

Clinical and Immunopathologic Effects of Swallowed Fluticasone for Eosinophilic Esophagitis

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Background & Aims: Eosinophilic esophagitis (EE) is a recently recognized clinical disorder that is understood poorly. We aimed to determine the efficacy of swallowed fluticasone propionate on the immunopathologic features associated with EE. **Methods:** A retrospective analysis was performed on 20 pediatric patients with EE. Inclusion criteria specified a peak eosinophil density of ≥ 24 cells per 400 \times field in the esophagus and treatment with swallowed fluticasone between 2 endoscopic assessments. Histologic specimens were examined for eosinophil and CD8⁺ lymphocyte infiltration, papillary lengthening, and proliferation of the basal layer as determined by monoclonal anti-Ki-67 (MIB-1) antibody staining. **Results:** The mean time interval between endoscopic assessments was 4.8 months. The patients were divided equally between allergic and nonallergic groups based on the results of skin-prick testing. All of the nonallergic patients responded to fluticasone propionate. The endoscopic appearance of the mucosa improved and microscopic evaluation showed markedly reduced eosinophil infiltration, reduced basal layer hyperplasia documented by a reduced number of MIB-1⁺ cells, and a reduced number of CD8⁺ lymphocytes. However, allergic patients were relatively refractory to therapy; 20% had a partial response, whereas 20% had no detectable improvement. Esophageal eosinophil levels before and after therapy in all patients strongly correlated with the level of epithelial cell proliferation as measured by MIB-1 staining. **Conclusions:** Collectively, these results suggest that patients treated with swallowed fluticasone have improved endoscopic, histologic, and immunologic parameters associated with EE. However, patients with identifiable allergies who fail dietary elimination may have a blunted response to treatment.

Eosinophilic esophagitis (EE) is an inflammatory disorder that is characterized by eosinophil accumulation specifically in the esophagus in association with a spectrum of clinical presentations.^{1,2} Patients with EE usually have symptoms that mimic gastroesophageal reflux disease (GERD), but the disease primarily is resistant to typical anti-GERD therapy.³ Accordingly, the

pathogenesis appears to be markedly different from GERD; for example, the esophagus has a greater number of eosinophils compared with GERD, pH probe studies typically are normal in EE, and EE is more prevalent among men. Additionally, EE is associated strongly with atopic disease; most patients have immunoglobulin (Ig) E sensitization to a variety of foods and inhaled allergens, and coexisting asthma.⁴ Skin-prick and patch testing, as well as IgE-radioallergosorbent test levels for food antigens, may identify foods to which an individual is sensitized, but the significance of food sensitization is not clear. Notably, only a minority of patients with EE and food sensitization have anaphylactic responses to the implicated foods. Additionally, it remains unknown whether compulsive dietary elimination of the implicated foods leads to disease remission. Although not extensively studied, it has been reported that successful treatment options for EE include a strict elemental (amino acid) diet,⁵⁻⁷ systemic glucocorticoids,⁸ and swallowed fluticasone propionate (FP).^{9,10} Only a limited number of EE patients treated with FP have been reported in the literature. Furthermore, only treatment successes have been described, raising the critical question of whether this is a universally efficacious therapy. We now report a series of 20 EE patients treated with FP. We show that FP is safe and highly efficacious with a response rate of 90%. However, we show that patients with the allergic variant of EE appear to be relatively refractory to swallowed glucocorticoids compared with the nonallergic variant.

Patients and Methods

Patients

Twenty nonsequential patients were identified retrospectively from our patient population as having EE based on

Abbreviations used in this paper: EE, eosinophilic esophagitis; FP, fluticasone propionate; GERD, gastroesophageal reflux disease.

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Table 1. Patient Characteristics

Patient	Sex	Age ^a	Symptoms at presentation	FP ^b	Acid suppression ^c	Response to Therapy ^d
1	M	8	Emesis, chest pain, abdominal pain	880	No	Yes
2	M	9	Emesis, chest pain	880	No	Yes
3	M	10	Abdominal pain	880	Yes	Yes
4	F	2	Emesis	880	Yes	Yes
5	M	1	Emesis, irritability	440	Yes	Yes
6	M	13	Emesis, dysphagia, food impaction	880	Yes	Yes
7	M	12	Chest pain	880	Yes	Partial
8	M	2	Emesis	880	Yes	Partial
9	F	5	Emesis, poor weight gain, poor appetite, dysphagia	1320	Yes	No
10	M	11	Food impaction, dysphagia, abdominal pain, chest pain	880	No	No
11	M	2	Emesis	880	Yes	Yes
12	M	11	Emesis, decreased appetite, nausea, heartburn	880	No	Yes
13	F	9	Emesis, dysphagia, food impaction, abdominal pain	880	No	Yes
14	F	1	Emesis, airway problems	880	Yes	Yes
15	M	11	Chest pain, dysphagia, emesis	880	No	Yes
16	M	9	Cough, emesis	440	Yes	Yes
17	M	6	Emesis, airway problems	880	Yes	Yes
18	F	7	Emesis	880	Yes	Yes
19	M	4	Emesis	880	Yes	Yes
20	M	5	Cough, emesis	880	Yes	Yes

^aAge in years at diagnosis.

^bFluticasone propionate daily dose (μg).

^cIndicates whether patient received concomitant acid suppression.

^dDenotes whether patient treatment with FP was associated with resolution of tissue eosinophilia on follow-up biopsy examination.

the presence of endoscopic esophageal thickening, often with furrowing/vertical lines¹¹ and microscopic analysis revealing mucosal eosinophil infiltration and basal proliferation. In particular, patients had a peak eosinophil count of ≥ 24 cells per $400\times$ microscopic field in at least one biopsy specimen from the distal esophagus after careful examination of all microscopic fields. Neither marked esophageal narrowing nor benign strictures were noted in any patient. All patients were treated with twice-daily FP as an isolated intervention during the complete period between 2 endoscopic evaluations. Fourteen patients also received concomitant acid suppression for either ongoing emesis, or as an empiric measure to maximize resolution of esophagitis. Patients with food allergies (patients 1–10) previously had failed dietary therapy with elimination of relevant antigens; the remaining patients had no food sensitivities identified by skin-prick testing. Knowledge of outcome or atopic status was not available before the patient selection process.

Skin-Prick Testing

Skin-prick tests were performed with commercially available extracts to a variety of food antigens and common aeroallergens (Hollister-Stier, Spokane, WA). The allergen extracts were applied intracutaneously with a DermaPIK device (Greer, Lenoir, NC). Reactivity was determined 20 minutes later by measuring the wheal-and-flare response compared with a positive histamine control and a negative control saline. Positive reactivity was defined as reactions unequivocally greater than the saline control. Aeroallergen extracts included cat, cockroach, dog, feather, grass, mite mix, mold mix, rabbit, ragweed, tree mix, and weed mix. Food extracts included

almond, apple, banana, barley, bean (string), beef, cacao bean, cantaloupe, carrot, cashew, celery, chicken, cinnamon, corn, egg, fish, flounder, haddock, halibut, hazelnut, lamb, lemon, lettuce, milk (cow's), mustard, oat, orange, pea, peach, peanut, pear, pecan, pork, rice, shrimp, soy, strawberry, tomato, tuna, walnut (English), watermelon, and wheat.

Medication

Patients were treated with swallowed FP as a sole intervention between 2 endoscopic assessments, except for acid-suppression therapy (Table 1). Patients were prescribed metered-dose inhalers of FP. They were instructed not to use a spacer. Additionally, they were instructed to spray the medication into the pharynx, and not to rinse, eat, or drink for 30 minutes after administration. All patients and/or guardians expressed understanding of instructions and assured compliance.

Analysis of Histologic Specimens

Retrospective data were examined after approval of the Cincinnati Children's Hospital Institutional Review Board; control specimens also were obtained from individuals undergoing endoscopy but showing no identifiable endoscopic or microscopic pathology. Formalin-fixed tissue was embedded in paraffin and $10\text{-}\mu\text{m}$ sections were stained with H&E. Slides were assessed for eosinophil density, layering of eosinophils on the mucosal surface, the presence of basal layer hyperplasia, and papillary lengthening. All $400\times$ fields were counted in each specimen. An average of 10 ± 4.1 (mean \pm SD) fields were assessed per specimen and the peak eosinophil count was recorded. Specimens were graded as resolved if all abnormal

Table 2. Allergic Phenotype of Patients

Patient ^a	Asthma ^b	Dermatitis ^b	Rhinitis ^b	Aeroallergens ^c	Family ^d	Food allergies	Eosinophils ^e
1	No	No	Yes	Yes	Yes	Almond, cantaloupe, carrot, celery, corn, halibut, hazelnut, pea, soy, wheat ^f	N/A
2	Yes	Yes	Yes	Yes	No	Peanut, egg ^f	N/A
3	Yes	No	Yes	No	Yes	Egg, fish, milk, peanut, soy	N/A
4	No	No	No	No	Yes	Egg, milk, peanut ^f	N/A
5	No	No	No	Yes	Yes	Almond, beef, pea, rice, soy, string bean	N/A
6	No	No	Yes	Yes	No	Almond, barley, carrot, chicken, egg, lettuce, mustard, orange, pea, pecan, peanut, pork, soy, strawberry, watermelon ^g	200
7	No	No	No	Yes	No	Beef, lamb, milk, wheat ^f	320
8	Yes	No	Yes	Yes	No	Chicken, egg, milk, mustard, peanut, wheat ^h	300
9	No	Yes	Yes	Yes	No	Tomato, peanut, string bean, wheat	200
10	No	No	Yes	Yes	Yes	Tomato, string bean	600

N/A, not available.

^aPatients correspond to patients 1–10 in Table 1.

^bA history of asthma, atopic dermatitis, or allergic rhinitis at any time point in the life of the patient is indicated.

^cThe presence of skin prick test reactivity to any aeroallergen tested is indicated.

^dThe presence of atopic disease in any immediate family member is indicated.

^eBlood eosinophil levels were determined before initiation of FP (eosinophils per mm³)

^fCashew and haddock not tested.

^gHaddock not tested.

^hCashew and tomato not tested.

features disappeared after FP treatment. Specimens were graded as partially resolved or unchanged based on eosinophil density and/or basal layer hyperplasia. Biopsy specimens with adequate tissue were stained for Ki-67 antigen with monoclonal anti-Ki-67 (MIB-1) antibody (Dako, Carpinteria, CA), and for CD8⁺ lymphocytes with monoclonal anti-CD8 (Novacastra, Newcastle upon Tyne, UK). Both antibodies were prediluted by the manufacturers and used according to their specifications. These specimens were analyzed by digital microscopy with MagnaFire Camera Imaging and Control Ver 1.1 (Optronics, Goleta, CA), and the number of immunostained cells was normalized to area using Image-Pro Plus 4.1.0 (Media Cybernetics L.P., Carlsbad, CA). Statistical tests were performed using SAS, version 8.1 (SAS Institute, Cary, NC).

Statistical Analysis

Effect of FP was analyzed using the paired Student *t* test. Pearson correlation was used to compare eosinophil infiltration with epithelial proliferation.

Results

Patient Characteristics

The patient population was 70% male with an average age of 6.9 ± 3.9 years (mean \pm SD), similar to the demographics described previously (Table 1).^{7,8,10–16} There was a prolonged time between onset of symptoms and time of diagnosis (4.5 ± 3.5 yr, mean \pm SD). The most common symptoms at presentation were emesis (70%), dysphagia (25%), chest pain (20%), abdominal pain (20%), and food impaction (10%). Patients with

food impaction were not allergic to the impacted food. Fourteen patients with severe vomiting also received acid-suppressive medication. All patients underwent an evaluation for food allergen sensitization. Ten patients had food allergies shown by skin-prick testing (Table 2). Of the panel of 42 food antigens tested, the mean number of reactivities was 6 ± 4.2 (mean \pm SD). In these 10 patients, the most common positive antigens were peanut (60%), egg (50%), soy (40%), milk (30%), and string beans (30%). Patients also underwent skin-prick test evaluations for sensitization to aeroallergens. Notably, 90% of patients showing positive skin-prick test reactivity to food antigens also were reactive to at least one aeroallergen (Table 2). Of the other 10 patients, only 4 had sensitivities to an aeroallergen. Consistent with these skin test results, 70%, 20%, and 30% of the skin test–positive individuals had a history of allergic rhinitis, atopic dermatitis, and asthma (Table 2). In contrast, 20%, 10%, and 10% of the skin test–negative individuals had a history of allergic rhinitis, atopic dermatitis, and asthma. When available, peripheral eosinophil counts are noted in Table 2.

Esophageal Histology

We first examined the general appearance of the esophagus after endoscopy before therapy with FP. Endoscopically, all patients had abnormal findings, including longitudinal furrows and mucosal thickening with loss of visible vascularity in the distal and/or proximal esophagus. A representative photomicrograph of the dis-

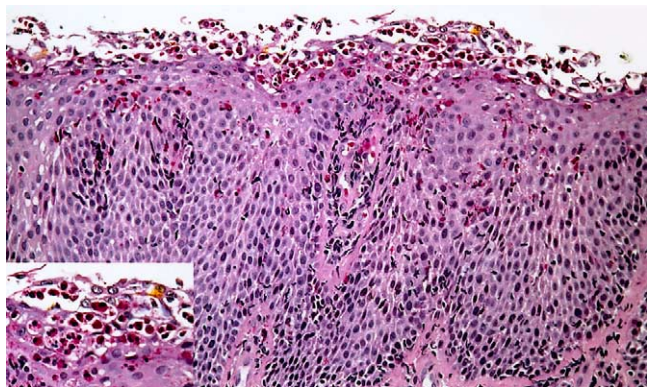


Figure 1. Esophageal histology. Histologic examination of distal esophagus from a typical patient with EE shows a dense eosinophilic infiltrate, thickening of the basal layer, and lengthening of the papillae. Eosinophils layer and form microabscesses at the mucosal surface (inset). Original magnifications for the photomicrograph and inset are 200 \times .

tal esophageal histology in a representative patient (patient 20) is shown in Figure 1. Biopsy specimens from the proximal and distal esophagus were examined for the maximum number of eosinophils by examination of all high-power fields (400 \times). Accordingly, maximum eosinophil levels were 43.4 ± 3.8 and 23 ± 4.3 in the distal and proximal esophagus, respectively (mean \pm SEM). All patients had marked mucosal eosinophilia, with peak eosinophil counts of ≥ 24 cells/400 \times field in the distal esophagus (Figure 2). Notably, there was a large variability in eosinophil density between different high-power fields within the same biopsy sample. For example, a typical patient (patient 16) had a mean eosinophil density of 14.4 ± 21 (mean \pm SD), but a range of 0 to 86 eosinophils per 400 \times field.

Esophageal Ki-67 Staining

EE has been associated with marked expansion of basal layer thickness. We hypothesized that the increased thickness of the basal layer was a result of increased epithelial cell proliferation. To test this hypothesis, we analyzed the level of expression of the nuclear antigen Ki-67, a nuclear protein that has been identified as a marker of cellular proliferation.¹⁷ Immunohistochemical analysis with monoclonal anti-Ki-67 (MIB-1) revealed a marked increase in immunopositive cells in EE patients compared with esophageal control tissue (Figure 3). EE samples showed Ki-67 staining in excess of the 3 cell-layer thickness considered to be normal in the esophagus (Figure 3A and 3B).¹⁸ This immunohistochemical technique proved most useful in assaying epithelial proliferation in improperly oriented tissue (Figure 3B). The level of Ki-67 staining in atopic and nonatopic EE was similar (Figure 4A). Because eosinophils have been implicated in

the cause of epithelial hyperplasia in an experimental murine model of EE,¹⁹ we hypothesized that there would be a direct correlation between the level of eosinophils and Ki-67⁺ staining. Indeed, there was a statistically significant correlation between these 2 variables of 0.695 ($P < 0.001$). Analyzed alone, nonallergic and allergic individuals had correlations of 0.755 ($P = 0.002$) and 0.649 ($P = 0.007$), respectively (Figure 4B).

Effect of Fluticasone Propionate on Eosinophilic Esophagitis

Overall, 16 patients showed resolution of endoscopic and microscopic pathology; this included normalization of eosinophil counts (to that reported in the normal esophagus) and basal layer thickness as compared with control specimens from patients without identifiable pathology.^{20,21} The effect of FP on esophageal eosinophil levels is shown in Figure 2. The responding individuals included all 10 nonallergic patients and 6 patients with identified food allergies. The remaining 4 allergic patients (Table 2, patients 7–10) included 2 patients (patients 9 and 10) who were completely resis-

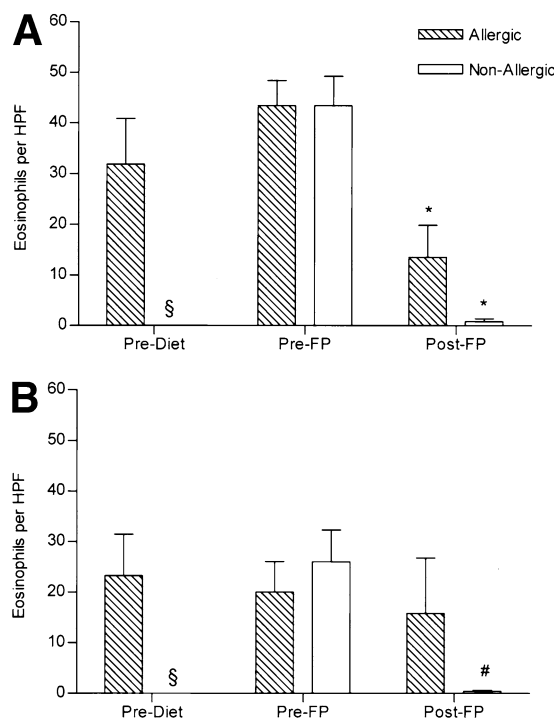


Figure 2. Esophageal eosinophil levels before and after FP therapy. A and B represent the distal and proximal esophagus, respectively. Patients with food allergies had failed dietary elimination as guided by skin-prick testing; nonallergic patients were not offered dietary elimination (§). Both allergic ($n = 10$) and nonallergic ($n = 10$) patients responded to fluticasone (* $P < 0.001$), however, the response was more pronounced in the nonallergic population. In the proximal esophagus (B), only the non-allergic population had a significant decrease in tissue eosinophils (#, $P = 0.003$) in response to therapy with FP.

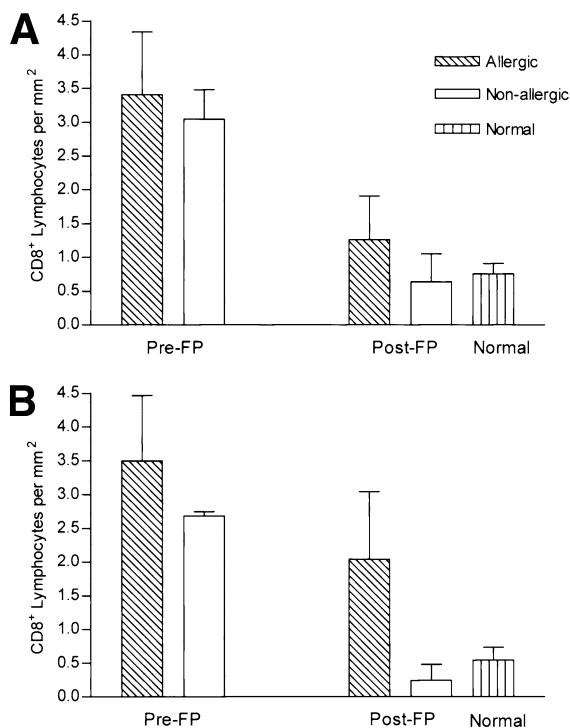


Figure 5. Quantification of CD8⁺ lymphocyte infiltration. CD8⁺ lymphocyte infiltration was assayed by immunostaining of the (A) distal and (B) proximal esophagus of patients with nonallergic and allergic variants of EE. Nonallergic patients had a marked decrease in CD8⁺ lymphocytes in the distal esophagus ($P < 0.01$) ($n = 6$) and proximal esophagus ($P < 0.001$) ($n = 4$). Allergic patients had a modest decrease in the distal esophagus ($P = 0.03$) ($n = 7$), but no significant decrease in the proximal esophagus.

ophilic gastroenteritis, and primary EE.^{22,23} Importantly, recent clinical studies have suggested that these disorders (especially primary EE) are occurring with increasing frequency. A recent study also has shown that the level of eosinophils in the esophagus negatively correlates with response to conventional gastroesophageal reflux therapy.⁸ Furthermore, a causal role for esophageal eosinophils in the development of epithelial hyperplasia, a cardinal feature of primary EE, has been shown in experimental models of aeroallergen- or interleukin-13–induced EE,²⁴ raising the possibility of this association in human EE. Collectively, these studies highlight the importance of further dissecting the pathogenesis and best treatment strategies for EE. Accordingly, the present study was designed to retrospectively analyze patients with primary EE to determine basic demographic and histologic characteristics of these poorly understood patients, to verify if topical glucocorticoids (e.g., FP) are effective therapy, and to determine if potential nonresponders had distinguishing characteristics not found in responding patients. Our results showed several important findings concerning EE and therapeutic intervention with FP. First, we report that FP treatment is associated with an

efficacious response in the majority of patients with EE. Second, we show that a subgroup of patients with EE failed to respond to this intervention, indicating that although FP is an attractive therapy, it is not universally effective. Third, we present evidence suggesting that patients with the allergic variant of EE may be particularly resistant to FP. These results are consistent with a report describing nonallergic and allergic variants of eosinophilic gastroenteritis, the latter group having increases in symptoms, peripheral eosinophilia, and serum IgE levels.²⁵ There are several possible explanations that may account for the apparent resistance of patients with allergic EE to FP. Notably, although skin-prick testing appears to identify allergic hypersensitivity, it is likely that this finding is merely a marker for the allergic variant of the disease, rather than an indication that the identified antigen(s) is related causally to the pathogenesis of the disease. Indeed, dietary elimination of food antigens identified by skin-prick or radioallergosorbent testing only infrequently results in improvement of symptoms. A recent preliminary study has suggested that a combination of skin-prick and patch testing may be better than either alone to formulate a successful elimination diet.¹⁶ The allergic patients examined in our study all failed to respond to dietary elimination of food antigens detected by skin-prick testing. As such, these patients may be refractory to FP because they are being exposed to other food antigens that they are sensitive to, but not identified with the testing used. Another explanation for these results concerns the possible role of aeroallergens in the development of EE. Well-controlled animal experiments have documented that respiratory inflammation can result in EE, proposing a role for swallowed aeroallergens and respiratory cytokines.¹⁹ It should be noted that the 4 patients not fully responsive to fluticasone all had significant environmental allergies with allergic rhinitis and/or asthma. Notably, a patient with EE apparently triggered by respiratory allergens recently has been described.²⁶ Accordingly, it will be important to determine the relative role of antigens derived from commensural enteric pathogens, dietary antigens, and aeroallergens in the pathogenesis of EE. It remains possible that the observed results may be explained by a possible selection bias for more severe patients in our allergic subgroup. Finally, the results may be explained by inadequate dosing or noncompliance with prescribed medication. Regardless of the explanation for these results, these data strongly suggest that some patients are refractory to FP therapy.

Swallowed FP is a potentially attractive therapy for EE for several reasons. In particular, it does not require

nasogastric feeds, does not involve unpalatable formulas, and only rarely is associated with adrenal suppression, even at high doses.²⁷ Swallowed FP is likely to mediate therapy by topical absorption in the esophagus. Pharmacokinetic studies have shown that approximately 1% of the drug is absorbed systemically and then metabolized efficiently in the liver at a rate approaching that of portal-hepatic circulation.²⁸ No alteration of morning serum cortisol level has been seen in volunteers after ingesting as much as 18 mg of FP.²⁸

Our study has several clinical implications. First, our data support the value of allergen evaluation in all patients diagnosed with EE. Removal of specific antigens from the diet may abate symptoms without requiring medications or institution of an elemental diet. It is noteworthy that all 4 of our patients who did not respond to FP had environmental allergies with allergic rhinitis and/or asthma; this suggests a causative role for aeroallergens in EE and that exposure avoidance may be helpful in refractory patients.

Second, without an understanding of the natural clinical course of EE, the response of each patient to a particular therapy must be followed-up closely and be documented by endoscopic assessment with biopsy examinations. Failure to respond to a particular dietary or pharmacologic intervention should prompt a modification in therapy because untreated inflammation is presumed to lead to esophageal stricture or narrowing; this recommendation is based on both literature reports^{29,30} and on our unpublished experience. For example, we have found that it is not uncommon for parents of EE pediatric patients to have a history of esophageal dilations, only to be later diagnosed with EE.

Third, because this disease ultimately is evaluated by histologic analysis, aggressive endoscopic surveillance with multiple esophageal biopsy examinations is indicated to assess treatment response, or lack thereof. Based on these collective clinical findings, our approach to patients with EE consists of having all patients initially undergo a comprehensive allergy evaluation. Patients without sensitivities to foods or aeroallergens on skin-prick testing are offered fluticasone therapy. Individuals with food or aeroallergen sensitivities are offered a specific antigen elimination (e.g., dietary and/or environmental changes). Regardless of specific therapy, all patients undergo a surveillance endoscopy with biopsy examinations approximately 3 months later to assess response to therapy properly. If required, therapy then is escalated to an elemental diet, systemic glucocorticoids, or both in some cases. If responsiveness to therapy is documented, we recommend prolonged therapy (the full

duration is not yet known). Finally, if therapy is reduced or discontinued, we recommend continuation of surveillance biopsy examinations because clinical symptoms and disease pathology often are dissociated.

Last, this study describes a population of patients with EE in which acid suppression was used empirically in some patients. Although justified in some cases for esophageal protection in patients with recurrent vomiting associated with EE, this practice merits further investigation because its use, even when justified by a patient's recurrent vomiting, may be associated with the development of side effects, possibly candidiasis when given with swallowed FP. Furthermore, the use of acid suppressants in this population did not clearly improve the patients' outcome because only 1 of the 6 patients without acid suppression failed to improve (patient 10). This is consistent with studies that have used pH probe studies meticulously to rule out pathologic esophageal acidification in patients with EE.³

In summary, we have found that although FP is safe and effective for the treatment of EE, patients with the allergic variant of EE are relatively refractory to this therapy. Our findings are derived from several lines of evidence including clinical symptoms (data not shown), endoscopic appearance, histopathology, and immunohistochemistry. Notably, our study reports the overexpression of the Ki-67 antigen in esophageal tissue of patients with EE; as such, we present evidence that the epithelial thickening in this disease is indeed caused by epithelial cell proliferation. Furthermore, we present evidence that eosinophil levels strongly correlate with Ki-67 immunoreactivity. This finding extends preclinical experimental EE studies in mice that have shown that esophageal eosinophils are involved in the cause of epithelial cell hyperplasia.¹⁹ Indeed, eosinophils produce a variety of epithelial cell growth factors, including members of the transforming growth factor family.³¹ Recognizing the limitations of retrospective analyses (which only permit us to make associations rather than conclusions about outcome), these results draw attention to the need for further studying of the pathogenesis and treatment of EE in prospective studies.

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