their analysis, slides and part of the uncut blocks will be sent to Dr. Rebecca Fitzgerald's lab looking at reproducibility of results, evaluation of the mucosal microbiome by sequencing and additional biomarker studies including RNA, methylation and protein analyses.
- We will send samples from a cell block made from the cytology fluid from the cytosponge and a portion of the primary cytosponge placed in RNAlater solution. For extra-cellular RNA signature analysis with possible detection of EoE specific lumen protein products proposed to Dr. Rodenberg's lab at the University of Cincinnati.
- FFPE RNA form the concurrent biopsies at the time of the EGD if available

as biopsy tissue represents the source of the dislodged cells/tissue pieces by the cytosponge. Parallel comparison will provide key clue to validate the new diagnostic approach.

- H+E sections will be digitized. Cytosponge and esophageal biopsy sections will be stained for EPX and analyzed.

Data Collection:

Several types of data will be collected. The first includes demographic data on patients including age, sex, allergy history, history of EoE. Routine pathology data will be collected based on biopsy findings. Cytology specimens will be analyzed both at Mayo Clinic, Rochester and Dr. Rebecca Fitzgerald's lab at the University of Cambridge, looking at reproducibility.

Two center site for conduction of the Cytosponge and eosinophilic esophagitis trial, Dr. Evan Dellon at the University of North Carolina and I have agreed that we will pool our data for this study. This is thought best as we will use the same methodology and we will each collect mutually needed data to perform the study. We will not be using the exact same data collection forms, however, as both Mayo and UNC have their established data bases for eosinophilic esophagitis where there are subtle differences in some of the types of data collected. On the other hand, the data that is needed for this two center study will be collected in an identical fashion.

When the Cytosponge is taken out of the patients' mouth, washed with sterile PBS, followed by centrifugation. The supernatant will be stored at -80oC for extra-cellular RNA signature analysis with possible detection of EoE specific lumen protein products proposed. The pellet will also be subjected to total RNA extraction immediately after the supernatant aspiration. The resulting RNA will be used for microRNA array and for a qPCR based array on representative EoE mRNA signature transcripts. Both methods are mature approaches regularly performed in the Rothenberg laboratory[1-3] and have been validated with multiple cohorts, forming the foundation for diagnostic purposes.

We will extract RNA from the remaining of FFPE sponge, aiming to acquire the RNA from the residue tissue after histological examination. The RNA isolated this way will be subjected to the same experiments as the fresh wash pellet cells.

In order to validate the proposed new method, we also aim to acquire FFPE RNA from the concurrent biopsy taken by EGD if available, as biopsy tissue represents the source of the dislodged cells/tissue pieces by the cytosponge. Parallel comparison will provide key clue to validate the new diagnostic approach.

We will make parallel comparisons of EPX staining from the Cytosponge and esophageal biopsy specimens. We will also assess correlations between EPX stains and peak eosinophil counts from the corresponding H+E stain.

Data Handling

A study coordinator will be responsible for collating and handling the above data into a computer accessible only though password entry by the coordinator and principal investigator of this study.

Data Analysis

A scoring system will be devised for cytology scoring of eosinophil density. EPX will be quantified by a pixel count algorithm using Aperio ImageScope software and a previously validated scoring system.

Feasibility and Time

It is expected to take six months to complete this study. For recruitment of patients, the Mayo Clinic has a long track record in studying patients with EoE and has over four hundred patients with this disease currently in their data base.

Strengths

The strengths of this study are the relatively small group of patients required, the non-invasive and well tolerated nature of this test relative to endoscopy and biopsy and the potential efficacy of this test. Other strengths include the expertise of the investigators in eosinophilic esophagitis and the collaboration with Dr. Rebecca Fitzgerald, the inventor of the cytosponge, whose team has great familiarity with the administration and interpretation of the test and its results.

Weaknesses

The main weaknesses of this study will be the potential inability of a patient to swallow the capsule or risk of impacting (though the capsule will dissolve and it will be easily removable).

References:

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