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# Airway Manifestations of Pediatric Eosinophilic Esophagitis: A Clinical and Histopathologic Report of an Emerging Association

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**Objectives:** Pediatric eosinophilic esophagitis (EE) typically presents with dysphagia, vomiting, dyspepsia, or food impaction. The purpose of this study was to highlight the emerging association of pediatric EE and airway disease. An additional goal of this study was to describe the unique histopathologic findings found in EE and specifically explore the potential role of the cytotoxic protein called eosinophil major basic protein (MBP) in the pathophysiology of the disease.

**Methods:** A retrospective review of 3 children with EE and airway symptoms included symptom presentation, aerodigestive tract endoscopic findings, ambulatory 24-hour dual pH-metry, allergy tests, treatment modalities, and treatment response. Esophageal tissue obtained from biopsies of each patient was evaluated by hematoxylin and eosin to determine the number of eosinophils per high-power field, by immunofluorescent anti-MBP staining to determine the presence of MBP, and by standard light and transmission electron microscopy to evaluate eosinophil migration patterns.

**Results:** All patients had airway inflammation that included nonspecific laryngeal edema and grade I or II subglottic stenosis. Allergy testing was positive in the 2 patients who were tested. All patients had symptoms refractory to standard reflux therapy. Ambulatory pH-metry findings were normal in 2 patients and abnormal in 1 patient despite maximum treatment. Two patients had visual abnormalities seen during esophageal examination. The number of eosinophils ranged from 20 to 45 per high-power field. Intracellular and extracellular MBP deposition was found in all esophageal biopsy specimens. All patients were treated with swallowed fluticasone, and 2 had symptom relapses that required repeat treatment.

**Conclusions:** The spectrum of pediatric EE can include upper airway disease. Intracellular and extracellular MBP deposition is present in EE, which potentially releases cytotoxic mediators that explain the esophageal and airway clinical symptoms seen in those with the disease. Eosinophilic esophagitis should be considered in patients with a history of atopic diseases and unexplained upper airway findings refractory to reflux treatment. Treatment with swallowed fluticasone is successful; however, relapses are common and require repeat treatment and close follow-up.

**Key Words:** dysphagia, eosinophil, esophagitis, fluticasone, major basic protein, pediatrics, reflux esophagitis, subglottic stenosis.

## INTRODUCTION

Eosinophilic esophagitis (EE) is an inflammatory disorder of the esophagus characterized by an isolated dense eosinophilic epithelial infiltration of the esophagus. In children, typical symptoms are recurrent emesis, regurgitation, feeding problems, and food impaction.[1-3] Airway disease has been linked to EE. Orenstein et al[2] were the first to show that the spectrum of symptoms in patients with EE included wheezing, pneumonia, sinusitis, and nasal congestion. Eosinophilic esophagitis has been identified as an etiologic factor in a child with subglottic stenosis and chronic laryngeal edema refractory to traditional reflux therapy.[4]

The clinical criteria for establishing the diagnosis of EE are evolving. Clinicians have had difficulty establishing the diagnosis, because the histopathologic finding of eosinophilia can be seen in the setting of reflux esophagitis (RE) and interpreted as RE only. Additionally, the symptoms overlap with those of several other disorders, including gastroesophageal reflux disease (GERD), Schatzki ring,[5] and esophageal infection, resulting in a low index of suspicion among clinicians unfamiliar with the disease. There are several clinical trends that may support the diagnosis. The patients are disproportionately male (up to 81%), and there appears to be a bimodal distribution among children — with one peak in infancy and a second during adolescence.[6,7]

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There is a personal or family history of allergy or atopic disease such as allergic rhinitis, asthma, and atopic dermatitis in 62% to 85% of cases of EE.[2,6] Peripheral blood eosinophilia may be found in up to two thirds of patients, and serum immunoglobulin (Ig) E is sometimes elevated.[8] The diagnosis is typically suspected among patients in whom antireflux therapy fails[2,6,9,10] or who require repeat esophageal dilations for dysphagia and food impaction.[11,12] The diagnosis is then confirmed by histopathology, when a dense eosinophilic infiltrate is demonstrated in the esophageal epithelium.[8] Although no definite criteria have been established by pathology, a threshold of >15 to 20 eosinophils per high-power field (HPF) is often used to distinguish EE from RE.[2,6,13-16]

Eosinophils have figured prominently in many disorders of the aerodigestive tract. Eosinophil major basic protein (MBP), the predominant toxic protein found in eosinophil granules, has been established as a critical cytotoxin in the pathophysiology of asthma and chronic rhinosinusitis.[17-20] Major basic protein directly induces airway reactivity and smooth muscle contraction and augments the smooth muscles' responsiveness to agonists.[21] Deposition of MBP in esophageal mucosa in patients with EE has been described,[22,23] but its role in the pathophysiology of EE has not been explored.

The purposes of this report are to describe the otolaryngological airway signs and symptoms in 3 patients with EE and to describe the histopathology of EE, exploring the potential role of MBP in the pathophysiology of the disease. Increasing the awareness of the relationship between EE and airway disease among otolaryngologists and gastroenterologists will facilitate successful treatment strategies for affected children.

## METHODS

The study was approved by the Institutional Review Board at the Mayo Clinic, Rochester. We retrospectively reviewed the histories of 3 patients who presented to a pediatric otorhinolaryngology practice with mixed airway and gastrointestinal (GI) symptoms and a diagnosis of EE. The clinical information extracted included symptoms, airway endoscopy findings, esophagogastroduodenoscopy (EGD) findings, the number of eosinophils per HPF from esophageal biopsy pathology reports, pH probe results, allergy testing, and treatments used.

To determine whether MBP, the major cytotoxic protein present in eosinophils, is present in patients with EE and mixed airway complaints, we examined the pretreatment esophageal biopsy specimens from these patients by light and transmission electron microscopy, including an immunofluorescent anti-MBP staining technique. The tissue blocks from the Mayo Clinic pathology department tissue registry for each patient were obtained. For each patient tissue sample, the formalin-fixed, paraffin-embedded esophageal tissue was sectioned at 5-μm-thick serial slices and embedded onto 3 positively charged glass slides for each patient and stained with the following: 1) hematoxylin and eosin (H & E); 2) antibody to eosinophil MBP using rabbit anti-human MBP[24-26]; and 3) a negative control for MBP (normal rabbit IgG). To confirm the pathology report of eosinophilia, we subjected the first slide to traditional H & E staining and examined it for number of eosinophils per HPF. The other 2 slides were used for MBP evaluation as described below.

A slide from each patient was stained with normal rabbit IgG to serve as the negative control. The other slide was stained with rabbit anti-human MBP antibody to target MBP within the esophageal biopsy sample. The sections were deparaffinized, rehydrated, and digested with trypsin. After a partial tryptic digestion to unmask antigenic sites, the specimens were incubated overnight at 4°C in 10% normal goat serum to block nonspecific binding by the second-stage antibody. The next day, the first-stage antibody was applied to each slide. The slides were washed and overlaid with equal concentrations of either normal rabbit IgG, applied to the control slide, or a specific affinity-purified rabbit anti-human MBP antibody, applied to the other slide for each patient. After incubation at 37°C for 30 minutes, the sections were washed and incubated in 1% chromotrope 2R at room temperature for 30 minutes to block nonspecific binding of fluorescein dye to the eosinophils. To indirectly illuminate MBP in the esophageal tissue second-stage antibody, we applied fluoresceinated goat anti-rabbit IgG to each slide and incubated it at 37°C for 30 minutes. After a final wash, the slides were mounted and sealed under coverslips; we used a glycerol-phosphate–buffered saline mounting medium made 10 mg/mL in para-phenylenediamine to inhibit fluorescence fading.

## RESULTS

A summary of the patient symptoms, clinical evaluations, histopathologic findings, and treatments for the 3 pediatric EE patients is presented in the Table. The individual patient presentations, disease courses, and treatments are described below.

*Patient 1.* A 3-year-old boy with a history of macrocephaly and global developmental delay was treated by the pediatric otorhinolaryngology department for mixed apnea. He required a tracheotomy at 12 weeks of age for life-threatening mixed apnea, pharyngomalacia, and aspiration. At 2 years of age, he

SUMMARY OF THREE PEDIATRIC PATIENTS WITH EOSINOPHILIC ESOPHAGITIS

| | *Patient 1* | *Patient 2* | *Patient 3* |
|---|---|---|---|
| Symptoms | Mixed apnea, aerophagia, emesis | Coughing, choking, apneic spells | Dyspnea on exertion |
| Airway evaluation | Grade I-II subglottic stenosis, laryngeal inflammation | Tonsillar hypertrophy, grade I subglottic stenosis, tracheal inflammation | Posterior glottic stenosis, grade I subglottic stenosis |
| Esophagogastroduodenoscopy findings | Linear streaking | Discrete white patches throughout esophagus | Normal in appearance |
| Ambulatory 24-h dual pH-metry results* | 2.2% at proximal probe, 15.1% at distal probe | No proximal reflux, <0.1% at distal probe | No proximal reflux, 2% at distal probe |
| Allergy testing | Not performed | Multiple food allergies on in vitro serum immunoglobulin E test | Aeroallergen allergies on prick testing, foods negative |
| Histopathology | 20 intraepithelial eosinophils per HPF distally, occasional eosinophil proximally | 45 intraepithelial esophageal eosinophils per HPF | 20 intraepithelial eosinophils per HPF |
| MBP staining | Intracellular and extracellular MBP | Intracellular and extracellular MBP | Intracellular and extracellular MBP |
| Treatment | Omeprazole daily and swallowed fluticasone propionate | Food elimination and swallowed fluticasone propionate | Pantoprazole sodium twice daily and swallowed fluticasone propionate |

HPF — high-power field; MBP — major basic protein.
*Percentage of time pH was less than 4.

required an adenotonsillectomy, and he was successfully decannulated 3 months later. His primary gastrointestinal complaint was recurrent nausea and vomiting with aerophagia and eructation. He had been treated with antireflux and promotility agents without improvement. Multiple airway evaluations were performed between 1999 and 2002, which repeatedly showed laryngeal edema with effacement of the laryngeal ventricles (Fig 1) and a grade I-II subglottic stenosis. Inflammatory changes of the larynx and mild inflammation of the trachea were also noted. In 1999, an ambulatory 24-hour dual pH-metry showed a pH of less than 4 for 2.2% of the time at the proximal probe, including 2 episodes that lasted more than 5 minutes, and 15.1% reflux time at the distal probe despite maximal medical therapy. From 1999 to 2002, the child had multiple EGD examinations that did not reveal any visible abnormalities. However, an EGD examination in June 2002 showed mild linear streaking of the distal esophagus. Biopsies performed then showed 20 to 30 eosinophils per HPF. The patient began treatment with swallowed fluticasone propionate, 2 puffs of 44 μg 4 times daily for 6 weeks, and demonstrated dramatic improvement. A Nissen fundoplication had been considered as the next step in his care, but was deferred because of his dramatic response to this medical therapy. An endoscopic examination 8 months after medical therapy began showed a visually normal larynx and esophagus with no eosinophils found on any of the esophageal biopsies. The child is now 6 years old and has had 1 relapse with symptoms of dysphagia and food impaction that responded to another course of swallowed fluticasone.

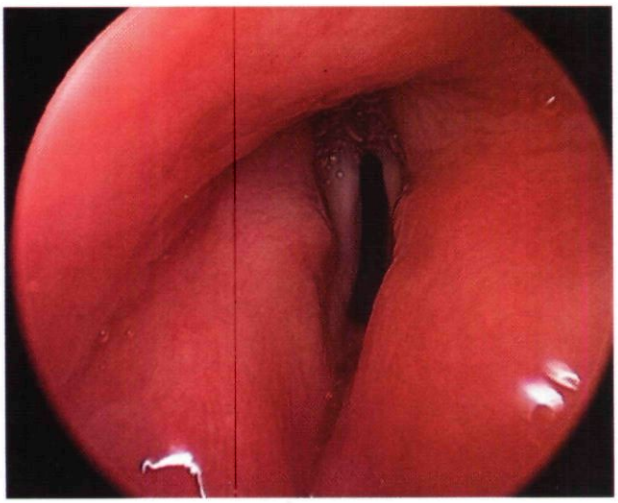

**Fig 1.** (Patient 1) Laryngoscopic evaluation shows glottic edema with effacement of laryngeal ventricles. Patient had mixed apnea, recurrent emesis, and eosinophilic esophagitis (EE).

*Patient 2.* A 2-year-old boy presented with the complaint of coughing and choking with feeding that resulted in "apneic spells." Additional symptoms included intermittent biphasic stridor, nausea, vomiting, wheezing, weight loss, and failure to thrive. Because of his long-standing problem of recurrent emesis, he had undergone a Nissen fundoplication 3 months before presentation to our institution. Diagnostic evaluation began with a complete rigid endoscopic evaluation of his airway and EGD. The

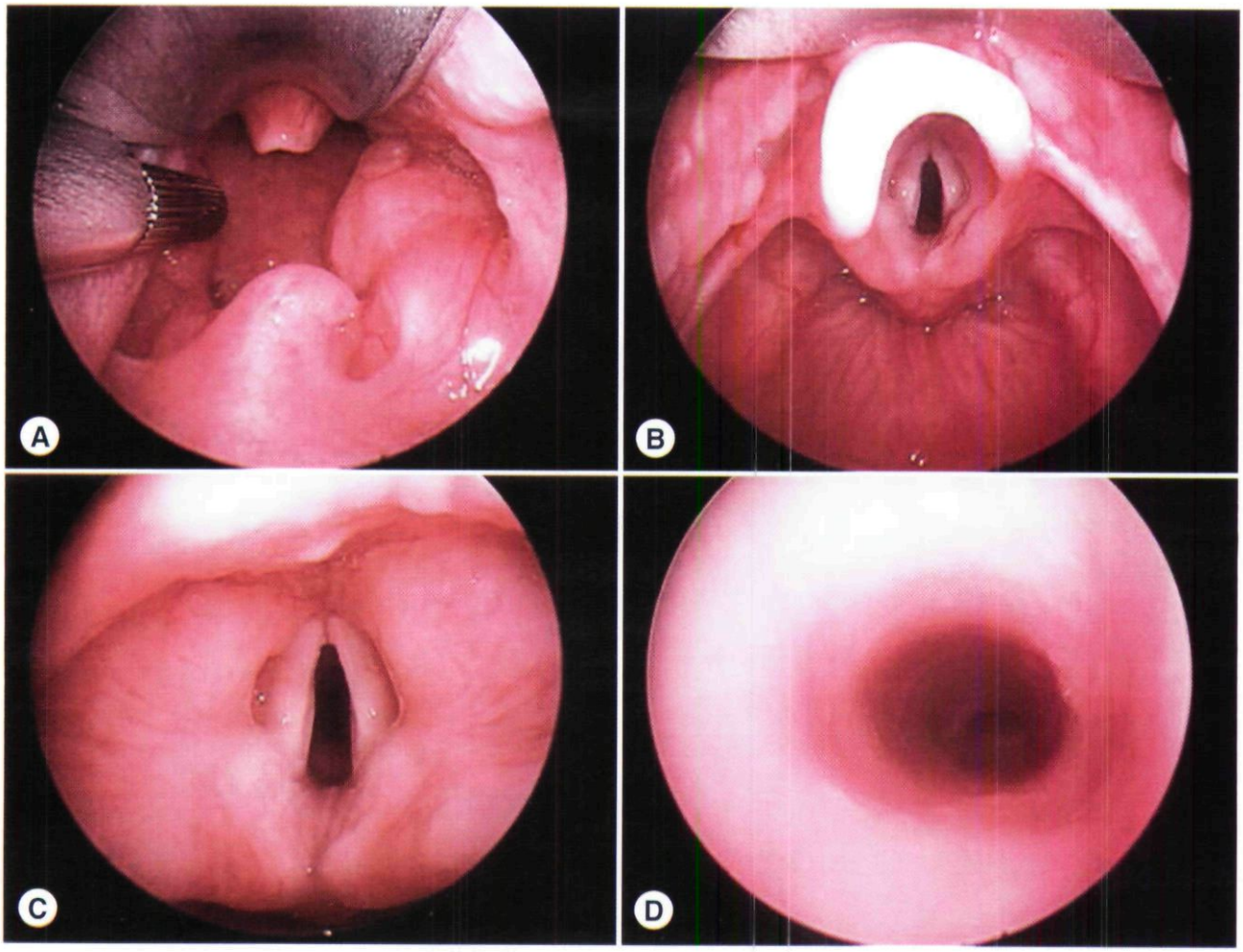


**Fig 2.** (Patient 2) Rigid endoscopic evaluation shows **A)** tonsillar hypertrophy, **B,C)** inflammatory changes of larynx with effacement of laryngeal ventricles and cystic changes in left false vocal fold, and **D)** mild subglottic narrowing with mucosal edema. Patient had recurrent emesis and apnea with EE.

findings included tonsillar hypertrophy, laryngeal ventricular effacement with a cobblestoning effect on the glottic surface of the epiglottis, grade I subglottic stenosis with mucosal edematous changes, and cobblestoning throughout the trachea and main stem bronchi (Fig 2). Discrete white patches were found throughout the esophagus. Biopsy specimens of the esophageal mucosa showed 45 eosinophils per HPF, consistent with EE; biopsy specimens of the trachea and postcricoid mucosa showed moderate inflammation with squamous metaplasia. Frozen biopsy sections revealed fungi; a culture grew out *Candida albicans*. Ambulatory 24-hour dual pH-metry was negative, demonstrating 2 brief reflux episodes in the distal probe only. The patient's serum showed elevated levels of IgE antibodies for cheese, egg white, milk, wheat, soy, peanut, beef, pork, and green peas. The patient was initially treated with an elimination diet and nystatin. After a 6-week post-treatment endoscopy showed no resolution of the eosinophilia, swallowed fluticasone propionate, 2 puffs of 44 μg 4 times daily for 6 weeks, was added to his regimen. He developed the side effect of thrush during his first treatment course. Overall, his treatment with swallowed fluticasone has dramatically improved his health; he had a 3.18-kg (7-lb) weight gain in 12 months. He has had a relapse, presenting at 8 months with the symptom of recurrent emesis that required another 6-week course of swallowed corticosteroid therapy. His stridor and "apneic spells" have resolved.

*Patient 3.* A 16-year-old girl presented with dyspnea on exertion. She had a complex airway history dating to the age of 3 years, at which she developed hemolytic uremic syndrome. During hospitalization at that time, she required a tracheotomy for prolonged intubation and ventilatory support. Subsequently, she underwent multiple airway procedures to assist with decannulation in the setting of severe acquired subglottic stenosis, including laryngotracheoplasty with placement of anterior and posterior cartilage grafts. This treatment permitted decannulation for only 1 month. Over the next 10 years, she underwent

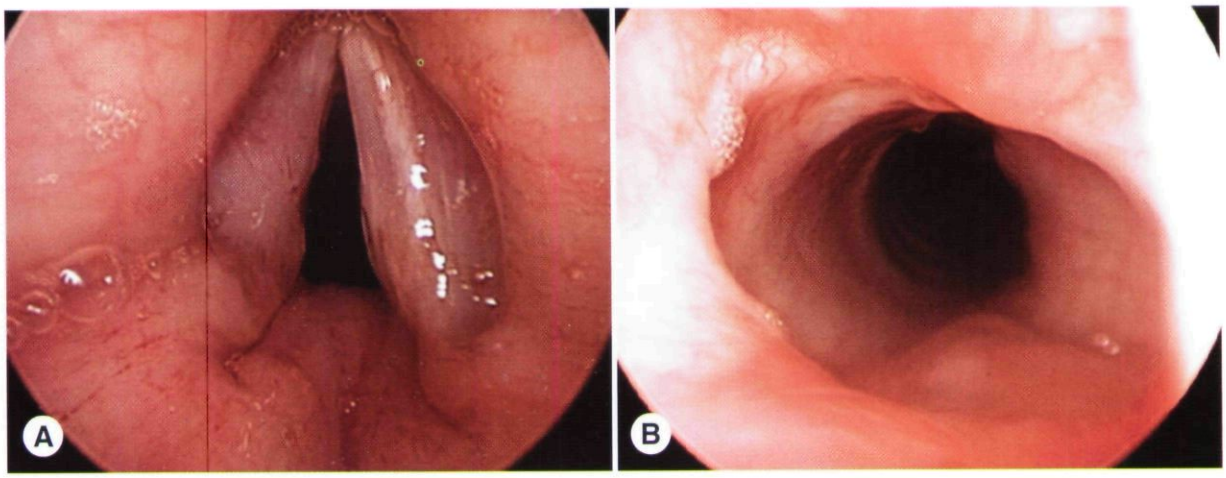


**Fig 3.** (Patient 3) Laryngoscopic examination shows **A)** posterior glottic stenosis with intra-arytenoid edema and **B)** subglottic narrowing. Patient had complex airway history, dyspnea on exertion, and EE according to esophageal biopsy.

repeated attempts at decannulation and 6 repeat tracheotomies. Finally, when she was 12 years old, decannulation was successful.

At 15 years of age, the patient began to experience shortness of breath that markedly restricted her activity. Direct laryngoscopy showed laryngeal ventricular effacement, intra-arytenoid mucosal edema, and posterior glottic stenosis with a 3-mm posterior glottic opening (Fig 3A). Bronchoscopy showed grade 1 subglottic stenosis with mucosal inflammatory changes (Fig 3B). Laryngotracheal reconstructive surgery with a posterior cartilage graft was recommended. The findings of the preoperative evaluation including an EGD were reported as visually normal. Biopsy specimens of the esophagus, however, showed 20 eosinophils per HPF in the distal esophagus and scattered eosinophils in the proximal esophagus, consistent with a diagnosis of EE. Skin prick allergy testing was positive for multiple grass pollens, house dust mite, and short ragweed pollen. Her blood eosinophil count was normal. Ambulatory 24-hour dual pH-metry study demonstrated a pH of less than 4 for 2% of the total time at the distal probe, and no significant proximal reflux. She had no classic symptoms of GERD, but was started on combined therapy with pantoprazole sodium 40 mg twice daily and swallowed fluticasone propionate, 2 puffs of 220 μg swallowed twice daily for 6 weeks, based on the presumption that both reflux and EE were contributing to her airway inflammation and scarring. Nine months after completion of fluticasone therapy, EGD showed a visually normal esophagus with eosinophils rarely seen in biopsy specimens. Nine months after completion of the fluticasone therapy, laryngoscopy showed no inflammatory changes and a patent subglottis and posterior glottis. In the interim she became symptomatic for allergic rhinitis and was treated with nasal fluticasone. At 19 years of age, she has no laryngeal inflammation and has had no relapse in airway symptoms. She continues to require treatment with nasal fluticasone for allergic rhinitis.

*Histopathologic Analysis.* Eosinophil infiltrates were noted on H & E and anti-MBP staining in esophageal biopsy specimens taken from all 3 patients. Histomicrographic evaluation of H & E–stained tissue showed accumulated eosinophils within the superficial layer of the epithelium, with less intense staining and less eosinophilia in the submucosal tissues (Fig 4). Anti-MBP staining demonstrated intracellular and extracellular MBP granules within the tissues. Transmission electron photomicrography showed intact eosinophils and extracellular granules in all 3 patients (Fig 5). These images also suggested migration of the eosinophils toward the epithelial surface. This finding suggests that the process may start deeper in the esophageal mucosal layers, with subsequent migration of the eosinophils to the epithelial surface and inflammatory changes present throughout all of the tissue layers traversed.

## DISCUSSION

*Clinical Symptoms and Diagnostic Testing for Eosinophilic Esophagitis.* The link between EE and upper airway symptoms in children was highlighted by Orenstein et al,[2] who noted that 62% of patients with EE also had wheezing, pneumonia, sinusitis, or congestion. The first description of EE that appeared in the otorhinolaryngology literature[4] was of a 2-year-old girl with stridor, choking, vomiting, chronic laryngeal inflammation, and subglottic stenosis refractory to surgery. When antireflux therapy failed to prevent recurrence of her symptoms, she was treated with oral corticosteroids, improved dramatically, and was eventually decannulated. Aside from the dif-

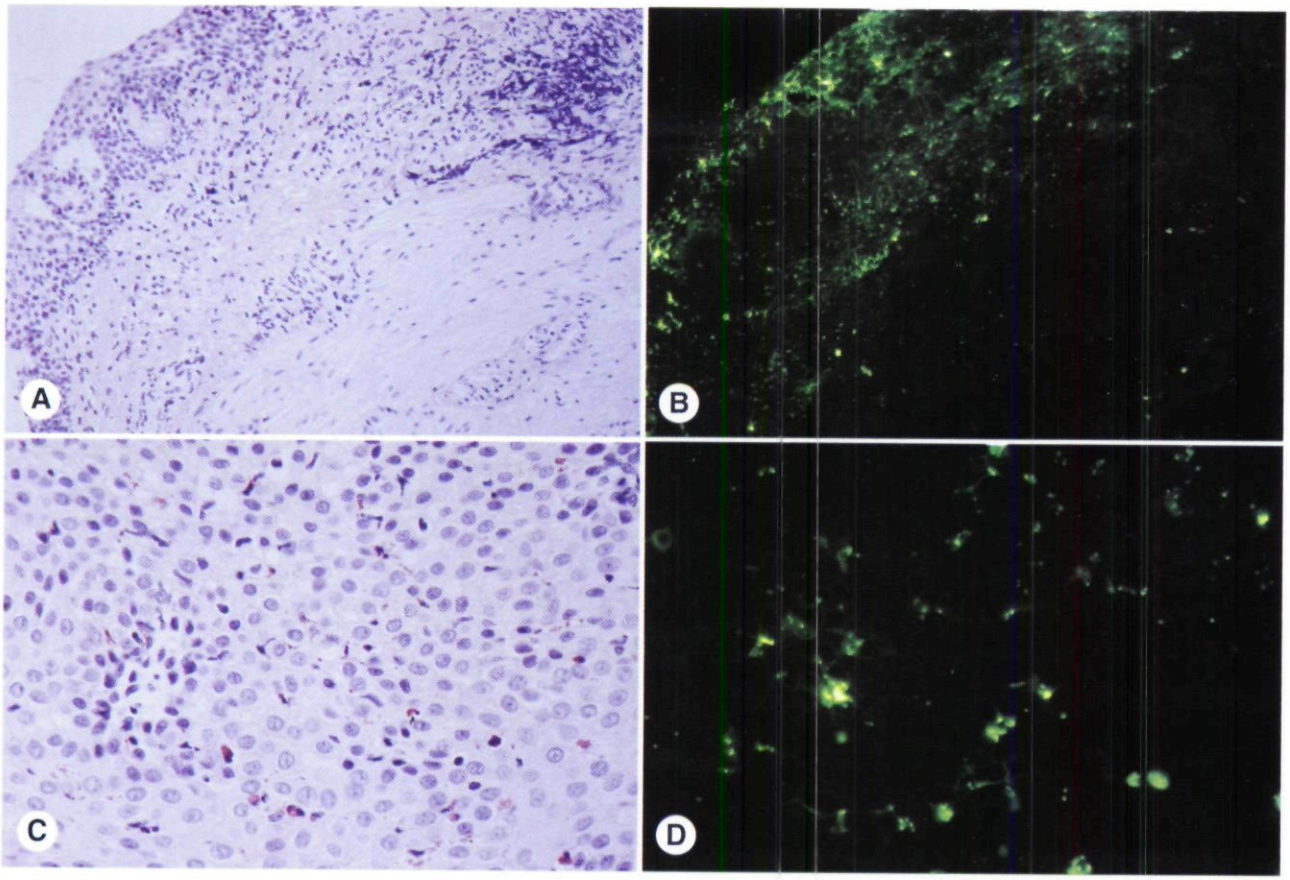


**Fig 4.** (Patient 1) Photomicrographs of esophageal biopsy specimens from patient with EE. **A,B)** Original ×160. **A)** H & E staining shows intraepithelial eosinophils that appear sparsely distributed. **B)** Eosinophils are much more visible with anti–major basic protein (MBP) immunofluorescence staining. MBP stain is densely concentrated along superficial surface of epithelium, with few eosinophils in underlying submucosa. **C,D)** Original ×400. Higher magnification shows **C)** H & E staining and **D)** anti-MBP immunofluorescence staining and demonstrates intact eosinophils (intracellular MBP) and punctate granules not in cells (extracellular MBP), but no diffuse staining of MBP. Note that these images may not seem to directly correspond, as images are from different slices of same tissue sample, taken 5 μm apart.

ferent treatment modalities (systemic versus topical administration of corticosteroids), the parallels between the histories of this child and our patient 3, in whom multiple attempts at airway reconstruction also failed before her diagnosis of EE, are striking. The findings of chronic laryngeal edema and mild subglottic stenosis in patients 1 and 2 suggest that EE may be another esophageal disease with an etiologic role in the development of chronic laryngeal inflammation and subglottic stenosis.

The presenting symptoms of EE in children are usually GI in nature. Two of the 3 children in this series had feeding symptoms. The typical symptoms in children include vomiting, regurgitation, epigastric pain, and poor eating.[1-3] Young children may demonstrate food refusal and poor weight gain, whereas adolescents experience dysphagia. Less common symptoms include growth failure and failure to thrive, hematemesis, and water brash.[3] There is substantial overlap between the symptoms of EE and RE. Previous reports emphasize that EE should be considered when patients presumptively treated for RE symptoms fail to respond to conventional therapy.[2,6,9,10] Similarly, our patient 1 had persistent symptoms despite maximal reflux treatment, and patient 2 had persistent symptoms despite Nissen fundoplication. Whether EE and GERD coexist in patients is controversial. Among the 3 patients we describe, the extent of reflux was variable according to ambulatory pH-metry studies. The results of ambulatory 24-hour pH-metry are typically reported as normal in patients with EE,[6,27] although EE and reflux may coexist, due to abnormal tone of the lower esophageal sphincter from severe inflammation.[6] Because of contrasting data from ambulatory 24-hour pH-metry studies in patients with EE, its role in the diagnosis and management of EE is yet to be determined. A contrast esophagram is less specific for EE, but an abnormality such as a Schatzki's ring,[5] esophageal strictures, and motility disturbances,[28,29] particularly in a symptomatic patient in whom reflux therapy has failed, should heighten the possibility of a diagnosis of EE.

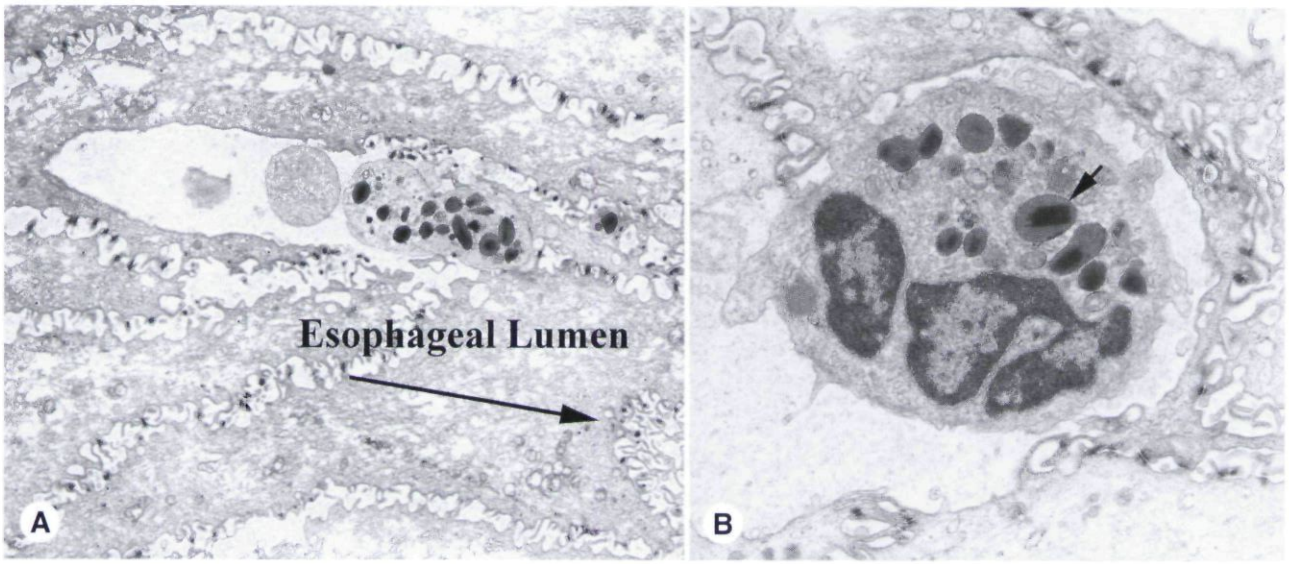


**Fig 5.** (Patient 2) Transmission photomicrographs of eosinophils in esophageal biopsy. **A)** Intact eosinophil is visible between epithelial cells (original ×3,000). Movement toward esophageal lumen is suggested by separation of tight junctions between epithelial cells and open space in vicinity of eosinophil. **B)** Intact eosinophil (arrowhead) with intact secondary granules demonstrates less electron-dense granule matrix with hyperelectron-dense MBP granule core (original ×7,500).

Many children with EE have atopic conditions, including asthma, atopic dermatitis, and environmental allergies. Aeroallergens and specific foods are the antigenic triggers. The most commonly implicated food allergies are milk, peanuts, and soy.[30] Whether the mounted response is IgE-mediated or non–IgE-mediated is debatable, but the literature suggests that both forms have a role in causing an eosinophilic response. For those with IgE-mediated allergic disease, radioallergosorbent tests (RASTs) and skin prick testing for specific antigens may provide useful information. Most authors show that many children who have EE have IgE-mediated allergies that can be diagnosed with skin prick testing and RASTs.[1,2,6,13,31,32] The 2 children in our series who had allergy testing demonstrated IgE-mediated allergies (to food in patient 2 and to aeroallergens in patient 3). However, negative RASTs or skin testing do not preclude the possibility of allergy antigen–induced EE. An additional group of children may have food antigen–induced EE hypersensitivity in the form of a type IV (cell-mediated) reaction. This subgroup of children with EE may have negative skin prick tests and RASTs for allergies (both IgE-dependent) but positive skin patch testing to food antigens (non–IgE-dependent) that show a delayed reaction (type IV).[14,30] Patch testing in those children who have no allergies detected by RAST or skin prick testing may help increase the identification of potential food allergies in the causation of EE and aid in its treatment.

Esophagoscopy with biopsy and histologic determination of the number of eosinophils per HPF is required to diagnose EE. Visual examination of the esophagus will often show unique features, including esophageal mucosal granularity, vertical linear furrowing as found in patient 1, concentric rings, and strictures.[2,6] A white pinpoint exudate on the esophageal mucosa, as found in patient 2, may also be present, corresponding to microscopic aggregates of eosinophils.[8] Strictures may be present in severe cases of EE, and may be more fragile than those seen in peptic disease, placing patients at greater risk for deep mucosal tears and perforation during examination and dilation.[6] As found in patient 3, visual abnormalities are not always present in EE, as dense eosinophilic infiltrates have been reported in cases that were visually normal on esophageal examinations.[13,30] Because of these inconsistencies between the gross appearance of the esophagus and abnormal histologic findings, multiple biopsy specimens from different levels of the esophagus (distal, middle, and proximal) are necessary to increase the diagnostic yield. Nevertheless, in the visually abnormal esophagus, directed biopsy sampling in areas of plaque, granularities, ring formation, and furrowing likely provides the greatest diagnostic yield.[13]

*Pathophysiology of Eosinophilic Esophagitis.* The pathophysiology of EE is not clearly understood, but the association between EE and other atopic diseases suggests immune dysregulation.[1] Two of our 3 patients underwent allergy testing. Patient 2 had significantly elevated serum IgE levels to multiple foods and seemed to respond well to both elimination of the implicated foods and swallowed

topical steroid therapy. Other studies describe similar findings; in general, more than half of patients with EE have food allergies according to in vitro serum tests for IgE or in vivo skin prick or skin patch testing.[1,2,31] Open challenges with implicated foods can produce nausea, vomiting, and other EE-associated symptoms in sensitized patients from 30 minutes to 8 hours after ingestion.[31] There still remains a substantial subset of patients with negative food tests in whom the clinical indications for EE are numerous. So, what are the other potential inciting factors?

Aeroallergens have been implicated in a murine model of EE. Mishra et al[33] demonstrated that induction of EE occurred as a result of intranasal allergen sensitization followed by topical delivery to the esophagus. Interestingly, when sensitization was attempted via application through the mouth or stomach, no significant esophageal eosinophilia was induced. The findings in patient 3 of our series support this hypothesis, as she had negative skin prick results to all foods tested, but positive responses to multiple common grass and weed pollen aeroallergens and symptoms of allergic rhinitis.

*Histopathology of Eosinophilic Esophagitis*. Histopathology is critical to the diagnosis of EE, with the diagnosis hinging upon the number of eosinophils per HPF. Therefore, esophageal endoscopy with biopsy should be obtained before treatment in patients in whom EE is a strong diagnostic possibility. Histopathologic findings in EE frequently overlap with those seen in RE. Increased mean papillary height and basal layer thickness may be seen in both diseases, but several features are more suggestive of EE, including the following: 1) eosinophils in both proximal and distal biopsy sites, 2) eosinophil aggregates or microabscesses, and 3) superficial layering of eosinophils close to the esophageal lumen, as seen in Fig 4.[2,34]

The number of eosinophils per HPF has also become important in distinguishing EE from RE. In 1993, Attwood et al[35] compared 12 adult patients with dysphagia and negative pH probe results to a reference group of 12 patients with GERD symptoms and positive pH probe results. In the group with dysphagia only, the average number of eosinophils per HPF was 56, whereas the group with GERD (based on pH results and symptoms) had only 1 eosinophil per HPF. Thus, these authors demonstrated a link between esophageal acid exposure and low-grade eosinophilia, but high-grade eosinophilia seemed to represent a different clinicopathologic entity.[35] At that time, little had been published on EE. Since that study, other authors have sought to establish a numerical threshold to distinguish the eosinophilic infiltration of EE from that seen in RE. Most studies agree that EE is diagnostic if the eosinophil infiltration is greater than 20 eosinophils per HPF, as seen in the example from our patient 1 (Fig 4).[2,3,14-16,36] However, typical EE symptoms and endoscopic findings are also reported in patients with fewer than 15 eosinophils per HPF.[13,30] Ruchelli et al[37] found that a level of at least 7 eosinophils per HPF provides 85% accuracy for diagnosing EE and 86% predictive value for failure of conventional antireflux and promotility therapy. The determination of a number of eosinophils per HPF that is diagnostic for pathologic EE is still evolving.

Features of EE in transmission electron photomicrographs were described by Justinich et al[38] in esophageal biopsy specimens from 12 patients with EE and compared to 3 controls. In EE specimens, the eosinophils showed signs of activation and movement through the vascular endothelium and into the mucosa. Eosinophils were absent from the biopsy specimens of the controls. No mechanistic interpretation of this finding in relation to the pathophysiology of EE was offered. As demonstrated in Fig 5, biopsy specimens taken from our 3 patients also showed signs of eosinophil activation with apparent movement of the eosinophils through the vascular endothelium toward the superficial layers of the esophageal mucosal tissue. We postulate that this migratory pattern of the eosinophils suggests that the antigen is present in the esophageal epithelial layer and that the eosinophil activation and migration in that direction is in response to the antigen. The effect on the tissue of this migratory response has yet to be determined, but it is very possible that it causes some type of chronic inflammatory response. This notion is supported by the findings from high-resolution esophageal ultrasound studies that show significant expansion of the esophageal wall and individual tissue layers, including the combined mucosa and submucosa and the muscularis propria, in children with EE in comparison with healthy control patients.[39]

*Major Basic Protein and Its Pathophysiologic Significance*. Eosinophil MBP deposition in esophageal tissue of patients with EE has been described. Furuta et al[22] reported an immunohistopathologic comparison between 17 pediatric patients with EE and a control group of 15 patients with chronic abdominal pain. Using an anti-MBP polyclonal antibody, they found that all patients with EE had both intracellular and extracellular MBP, whereas MBP was absent in the specimens from the controls.[22] A similar study demonstrated that extracellular MBP deposition was

seen in adults with EE and food impaction as compared to adults with food impaction without EE.[23] The significance of MPB in the pathophysiology of EE had not been explored. The potential pathologic role of eosinophils in EE may be inferred from extensive studies on the role of eosinophils in asthma and chronic rhinosinusitis. Eosinophils degranulate and release MBP, which likely plays an important role in the clinical manifestations of these diseases. Both eosinophils and MBP have been found to induce bronchial hyperreactivity, cause bronchoconstriction, and directly damage epithelium.[17,18] Major basic protein also appears to act as an allosteric antagonist to the M2 subtype of muscarinic receptors in presynaptic parasympathetic nerve terminals.[21] These receptors normally bind to acetylcholine (ACh) and signal the cell to decrease the amount of ACh released, in a negative feedback loop. By blocking the binding of the receptors to their normal substrate, MBP interrupts normal feedback inhibition. The consequence is excessive smooth muscle contraction secondary to excessive release of ACh. We hypothesize that similar mechanisms may be at work in EE, whereby MBP-induced smooth muscle contraction elicits esophageal dysmotility and the symptoms of abdominal pain, dysphagia, nausea, and vomiting. Major basic protein also incites tissue injury and edema, and it may further compromise the esophageal lumen, leading to strictures and other visible abnormalities.

*Treatment Strategies for Eosinophilic Esophagitis.* Swallowed topical corticosteroids have emerged as a cornerstone of treatment since 1998, when Faubion et al[40] described 4 pediatric patients with EE who were successfully treated with this therapy. Subsequently, Teitelbaum et al[1] demonstrated the relative safety and efficacy of this treatment in a prospective study of 15 children with EE. The patients enrolled in their study were divided into 2 treatment groups. The first group of 11 patients had food allergies and initially began diet restriction; the second group of 4 patients had no food allergies and began taking swallowed fluticasone propionate. None of the 11 patients on diet restriction experienced symptomatic relief; 9 of these 11 were moved into the swallowed fluticasone arm. All 13 patients ultimately treated with swallowed fluticasone experienced resolution of their EE symptoms. Posttreatment endoscopy, performed on 11 of these 13 patients, demonstrated resolution of the eosinophilic infiltrate. There were no major complications, and only 1 minor complication, esophageal candidiasis, which was easily treated.[1]

Fluticasone is the agent most commonly used in swallowed topical corticosteroid therapy, and it is delivered by a standard metered-dose inhaler. The patient is taught an inhaler technique in which a spacer is not used and the goal is to swallow at least 80% of the medication. The usual dosage range (depending on the child's size and disease severity) is 44 to 220 μg per puff, 2 to 4 puffs twice daily for 6 weeks. This technique delivers the active agent directly to the inflamed area with little GI absorption and with rapid first-pass hepatic metabolism, and it avoids the side effects associated with oral corticosteroids. The drawbacks of swallowed topical corticosteroids include occasional intolerance of the medication, oral thrush or esophageal candidiasis, and inadequate potency if deeper tissue layers are involved (in more severe cases). Patients are instructed to rinse the mouth with water after taking a dose to decrease the risk of thrush and to avoid any other oral intake for half an hour to prevent dilution of the medication. All 3 of our EE patients began swallowed topical corticosteroid treatment and achieved a positive response. Patient 2 was the only one with a minor adverse reaction; he developed esophageal candidiasis that responded to conventional antifungal therapy.

Oral corticosteroids have been central to the treatment of many diseases of immune dysregulation and may prove beneficial in children with EE who are intolerant of, or unresponsive to, swallowed topical corticosteroids. In a prospective trial of a 4-week course of methylprednisolone for EE, dramatic histopathologic improvement (from a mean of 34 to a mean of 1.5 eosinophils per HPF) was noted in 19 of 20 pediatric patients with EE.[41]

Elimination diets may be a primary or adjunctive treatment in the management of EE, particularly among patients with food allergies. In contrast to the poor response to diet restriction previously described, Orenstein et al[2] demonstrated that 10 of 12 EE patients with food allergies who were placed on elimination diets benefited from this therapy. Finally, for severely refractory cases, elemental formulas such as Neocate have been successful with stepwise reintroduction of suspect foods.[32] Unfortunately, the formulas are notoriously bad-tasting, compromising compliance markedly. Alternatively, patients may be fed the formula through a transnasal or gastric feeding tube, but this is the most onerous of all treatment options. As seen in our patient 2, not all respond to elimination diets alone, and some patients require other treatment modalities.

Antireflux medications have usually been tried in this patient population by the time EE is considered a diagnostic possibility. Nevertheless, antireflux medications may be used concurrently with one of the EE-directed therapies just reviewed, if patients

have had some (albeit incomplete) improvement on them, or if pH studies have been positive. Regardless of the treatment, symptom relapse after completion of therapy may occur in more than half of patients afflicted with EE, so there is a need for good patient and parent education and close follow-up.[8] As in the cases of patients 1 and 2, symptom relapse after treatment suggests that EE is a chronic disease.

## CONCLUSIONS

Eosinophilic esophagitis is a unique clinicopathologic entity that has gained attention over the past decade, but has not been well recognized among otorhinolaryngologists. Additionally, there has been a general lack of consensus on the histopathologic criteria used to establish a diagnosis of EE. Thus, EE is likely underrecognized and underdiagnosed.

Patients with EE may first consult an otorhinolaryngologist because of concomitant swallowing and airway complaints. All 3 of our patients had some degree of airway inflammation or subglottic narrowing on microlaryngoscopy and had more than 20 eosinophils per HPF on esophageal biopsies. The histopathologic findings described here closely parallel the findings in other reviews of EE, as well as the findings in the related disorders of asthma and chronic rhinosinusitis, with intact eosinophils and eosinophilic granules densely infiltrating the superficial esophageal epithelium. The cytotoxic protein eosinophil MBP present in the intracellular and extracellular tissue may play a role in the pathophysiology of the disease and its symptoms.

Awareness and recognition of EE is critical, as successful treatment regimens are available, including swallowed topical steroids, oral corticosteroids, elimination diets, and elemental formulas. Regardless of the treatment employed, relapse is common, so close follow-up is highly recommended.

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