A PHASE II, RANDOMIZED, PLACEBO-CONTROLLED STUDY EVALUATING THE EFFICACY OF ANTIHISTAMINES IN THE TREATENT OF EOSINOPHILIC ESOPHAGITIS (THE ATEE STUDY)

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Study Product: *Famotidine and loratidine*

Protocol Number: (IRBe) 19-005510

Initial version: [11/22/19] Version **(1.2)**

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List of Abbreviations

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IND	Investigational New Drug Application
IRB	Institutional Review Board
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
EoE	Eosinophilic esophagitis
EGD	Esophagogastroduodenoscopy
DSQ	Dysphagia Symptom Questionnaire
EREFS	EoE endoscopic Reference Score

Study Summary

Title	A phase II, randomized, placebo-controlled study evaluating the efficacy of antihistamines in the treatment of eosinophilic esophagitis (the ATEE study).
Running Title	Antihistamines in eosinophilic esophagitis
Protocol Number	19-005510
Phase	phase II
Methodology	Prospective, randomized, placebo-controlled trial
Overall Study Duration	Two years
Subject Participation Duration	14 weeks
Single or Multi-Site	Single
Objectives	To assess the efficacy of antihistamines in the treatment of eosinophilic esophagitis. Primary outcomes are the number of eosinophils on histology and safety. Secondary outcomes are changes in dysphagia symptom questionnaire score, EoE endoscopic reference score, and the percentage of patients with histologic response of ≤15 eos/hpf on biopsy.
Number of Subjects	The planned sample size is 20 patients in each arm, for a total of 40 study patients. Assuming a dropout rate of 20%, the goal is to enroll 50 patients.
Diagnosis and Main Inclusion Criteria	Consentable, nonpregnant adults with a diagnosis of eosinophilic esophagitis
Study Product, Dose, Route, Regimen	Famotidine 40 mg tab twice daily by mouth and loratadine 10 mg tab once daily by mouth for 12 week duration.
Duration of Administration	12 weeks
Reference therapy	Placebo
Statistical Methodology	Patients will be randomized in a 1:1 fashion by a computer generated program. The changes in peak counts and questionnaire scores in the treatment arm from baseline to 12 weeks will be compared using a paired t-test or signed rank test. Categorical demographic variables and percentage of patients with histologic response of ≤15 eos/hpf at 12 weeks will be compared between the two arms using Chi-squared test or Fisher's exact test.

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures.

1.1 Background

Eosinophilic esophagitis (EoE) is a clinicopathologic condition generally occurring in children and young adults characterized by symptoms of esophageal dysfunction and histologically by ≥ 15 eosinophils per high power field. It is hypothesized to be an immune and antigen-driven effect, which is limited to the esophagus. [1] First characterized less than 30 years ago, it is the most prevalent cause of esophageal symptoms among children and young adults. [2] The most prominent symptom in adults is the feeling of food sticking in the chest after swallowing, and EoE is the most common cause of food bolus obstruction.[1] Prominent symptoms of dysphagia are secondary to inflammation leading to progressive esophageal fibrosis, strictures, and narrowing.[3]

Guidelines on the diagnosis and management of EoE were first published in 2007, and updated in 2011. [4] These guidelines required clinical and/or histologic unresponsiveness to a PPI trial for the diagnosis of EoE, with the elimination of other causes of esophageal eosinophilia.[2] EoE and GERD were felt to be mutually exclusive. [4] A new condition was defined in guidelines published in 2011, 2013, and 2014 describing PPI-responsive esophageal eosinophilia (PPI-REE). [4] These patients had evidence of esophageal dysfunction and lacked the typical features of GERD, however responded to treatment with a high dose PPI trial. More recently, consensus guidelines have advocated for the removal of a PPI trial in the diagnosis of EoE. [5] Recent studies have shown that the clinical, histologic, and endoscopic features of EoE and PPI-REE are indistinguishable, and that GERD and EoE may share a complex relationship and can occur together.[4]

To date, the optimal therapy for EoE is undetermined, and limited by a lack of approved medications. [4] Treatment options include PPIs, corticosteroids, and diet modifications. Currently, PPIs are the first line treatment option. In a systematic review and meta-analysis, PPI therapy was found to have a clinical response in 60.8% of patients and histologic remission in 50.5%. [2] Proton pump inhibitors have two hypothesized mechanisms of action. First, they reduce the acidity of stomach fluids, and may reduce symptoms when reflux occurs. However, the role of reflux in the pathogenesis of EoE is unclear. Secondly, PPIs may also reduce inflammation in the esophageal epithelium by direct effects on the eotaxin-3-eosinophil pathway, reducing the eosinophil response. [6] The response to PPI therapy is higher in patients with documented GERD (70%) versus those with negative esophageal pH monitoring (29%).[2]

In patients who do not respond to a PPI, corticosteroids are the next preferred treatment. Topical corticosteroids have established efficacy in EoE, however their use remains off-label which limits insurance coverage. [7, 8] Additionally, there are no formulations specifically for esophageal delivery, and asthma preparations such as fluticasone inhalers or aqueous budesonide are used. For inhalers, the medication is swallowed rather than inhaled to coat the esophagus and

provide an anti-inflammatory effect. [9] The dosing and delivery method may be confusing for patients when compared to the instructions on the package insert. [5] Side effects of corticosteroids include oral and esophageal candidiasis. [5] Other long-term side effects include bone thinning, weight gain, and diabetes. Although studies have evaluated the ability of corticosteroids to induce remission, EoE is a chronic disease and further studies are needed to assess long term management. Symptoms of EoE generally relapse within several weeks of discontinuation of topic corticosteroid treatment. [10] Other medications under evaluation include prostaglandin receptor antagonists, immunomodulators such as azathioprine and 6-mercaptopurine, and biologic agents. [10]

Treatment also includes diet modification. The "six food elimination diet" is the most common dietary approach, with elimination of dairy, wheat, eggs, soy, nuts, seafood, and shellfish. [1] If symptoms improve, repeat endoscopy is performed after 12 weeks to document resolution of inflammation. This strategy is resource and cost intensive.[1] With the use of the six food elimination diet, triggers are identified, however long-term adherence to a specific diet is difficult. [5]

Allergists are well-established in the understanding and pathogenesis of EoE. They play a role in the management of atopic comorbidities, including asthma, allergic rhinitis, atopic dermatitis, and food allergies. Although the pathophysiology of EoE suggests an immune, antigen-mediated reaction, no study has evaluated the efficacy of antihistamines for treatment. Histamine is well characterized in the acute inflammatory and allergic response, and histamine receptors are present on eosinophils, T cells, monocytes, and dendritic cells. All are capable of producing and secreting histamine.[11] The histamine 1 and 2 receptors (H1R and H2R) are expressed not only on these immune cells, but ubiquitously throughout the gut wall. [12] In the gastrointestinal tract, histamine has three major functions; the enhancement of gastric acid production, modulation of gastrointestinal motility, and alteration of mucosal ion secretion. [12] There is a strong hereditary pattern of EoE with multiple implicated genes, which are more closely related to those involved in allergen sensitization and squamous epithelial cell dysfunction.[13] Based on the evidence that the allergic response plays an important role in the pathogenesis of EoE, anti-histamines may provide treatment benefit.

The purpose of this study is to evaluate the efficacy of anti-histamines in the treatment of EoE.

1.2 Investigational Agent

The investigational agents are famotidine 40 mg tablet twice daily by mouth and loratadine 10 mg tablet once daily by mouth for 12 week duration. Famotidine is an H2-receptor antagonist that inhibits the action of histamine at the histamine H2-receptors. Famotidine is FDA approved for the treatment of duodenal ulcer disease, erosive esophagitis, gastric hypersecretion, gastric ulcer, gastroesophageal reflux disease, and indigestion. Loratadine is a long-acting tricyclic antihistamine, which selectively blocks histamine H1 receptor activity. FDA approved uses include idiopathic urticaria and seasonal allergic rhinitis.

1.3 Clinical Data to Date

Although there is published data supporting the use of famotidine in peptic ulcer disease and gastroesophageal reflux disease, there currently is not study data for its use in EoE. Loratadine has published data for the treatment of urticaria and seasonal allergies, however has not been used in the treatment of EoE.

1.4 Dose Rationale

Loratadine 10 mg tablet daily is the standard dosage for the treatment of seasonal allergic rhinitis and idiopathic urticara. As EoE is an immune and allergen-mediated disease, we hypothesize the standard dosage will be effective in EoE. Similarly, famotidine 40 mg tablet twice daily is the standard dose for the treatment of peptic ulcers and gastroesophageal reflux disease. As stated in the background, gastroesophageal reflux may play a role in the pathogenesis of EoE. We hypothesize the standard dose of famotidine will be effective in EoE.

1.5 Risks and Benefits

Both famotidine and loratadine are over-the-counter medications, which are available to the general population without a prescription. Risks associated with these medications include their known common side effects, such as headache, dizziness, constipation, diarrhea, dry mouth, fatigue, somnolence, esophagitis, abdominal pain, constipation, and diarrhea. Other rare but serious side effects include rash (including but not limited to Stevens-Johnson syndrome and toxic epidermal necrolysis), arrhythmia, pancreatitis, bone marrow suppression (including but not limited to agranulocytosis, aplastic anemia, pancytopenia, thrombocytopenia), liver injury/failure, rhabdomyolysis, seizure, anaphylaxis, angioedema, and pneumonia. The risks are reasonable in relation to the anticipated benefits of a therapeutic response as these medications are generally well tolerated, over-the-counter, and used at the same dosing in other allergic and gastrointestinal disorders. The risks will be stated in the consent form.

2 Study Objectives

Primary Objective

To assess the efficacy of H1 and H2 antihistamines in the treatment of EoE through a decrease in the esophageal eosinophil count on biopsy. To assess the safety and tolerability of H1 and H2 antihistamines in subjects with EoE.

Secondary Objective

To determine whether loratadine and famotidine are effective for improving symptoms of EoE as measured by the Dysphagia Symptom Questionnaire (DSQ). To assess the percentage of patients with histologic response of ≤15 eos/hpf on biopsy. To assess the percentage of patients with endoscopic response as measured by the EoE Endoscopic Reference Score (EREFS).

3 Study Design

3.1 General Description

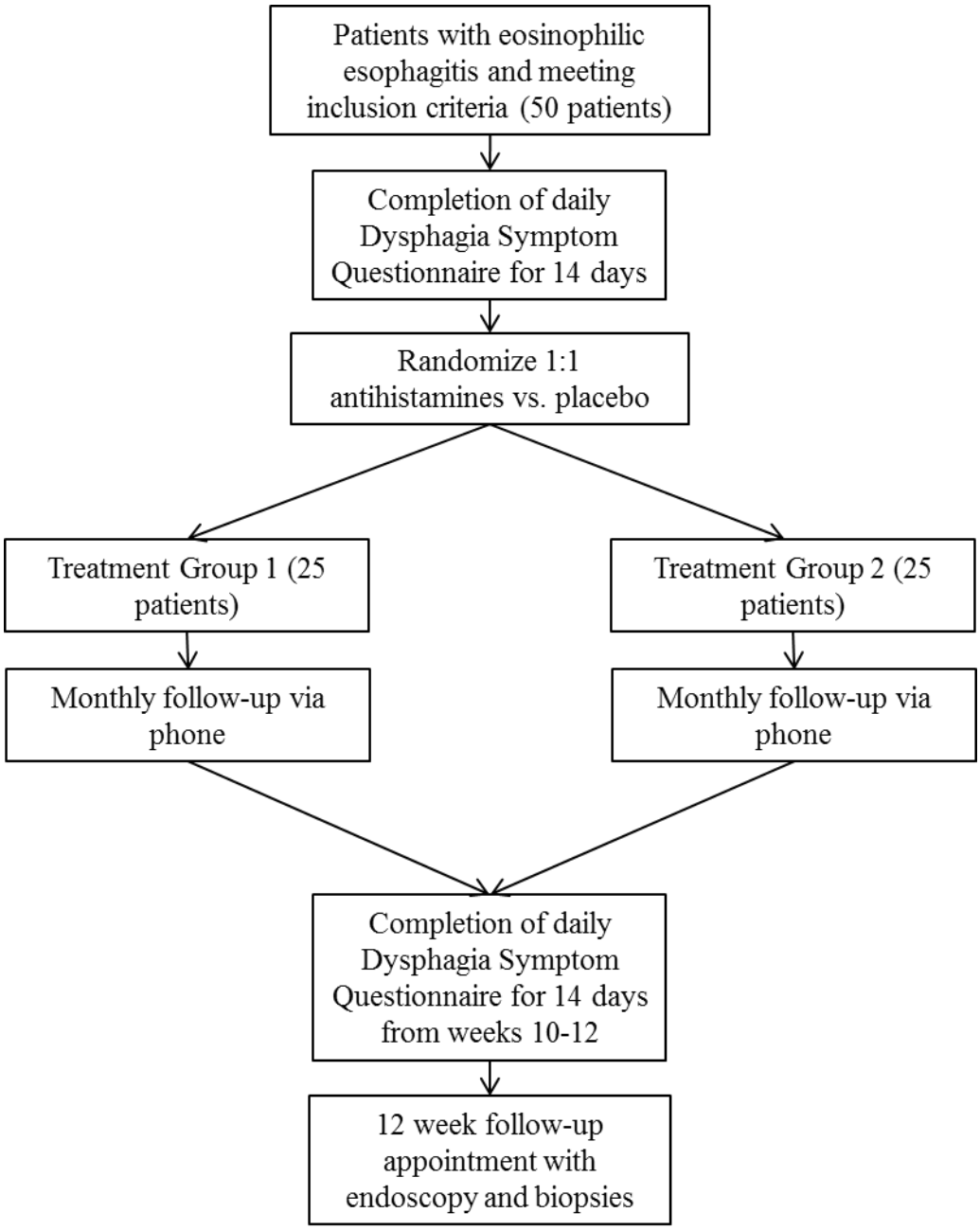
This is a randomized, placebo-controlled phase II trial of the safety and efficacy of loratadine and famotidine in the treatment of EoE. Response to treatment will be assessed by questionnaires and endoscopy with histopathology. The expected duration of subject participation is 14 weeks. Subjects will be screened at outpatient clinic visit appointments and interested qualified subjects will be consented and offered participation in this trial. Once consent has been obtained, subjects complete the Dysphagia Symptom Questionnaire daily for two weeks. At week two, subjects will begin treatment and follow-up for the next 12 weeks. The study will be preceded by a washout period of at least 6 weeks followed by daily completion of DSQ for two weeks and active or placebo administration for 12 weeks. Participants will have monthly phone visits to monitor for adverse events and monitor compliance while on treatment. A final office visit for evaluation and repeat endoscopy will be conducted at the end of the study. During the final two weeks of treatment, subjects will again complete the DSQ daily. All data will be entered in a prospective automated institutional database (Redcap).

3.2 Number of Subjects

The planned sample size is 20 patients in each arm, for a total of 40 study patients. Assuming a dropout rate of 20%, the goal is to enroll 50 patients. The estimated baseline mean eosinophil count is expected to be between 80-110 eos/hpf, with an estimated standard deviation of change from baseline in eosinophil counts of 30-50 eos/hpf. Using a conservative standard deviation assumption of 50 eos/hpf, a sample size of 25 patients in each arm provides 80% power to detect a significant between group difference of 40%. Using a conservative standard deviation assumption of 50 eos/hpf, a sample size of 16 patients in each arm provides 80% power to detect a significant between group difference of 50%.

3.3 Duration of Participation

Each subject will participate for 12 weeks randomized to the study drugs or placebo.



3.4 Primary Study Endpoints

To determine whether loratadine and famotidine are effective for improving maximum eosinophil counts (eos/hpf) after 12 weeks of therapy with antihistamines in patients with EoE.
To assess the safety and tolerability of H1 and H2 antihistamines in subjects with EoE.

3.5 Secondary Study Endpoints

To determine whether loratadine and famotidine are effective for improving symptoms of EoE as measured by the validated Dysphagia Symptom Questionnaire after 12 weeks of therapy with antihistamines.

To determine whether loratadine and famotidine are effective for improving the percentage of patients with histologic response of ≤15 eos/hpf after 12 weeks of therapy with antihistamines.

To determine whether loratadine and famotidine are effective for improving the endoscopic manifestations of EoE as measured by the validated EoE Endoscopic Reference Score.

3.6 Primary Safety Endpoints

All adverse events will be reported. A safety assessment will be performed monthly via phone visits after initiation of treatment, and at the 12-week follow-up visit with vital signs and a physical examination.

The EGD with biopsies to be performed at 12 week follow-up carries the standard risks associated with this procedure: complications from conscious sedation, performing endoscopy and biopsies. These risks include cardiac or respiratory compromise, allergic reactions to the medications used for sedation, perforation, bleeding and aspiration. The most significant risk is that of cardiovascular events estimated at .003%.[14] Though these risks are present, they are very low.

3.7 Identification of Source Data

The DSQ and EREFS scores will be entered into a computer database along with the patients' age, gender, and endoscopy and pathology reports from the electronic medical record. The data will be stored on the Mayo network hard-drive through the principal investigator's account.

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

1. Patients over the age of 18, male and female.
2. Patients who carry the diagnosis of EoE based on esophageal biopsies obtained within 6 months prior to enrollment (>15 eosinophils per hpf on at least 2 esophageal levels)
3. Subjects must be able to give appropriate informed consent

4.2 Exclusion Criteria

1. Not willing or able to sign consent.
2. Patients who have used topical or systemic corticosteroid therapy for any reason in the previous 6 weeks.
3. Patients who have been treated with acid-suppressing medications (PPI or H2 receptor antagonists) in the previous 6 weeks.
4. Patients on immunosuppressive or immunomodulating medications in the previous 6 weeks.
5. Patients with known allergies or hypersensitivity to anti-histamines.
6. Patients who have contraindications to the procurement of biopsies including patients who have known bleeding disorders, a history of bleeding diathesis, or who are currently using warfarin or clopidogrel.
7. Patients who have a contraindication to the performance of an esophagogastroduodenoscopy (EGD) including previous cardiopulmonary arrest during an endoscopic procedure.
8. Patients who are pregnant.

4.3 Subject Recruitment, Enrollment and Screening

After institutional review board approval, all patients who present to the Mayo Clinic Florida Esophageal Diseases Clinic or General Gastroenterology Clinic with the diagnosis of EoE who meet enrollment criteria will be offered enrollment into the study. All enrolled patients will be entered by the physician through written informed consent.

The appointment schedule for the Esophageal Diseases clinic and the General Gastroenterology clinic at the Mayo Clinic Florida will be reviewed 1-5 days in advance by the study coordinator to determine which patients are coming for dysphagia or EoE. If the indication for the appointment is dysphagia, the study coordinator will review the patient's chart or outside records to determine if there is evidence of EoE. The physician that is scheduled to see any patient that is identified as having EoE will be contacted prior to the patients' appointment and asked if he or she agrees to have the patient approached for study enrollment.

We will also make general announcements to our Gastroenterology Division about the study in an attempt to recruit patients with EoE at our center.

Once patients are identified, they will be approached by our study coordinator and informed of the study. They will be provided with a consent form. The consent form will be documented by their signature on the consent form and this will be entered into the patient's permanent medical record.

Consent will either be performed in person face-to-face on the Mayo Clinic Florida Campus in a private meeting room, or via Zoom meeting, in the setting of the coronavirus pandemic. This will be per the patient preference. If consent is performed via Zoom meeting, the study coordinator or investigator will teleconference from a private exam or conference room on the Mayo Clinic Florida campus, and the study participant will teleconference from a private

location of their choosing. To confirm the study participant's identity by Zoom, the coordinator or investigator will ask them to confirm their name and date of birth. All included study participants must be willing and able to sign consent and be age 18 or older. The study will not include adults lacking capacity. The link for the Zoom meeting and consent will be emailed to the patient at an email of their choice, in the week prior to the Zoom meeting. If study participants request a hard copy of the consent, this will be mailed at the request of the study participant.

Women of child-bearing age (18-50) and potential will be given a urine pregnancy test prior to enrollment in the trial.

In the Mayo Clinic Florida Esophageal Diseases Clinic, we see approximately 3-5 patients with EoE per week. This amounts to an estimated 150 patients per year. Assuming we can enroll at least half of these patients in our study, the study will take approximately 12 to months complete.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subjects may be withdrawn from the study for safety issues, failure of subject to adhere to protocol requirements, and subject decision to withdraw from the study. Safety issues include adverse effects from the study drugs, or inability to tolerate the study drugs. Failure of subjects to adhere to protocol requirements includes adherence to the medication regimen, absence of follow-up visits, or not completing study questionnaires. As there is a 6 week wash out period prior to study inclusion, patients may have worsening of symptoms of EoE while off prior medications. Patients who have worsening or relapse of symptoms of EoE while enrolled in the trial will be removed from the study.

For patients who do not tolerate the study drug, other treatment alternatives will be offered, including PPIs, corticosteroids, and/or diet modification.

Data will be collected on withdrawn subjects up until the point of study withdrawal. A scheduled follow-up appointment will be ordered in clinic following study withdrawal.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

A follow-up appointment will be ordered at the 12 week period after initiation of the study drug or placebo.

5 Study Drug

5.1 Description

Loratadine is an oral non-sedating anti-histamine which is highly selective for H1-receptors. H1-antagonists such as loratidine compete with free histamine for binding at H1-receptor sites, which blocks the effects of histamine on H1-receptors in the GI tract through competitive antagonism. Compared to traditional sedating anti-histamines, loratadine is not associated with QT prolongation and has less central nervous system effects. Compared to first generation anti-histamines, loratadine has reduced penetration of the blood brain barrier and greater specificity for the H1 receptor with fewer anti-cholinergic effects. Loratadine is used in the pediatric and adult population for allergic rhinitis and chronic idiopathic urticaria. The standard dose is 10 mg PO once daily. After oral administration, the onset of action occurs within 1-3 hours with peak effects in 8-12 hours and duration of action greater than 24 hours.

Famotidine is an oral histamine type 2 receptor antagonist that inhibits the action of histamine at the histamine H2-receptors, including gastric cells, used for gastrointestinal disorders including gastroesophageal reflux disease and peptic ulcer disease. Oral absorption is approximately 40-45%, and the elimination half-life is 2.5-3.5 hours.

5.2 Treatment Regimen

Famotidine will be taken 40 mg twice daily by mouth for 12 weeks. Loratadine will be taken 10 mg daily by mouth for 12 weeks.

Patients will be randomly assigned to either the treatment or placebo group by a blocked randomization protocol from a computer generated program via the Mayo Clinic statistical team.

5.3 Preparation and Administration of Study Drug

Preparation of the study drug will be performed in the pharmacy for both famotidine and loratadine, and 12 week supplies given to the patient. Famotidine should be taken by mouth 30-60 minutes before the morning and evening meals, and Loratadine should be taken 30-60 minutes before the morning meal.

Similarly, the placebo drugs will be prepared by the pharmacist, with identical instructions for administration as the treatment drugs.

5.4 Subject Compliance Monitoring

In a diary, patients will record time of medication administration before breakfast and evening meal. At the follow-up visit following the 12 week intervention period, a pill count will be performed. Patients who are not more than 70% compliant will be withdrawn from the study.

5.5 Prior and Concomitant Therapy

During the study, participants may not take proton pump inhibitors, local or systemic corticosteroids, or other immunomodulatory or immunosuppressive medications (anti-TNF for example). Patients may not take other anti-histamines. For symptoms of dyspepsia or abdominal discomfort, patients may take over-the-counter medications including bismuth subsalicylate, sucralfate, and calcium carbonate.

No major dietary changes will be allowed during the study period.

5.6 Packaging

Patients will pick up the study drugs at the Mayo Clinic pharmacy. Loratadine will be dispensed as 10 mg tablets, count of 84. Famotidine will be dispensed as 40 mg tablets, count of 168. This is a 12 week supply of both study medications.

Patients randomized to the placebo arm will receive placebo pills dispensed in similar shape, size, and color.

5.7 Masking/Blinding of Study

Subjects will be masked as to the treatment allocation. The study PI and co-investigator AK will be unblinded to monitor for adverse events. The study pharmacy will be unblended for the allocation of the study medications. Patients will be randomized in a 1:1 fashion to the two study arms using a blocked randomization protocol with a computer generate program.

5.8 Receiving, Storage, Dispensing and Return

5.8.1 Receipt of Drug Supplies

Study participants will pick up the study medications, either treatment or placebo arm, at the Mayo Clinic pharmacy.

5.8.2 Storage

Medications (loratadine, famotidine, and/or placebo) should be stored at room temperature in a dry location and protected from light.

5.8.3 Dispensing of Study Drug

On the 12 week follow-up appointment, the treating physician will perform a pill count to document the amount of drug remaining. The pill count will be entered into the study database.

5.8.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug remaining at the 12 week follow-up visit. This reconciliation will be logged into the study database. Any discrepancies noted will be documented and investigated, prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

6.1 Visit 1 (Enrollment)

Once patients have met enrollment criteria and consented to enrollment in the study, they will be given the Dysphagia Symptom Questionnaire to complete daily for two weeks. All study subjects will be given famotidine 40 mg oral twice daily and loratadine 10 mg once daily for 12 weeks or placebo pills for 12 weeks duration dispensed from the Mayo Clinic pharmacy which will be started after completing the DSQ for two weeks. Subjects will receive a diary to track medication administration and symptoms. The patient consent can performed via Zoom per patient preference.

6.2 Visit 2 (4 week follow-up after starting study drugs)

This visit will occur per participant preference, either via phone call or in person by appointment, with the study coordinator and will occur at 4 weeks plus or minus a one week period after initiation of the study drugs. Study participants will review medication compliance and adverse effects.

6.3 Visit 3 (8 week follow-up after starting study drugs)

This visit will occur per participant preference, either via phone call or in person by appointment, with the study coordinator and will occur at 8 weeks plus or minus a one week period after initiation of the study drugs. Study participants will review medication compliance and adverse effects.

6.4 Visit 4 (12 week follow-up)

At the 12 week follow-up visit with a physician, we will review the results of the DSQ to be completed for weeks 10-12 of the study drugs. The 12 week follow-up appointment will occur no earlier than 12 weeks after initiation of the study drug and no later than 14 weeks after initiation. The patient will meet with one of the study investigators, and be consented for an EGD. An EGD with esophageal biopsies will be performed. Prior to EGD, a complete blood count and basic

metabolic panel will be ordered as routine per institutional policy. Either KD or DF will perform the EGD in the endoscopy suite, per our institution's standard protocol. Any macroscopic abnormalities seen in the esophagus will be documented and photographed. Any lesions in the upper digestive tract seen on EGD will be biopsied and treated as deemed appropriate by the performing endoscopist. Biopsy specimens will be labeled and placed in a container and labeled with the patient's ID number.

The eosinophil count will be determined by the study pathologist for the post-treatment exam using a previously validated protocol. [2, 3] Four esophageal biopsies will be sampled from both the distal esophagus (3cm above the GE junction) and proximal esophagus (at least 10 cm above the GE junction) to maximize sensitivity of detecting eosinophils.[1] On each biopsy fragment, 5 high-power fields will be examined and the overall peak eosinophil count determined from the field deemed to be most inflamed from all esophageal levels and all high power fields. [4]

Symptomatic improvement will be defined as at least 2 levels on the dysphagia questionnaire.

Study Activity	Week 0 (initial enrollment appointment)	Week 6	Week 10	Weeks 14-16 (follow-up appointment)
Study Agent(s) (weeks 2-14)		X	X	
Informed consent	X			
Physician office visit with medication review and physical exam	X			X
Phone or office visit with study coordinator		X	X	
Pill count		X	X	X
Drug diary		X	X	
Adverse event evaluation		X	X	X

B-HCG (non-menopausal females)	X			
Dysphagia Symptom Questionnaire (complete weeks 0-2 and 12-14)	X			X
CBC and BMP				X
EGD w/ biopsy				X

7 Statistical Plan

7.1 Sample Size Determination

The planned sample size is 20 patients in each arm, for a total of 40 study patients. Assuming a dropout rate of 20%, the goal is to enroll 50 patients. The estimated baseline mean eosinophil count is expected to be between 80-110 eos/hpf, with an estimated standard deviation of change from baseline in eosinophil counts of 30-50 eos/hpf. Using a conservative standard deviation assumption of 50 eos/hpf, a sample size of 25 patients in each arm provides 80% power to detect a significant between group difference of 40%. Using a conservative standard deviation assumption of 50 eos/hpf, a sample size of 16 patients in each arm provides 80% power to detect a significant between group difference of 50%.

Pilot studies evaluating other medications in EoE have used similar sample sizes. [15, 16]

7.2 Statistical Methods

Descriptive Statistics

Means and standard deviations will be used to summarize quantitative and continuous variables. These will include age, dysphagia questionnaire scores, and eosinophils per hpf on histology. Frequencies and percentages will be used to summarize categorical variables including gender; the proportion of patients that have symptomatic and histologic improvement in symptoms on placebo; and the proportion of patients that have symptomatic and histologic improvement in symptoms on anti-histamines.

Handling of Missing Data

We will perform data queries for missing dysphagia questionnaires and pathology reports.

Primary Hypothesis: Antihistamines are effective for the treatment of EoE and will result in symptomatic improvement as assessed by patient questionnaires and endoscopic and histologic improvement as assessed by endoscopic score and by eosinophil counts on biopsy.

Continuous demographic variables, peak eosinophil counts and dysphagia and GERD questionnaire scores at baseline and 12 weeks will be compared between the treatment and placebo arms using a two-sample t test or Wilcoxon rank sum test where appropriate. Categorical demographic variable and percentage of patients with histologic response of ≤15 eos/hpf at 12 weeks will be compared between the two arms using Chi-squared test or Fisher's exact test. The changes in peak counts and questionnaire scores in the treatment arm from baseline to 12 weeks will be compared suing a paired t-test or signed rank test

All tests will be two-sided with alpha level set at 0.05 for statistical significance.

Interim Analysis

7.3 Subject Population(s) for Analysis

- All-randomized population: Any subject randomized into the study, regardless of whether they received study drug
- All-treated population: Any subject randomized into the study that received at least one dose of study drug
- Protocol-compliant population: Any subject who was randomized and received the protocol required study drug exposure and required protocol processing
- All-completed population: Only subjects who completed ALL study related procedures and follow-up will be included

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event
An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Serious Adverse Event
Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization
- inpatient, new, or prolonged; disability/incapacity
- persistent or significant disability or incapacity
- birth defect/anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period

For this study, the study treatment follow-up period is defined as from the initiation of the study drugs or placebo until the 12 week follow-up appointment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator should instruct each subject to report, to the sponsor-investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if they are considered clinically significant or require therapy.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should not be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.

- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the event tracking log (see attached form). All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs during the Adverse Event Reporting Period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.4 Unmasking/Unblinding Procedures

The PI and co-investigator AK will be unblinded to monitor for adverse events. In the circumstance that an event occurs which may require unbinding of a study participant, the study team including the PI will meet and discuss the event. Unblinding will be reported in a timely manner using the same requirements for the reporting of serious adverse events.

8.5 Stopping Rules

Adverse events will be continuously monitored by the study team for patient safety. It is unknown how many adverse events will occur during this study. If an adverse event occurs, it

will be evaluated by the study team and PI with creation of a management plan, including stopping the study as applicable.

If a death is observed at any point in the trial, the study will stop. We will evaluate the study after the first 25 patients. If no more than 2 adverse events have occurred, the study will continue to its full enrollment of 50 patients.

8.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

Data Management

Data will be captured in an electronic fashion via institutional database (RedCap).

Data Security and Confidentiality

Access to the data will be provided to only necessary persons, those that collect the data, or need to analyze the data. Data will be stored in a password-protected folder behind the Mayo firewall (Redcap database). All data collection and analysis will be done behind the firewall.

Data Quality Assurance

All data will be entered by appropriately trained personnel. Questionnaires will be collected by the physicians.

Data Clarification Process

Incomplete or erroneous data will be corrected by analyzing the original patient electronic record and case report forms.

9.3 Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents.

The sponsor-investigator will retain the specified records and reports for;

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Retention of and Access to Research Data Policy" [redacted]

Whichever is longer

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to

all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

Please see the attached data safety and monitoring plan.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

Financing for this study will occur through Mayo Internal Funding and/or from grants from the National Institute of Health. Applications for grants will be submitted the 2019 application year.

12.2 Conflict of Interest

None.

Any study team member who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor-investigator prior to participation in this study.

13 Publication Plan

Subsequent to trial closure, data will be analyzed, and a manuscript will be submitted to the appropriate journal, after consensus among all the investigators. Dr. Dawn Francis holds the primary responsibility for publication of the results of the study. The study will be registered to clinicaltrials.gov prior to subject recruitment and enrollment, and the results posted to clinicaltrials.gov within 12 months of final data collection for the primary outcomes.

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