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ORIGINAL ARTICLE

Esophageal epithelial immunoglobulin G is an important marker for the diagnosis and management of pediatric eosinophilic esophagitis

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Abstract

Background and Aim: Our primary aim was to describe the prevalence of immunoglobulin G (IgG) and its subclass IgG4 in immunohistochemistry staining in esophageal biopsy specimens of children with eosinophilic esophagitis (EoE) compared with that of specimens from children with gastroesophageal reflux disease (GERD).

Methods: Esophageal biopsy specimens from children with EoE or GERD were stained prospectively for IgG and IgG4 antibodies. Subjects with EoE were divided into cohorts with either active EoE or EoE in remission. Active EoE cases were further divided into proton pump inhibitor responsive (PPI-r) and PPI-nonresponsive (PPI-nr) subgroups. Demographic, clinical, and histologic data were compared among groups, including quantified IgG and IgG4 staining, peak eosinophil count, eosinophil-derived neurotoxin levels, and EoE endoscopic reference score.

Results: Seventy-nine children (aged 10.6 ± 5.6 years; 68% male) were enrolled. IgG-positive cell counts were significantly elevated in those with active EoE ($n = 29$, 3 [interquartile range, IQR: 2–6]/high-powered field [HPF]), compared with those having EoE remission ($n = 25$, 1 [IQR: 0–2]/HPF; $P = 0.002$) and those with GERD ($n = 25$, 0 [IQR: 0–0.25]/HPF, $P = <0.0001$). IgG-positive cell counts were significantly higher in the PPI-r ($n = 15$, 5 [IQR: 2.5–11]/HPF) subgroup, compared with the PPI-nr subgroup ($n = 11$, 3 [IQR: 1.5–3.5]/HPF; $P = 0.041$) at baseline endoscopy.

Conclusion: Initial esophageal tissue biopsy specimens from pediatric subjects with active EoE showed a significant increase in IgG-positive staining compared with tissue from subjects in EoE remission or with GERD. There was higher positivity of IgG-stained cells in the PPI-r subgroup compared with the PPI-nr subgroup.

Introduction

Eosinophilic esophagitis (EoE) is a chronic disorder with eosinophil-predominant inflammation. It is frequently associated with atopic conditions, including food allergy, asthma, and atopic dermatitis.[1,2] Since the early 1990s, the prevalence of EoE has increased significantly in children and is currently 34.4 cases per 100 000.[3,4] Differentiating gastroesophageal reflux disease (GERD) from EoE is challenging because of overlapping clinical and histologic features. However, the pathogenesis, natural history, and treatments for these conditions differ considerably, so it is important to find diagnostic tools to improve differential diagnosis.

The main treatment modalities for EoE include diet modification, pharmacotherapy, and, in patients with advanced disease, esophageal dilation.[5] Medications include proton pump inhibitors (PPIs), biologic drugs, and topical steroids.[6,7] Frequently, PPI is given initially and followed by other therapies if there is no histologic remission.[6,7] Since EoE management in children requires multiple endoscopies with sedation, identifying specific biomarkers to predict PPI responsiveness may help reduce the number of procedures required and also differentiate EoE from GERD.

Potential biomarkers may include immunoglobulin G (IgG) and its subclass IgG4. Studies of tissue biopsies from adults with EoE show elevated esophageal IgG4-positive cells compared with biopsies from controls[8–10]; however, the diagnostic role of IgG4 in pediatric EoE remains inconclusive.[11–13] Currently, it is not known whether the presence and elevation of IgG4 indicates a humoral antibody response to chronic antigen exposure, or whether it indicates EoE pathogenesis. Therefore, our primary aim was to describe the prevalence of IgG and its subclass IgG4 immunohistochemistry staining in esophageal biopsies of children with EoE compared with biopsies from children with GERD. A secondary objective was to use IgG and IgG4 staining to help in detecting a response to PPI therapy in active EoE subjects.

Methods

We performed immunohistochemistry staining for IgG and IgG4 on previously collected esophageal biopsies from children ($n = 79$) with known EoE or GERD, between January 2018 and March 2020. Enrolled subjects included children with active EoE, GERD, and EoE in remission (mean age 10.6 ± 5.6 SD years; range 1–20; 68% male) who underwent esophagogastroduodenoscopy (EGD) for clinical diagnosis (Table 1). Patient data (demographic data, presenting symptoms, laboratory results, as well as medical history of eczema, allergic rhinitis, environmental allergies, asthma, and/or food allergies) were accessed from patients' electronic medical records (Table 1).

Patient data and tissue samples were anonymized. The study was approved by the Institutional Review Board of the Arnold Palmer Hospital for Children in Orlando, Florida.

Study cohorts. The diagnosis of active EoE ($n = 29$) was based on symptoms of esophageal dysfunction, and the presence of >15 eosinophils (eos) per high-powered field (HPF) in at least one biopsy of the esophagus on baseline endoscopy with histopathologic features of EoE including eosinophil micro-abscess formation, superficial layering of eosinophils, and extracellular eosinophilic granules, with some having subepithelial fibrosis of lamina propria. Within the active EoE, subjects were subdivided into PPI-responsive (PPI-r) and PPI-nonresponsive (PPI-nr) subgroups based on their response to high-dose PPI therapy (1 mg/kg per dose twice a day [BID]). PPI-r EoE was diagnosed in 15 patients based on histologic demonstration of ≥15 eos/HPF prior to PPI therapy, with histologic normalization following 8 weeks of PPI therapy.[5] PPI-nr EoE was diagnosed in 11 patients based on histologic demonstration of ≥15 eos/HPF prior to PPI therapy, without histologic normalization following 8 weeks of PPI therapy. The remaining three subjects were "unknown" for their response to PPI. These patients did not receive the proper treatment of 1 mg/kg BID dosing of PPI initially. Furthermore, because these patients had >15 eosinophils in at least one of the biopsies, they were included in the "active EOE" group.

The EoE in remission ($n = 25$) group consisted of subjects with a history of EoE on initial biopsy who were treated and became asymptomatic and had esophageal eosinophilia with ≤15 eosinophils/HPF.[5] Thirteen out of 25 subjects obtained remission following 8 weeks of high-dose of PPI therapy. The remaining 12 subjects obtained remission by topical steroids ($n = 8$), diet elimination ($n = 3$), or a combination of both ($n = 1$). The GERD group included 25 children who had eos/HPF <15 and responded well to acid suppression therapy. These subjects had histopathologic features of GERD including spongiosis and basal cell hyperplasia without any eosinophilic infiltration (eosinophilic micro-abscess formation, superficial layering of eosinophils, extracellular eosinophilic granules, subepithelial fibrosis of lamina propria). Additionally, these subjects had typical symptoms of GERD including acid reflux, regurgitation, heartburn, and/or chest pain with supportive evidence of esophagitis on endoscopy or in esophageal biopsy and correlated with intraluminal impedance-pH monitoring (MII-pH) or wireless (Bravo) capsule study results.

Immunohistochemical staining for IgG and IgG4. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue samples from esophageal biopsies. Sections of 4-μm paraffin-embedded tissue were mounted on coated slides, dewaxed, and rehydrated using standard techniques. Antibodies were prediluted, and staining was performed per manufacturer's specifications using a HiDef Detection horseradish peroxidase (HRP) polymer system on an open automated staining platform. Samples were stained with anti-IgG and anti-IgG4 antibodies (Cell Marque). Intracellular staining for B-cell lineage including plasma cells was considered positive staining. Nonspecific staining, such as extracellular staining of the epithelial cells, was considered negative staining and was not counted in the analysis. To quantify IgG and IgG4 positivity within any given tissue section, IgG and IgG-positive plasma cells were manually counted in five distinct HPFs at 400× magnification using an eyepiece with a 22-mm field of view. Normal reference values in the esophagus of IgG and IgG4 cells per HPF are limited, especially in pediatric literature. Similar to other studies, we have used GERD cohort as control[9,10] and expected minimal positivity of IgG and IgG4. Esophageal brushing samples were obtained and analyzed for eosinophil-derived neurotoxin (EDN), a well-established marker of disease activity, as reported by

Smadi *et al.*[14] Cases were blinded for patient groups, and clinical characteristics were reviewed by a pathologist with expertise in pediatric eosinophilic gastrointestinal disease.

TABLE 1 Patients' demographic, clinical, and laboratory variables

	EoE ($n = 54$)	GERD ($n = 25$)	*P*-value
Age, mean (±SD), years	10.6 ± 5.57	10.5 ± 5.9	0.245
Male (%)	80	56	0.117
Race/ethnicity, *n* (%)			
Non-Hispanic White	31 (57)	18 (72)	0.383
Hispanic or Latino	15 (27.7)	3 (12)	
African American or Black	4 (7.4)	1 (4)	
Other	4 (7.4)	3 (12)	
Medical history, *n* (%)			
Atopic disorders	47 (87)	15 (60)	0.002
Asthma	27 (50)	8 (32)	0.152
Food allergy	27 (50)	2 (8)	<0.001
Allergic rhinitis	12 (22)	6 (24)	1.00
Eczema[†]	7 (12.9)	0	
Environmental and seasonal allergies	3 (5.5)	1 (4)	

[†]Comparison not conducted; did not meet statistical assumptions.
EoE, eosinophilic esophagitis; GERD, gastroesophageal reflux disease.

Statistical analysis. Descriptive statistics are reported as mean (±SD), frequency, and percent. Statistical analysis included Chi-square test, Fisher exact test, independent *t*-tests, Mann–Whitney *U* test (reporting the median with interquartile range), and Pearson *r* test. All statistical significance is defined with a *P*-value of <0.05. Associations among the three groups with EDN, peak eosinophil count (PEC), and EoE reference score (EREFS) were identified using the Pearson correlation coefficient. Statistical testing was conducted using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

Results

Compared to the GERD cohort, the EoE showed an increased prevalence of atopic disorders (asthma and food allergy) (87%; $P = 0.002$) (Table 1). The median EREFS in patients with active EoE (EoE group, $n = 29$) was 3 [IQR: 2–4] compared with 0 [IQR: 0–2] for patients with EoE in remission ($P \leq 0.001$). EDN was higher in patients with active EoE than in those in remission (70 [IQR: 23–161] *vs* 0.4 [IQR: 0–4.1]; $P \leq 0.0001$) and PEC also differed significantly between these two groups (50 [IQR: 30–75] *vs* 0 [IQR: 0–3]; $P \leq 0.0001$ [Table 2]). Atopic disorders were less prevalent in patients with GERD. Subjects in this group had a mean DeMeester reflux score of 35.72 ± 32.43 (normal < 14.72) and Boix-Ochoa reflux score of 34.71 ± 26.70 (normal < 11.99).

Table 2 Median immunoglobulin G (IgG) staining (interquartile range [IQR]) between patient groups

				P-value	
	Active EoE ($n = 29$)	Remission EoE ($n = 25$)	GERD ($n = 25$)	Active *versus* remission EoE	Active EoE *versus* GERD
IgG	3 [IQR: 2–6]/HPF	1 [IQR: 0–2]/HPF	0 [IQR: 0–0.25]/HPF	<0.002	<0.0001
IgG4[†]	0 [IQR: 0]/HPF	0 [IQR: 0]/HPF	0 [IQR: 0]/HPF	0.4354	0.284
EREFS	3 [IQR: 2–4]	0 [IQR: 0–2]	NA	<0.0001	
PEC	50 [IQR: 30–75]	0 [IQR: 0–3]	NA	<0.0001	
EDN	70 [IQR: 23–161]	0.4 [IQR: 0–4.1]	NA	<0.0001	

[†]Comparison not conducted; did not meet statistical assumptions.
EDN, eosinophil-derived neurotoxin; EoE, eosinophilic esophagitis; EREFS, eosinophilic esophagitis reference score; GERD, gastrointestinal esophageal reflux disease; HPF, high-powered field; NA, not applicable; PEC, peak eosinophil count.

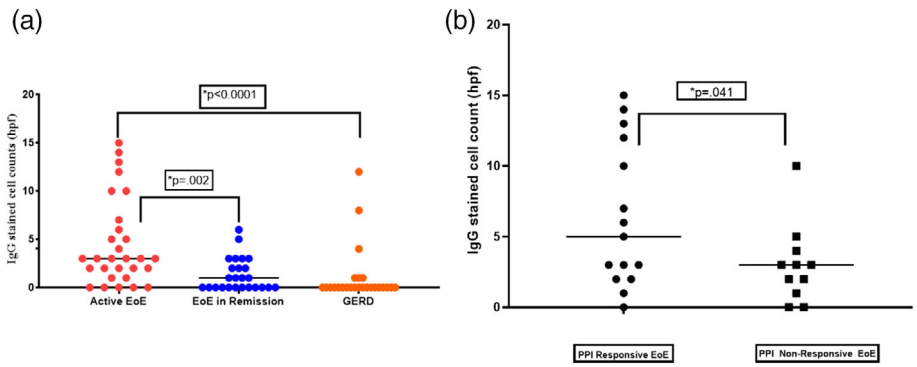

Figure 1 (a) Box and whisker plots showing immunoglobulin G (IgG)-positive staining was significantly elevated in active eosinophilic esophagitis (EoE) compared with EoE in remission and gastroesophageal reflux disease (GERD). (b) Box and whisker plots showing that in the active EoE group the IgG-positive cell counts were significantly higher in the proton pump inhibitor (PPI) responsive subgroup compared with nonresponsive subgroup at baseline endoscopy. HPF, high-powered field.

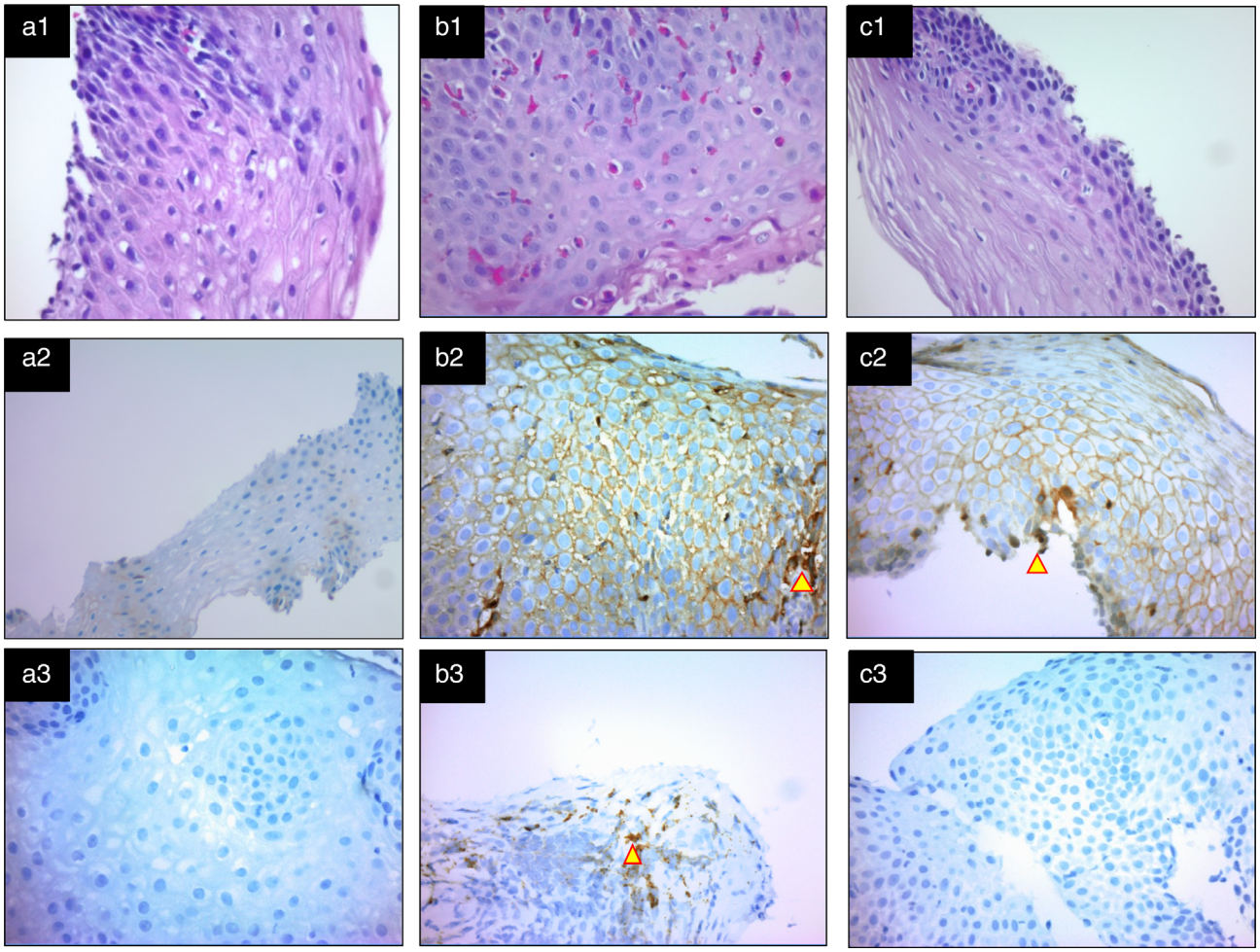

Figure 2 Epithelial immunoglobulin G (IgG) and IgG4 staining at 400× magnification showing increased IgG and IgG4 in active eosinophilic esophagitis (EoE) compared with EoE in remission and gastroesophageal reflux disease (GERD). (a1) GERD HE staining showing basal cell hyperplasia and lamina propria papillae elongation into upper epithelium. (a2) IgG staining in GERD. (a3) IgG4 staining in GERD. (b1) Active EoE (HE stain) showing increased eosinophils in squamous mucosa concentrated in the surface epithelium. (b2) IgG staining in active EoE showing positive lymphocytes located between epithelial cells. (b3) IgG4 staining in active EoE showing some background positive lymphocytes. (c1) Remission EoE (HE stain) showing absent eosinophils within the surface epithelium. (c2) IgG staining in remission EoE showing scant positivity in lymphocytes located between epithelial cells. (c3) IgG4 staining in remission EoE.

The median of IgG-positive cells was significantly elevated in patients with active EoE (3 [IQR: 2–6]/HPF) compared with that in EoE in remission (1 [IQR: 0–2]/HPF; $P = 0.002$), and GERD (0 [IQR: 0–0.25]/HPF, $P \leq 0.0001$) (Figs 1,2). Within the active EoE group, IgG-positive cell counts were significantly higher in the PPI-r subgroup ($n = 15$, 5 [IQR: 2.5–11]/HPF) compared with the PPI-nr subgroup ($n = 11$, 3 [IQR: 1.5–3.5]/HPF, $P = 0.041$) at the baseline endoscopy (Figs 1b,3).

Within the entire EoE subjects ($n = 54$), 14.8% tested positive for IgG4, five had PPI-r EoE, and three had PPI-nr EoE. Only one subject was positive for IgG4 staining in the GERD cohort. In subjects with active EoE, there was a moderate correlation between IgG-positive cell counts and the PEC (Pearson correlation 0.560) ($n = 29$, $r = 0.560$ and $r^2 = 0.314$, $P = 0.02$). Correlations of IgG-positive cell counts with PEC, EREFS, and EDN are summarized in Table 3.

Discussion

We found a significant increase in IgG-positive cells in pediatric patients with active EoE compared with patients in EoE remission or with GERD. We observed a decrease in both IgG and IgG4-positive cells in patients with EoE in remission and a statistically significant correlation between PEC and IgG positivity in all active EoE cases. A possible hypothesis is that both IgG and IgG4-positive cells are involved in EoE pathogenesis, with immune complex deposition in response to exposure to specific food antigens. However, how IgG4 contributes to EoE pathogenesis remains unclear. Production of IgG4 is a result of Th2 cytokines through the activation of the naïve B cells. After chronic exposure to the antigen, excess Th2 responses are known to induce Treg cells, which secrete interleukin 10 (IL-10), inducing class-switching to IgG4. Aside from Treg cells, B-regulatory

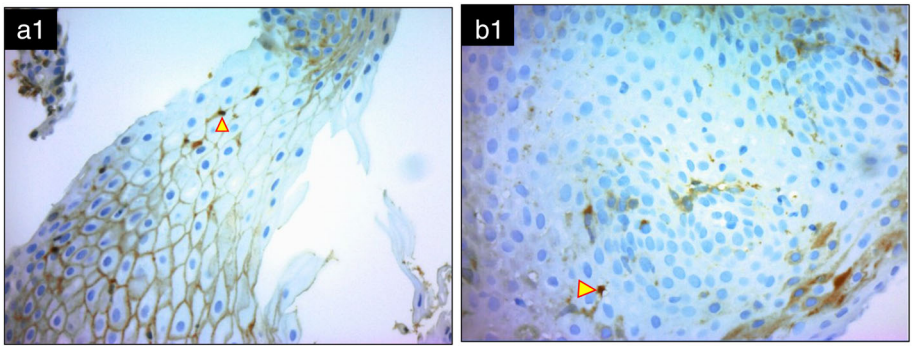

Figure 3 Immunoglobulin G (IgG) and IgG4 staining (400× magnification) showing increase IgG4 and IgG in proton pump inhibitor responsive (PPI-r) EoE at baseline endoscopy. (a1) Increased IgG expression in PPI-r eosinophilic esophagitis (EoE). IgG-positive lymphocytes are located between epithelial cells in a background of nonspecific staining. (a2) Increased IgG4 expression in PPI-r EoE. Occasional IgG4-positive lymphocytes are seen located between epithelial cells in a background of nonspecific epithelial staining.

Table 3 Correlation of immunoglobulin G (IgG)-stained cell counts with peak eosinophil count (PEC), eosinophilic esophagitis reference score (EREFS), and eosinophil-derived neurotoxin (EDN)

Group	*n*	*r*	r^2	*P*-value
PEC				
Active EoE	29	0.560	0.314	0.02
PPI-r	15	0.744	0.554	0.001
PPI-nr	11	−0.090	0.008	0.793
Remission EoE	25	0.549	0.301	0.004
EREFS				
Active EoE	29	0.273	0.075	0.076
PPI-r	15	0.352	0.124	0.099
PPI-nr	11	0.570	0.325	0.068
Remission EoE	25	0.485	0.235	0.007
EDN				
Active EoE	24	0.138	0.019	0.522
PPI-r	14	0.127	0.016	0.666
PPI-nr	8	0.756	0.572	0.03
Remission EoE	21	0.222	0.049	0.334

EoE, eosinophilic esophagitis; PPI, proton pump inhibitor; PPI-nr, PPI non-responsive; PPI-r, PPI responsive.

cells and eosinophils are potential sources of IL-10.[15,16] IgG4 may be a compensatory mechanism to dampen the ongoing response of type 2 inflammatory responses in EoE because of the structural properties of IgG4. IgG4 possesses a short hinge and low Fab arm flexibility and can undergo a process termed "Fab arm exchange," which precludes cross-linking of identical antigens and has a low affinity for the Fc-γ activating receptors.[17,18] Furthermore, additional clinical studies in both adult and pediatric EoE cohorts are needed to understand whether IgG4 directly contributes to the pathogenesis of EoE or it is a downstream response to excess Th2 cytokines and eosinophils.

We found a statistically significantly higher prevalence of IgG-positive cells in patients responsive to PPI therapy compared with those not responsive to PPI. We also found more IgG4-positive cells in EoE patients responsive to PPI compared with those not responsive to PPI, although the number of cases was small. To our knowledge, this is the first study to observe that IgG positivity may help in detecting response to PPI therapy in children, although it remains unclear why certain patients with EoE respond to PPI while others do not.[2,7] We speculate that PPI-r EoE patients initially may have an acid-induced injury to the epithelial barrier, which subsequently exacerbates food protein intolerance and leads to excessive elevation of IgG and IgG4.

The prevalence of IgG4-positive cells was very low overall and highest in patients with active EoE. We did not calculate the sensitivity and specificity of this result because of the small sample size. Although EoE and other IgG4-related disorders share some common characteristics (male dominance, steroid responsive, and progression to fibrosis if untreated),[8,19–21] EoE is not a typical IgG4-related disorder.[8,19–21] Unlike other IgG4-related disorders, total serum IgG4 is only minimally increased in EoE.[20] In contrast to similar studies of pediatric patients with EoE,[11–13] we were unable to obtain high sensitivity and specificity for our IgG4 results. A possible explanation may be that most of the biopsy samples contained only squamous epithelium, which has fewer IgG4 cells than the lamina propria.[8,13] We speculate that IgG4 plasma cells are further downstream for response, as they are usually seen in the lamina propria. However, IgG tends to have nonspecific antibody response and present in excess in the epithelium of EoE due to excess Th2 cytokines from chronic antigen exposure. In addition, the variability of IgG4 among other EoE studies may reflect the non-uniform nature of EoE disease distribution.[22]

Study limitations include the retrospective sample collection, the small sample size, and a single-center study. However, our overall sample size was larger than that of previous pediatric studies.[11,12] Larger prospective studies are warranted in the future to replicate our findings. In addition, we did not assess fibrosis markers, although none of the patients with EoE developed stricture. Further prospective controlled studies are needed to elaborate this relationship and explore the effects of therapy on IgG and IgG4 depending on treatment options.

Endoscopy with tissue biopsy remains the standard for diagnosing and monitoring EoE. Developing prognostic biomarkers to determine which cases will respond to PPI therapy, to predict the risk for fibrosis and to reduce the number of invasive

procedures required, is an important goal for managing pediatric EoE. Our results suggest that using IgG immunostaining may help differentiate EoE from GERD and identify patients with PPI-r EoE. Our results were inconclusive regarding the role of IgG4 immunostaining in differentiating EoE from GERD.

In conclusion, further study of IgG and IgG4 immunostaining of esophageal biopsies of pediatric patients with EoE is needed to clarify the use of specific immunoglobulin subclasses as disease-specific and potentially treatment-specific biomarkers. More studies are also needed to elucidate the mechanism.

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