

Increased Prevalence of Airway Reactivity in Children With Eosinophilic Esophagitis

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Summary. Rationale: Asthma is prevalent in children with eosinophilic esophagitis (EoE) estimated at 24–42% in prior studies versus 9% for the general population. However, pulmonary function and airway hyperresponsiveness (AHR) in children with EoE have not been previously defined. Methods: A cross-sectional prospective study was conducted of children ages 7–18 years with EoE and healthy controls. Methacholine bronchial challenge and exhaled nitric oxide were assessed. As measures of atopy and immune activation, peripheral blood was analyzed for total IgE, specific IgE to selected aeroallergens, eosinophil count, and serum cytokines including eotaxin. Results: EoE subjects ($n = 33$) and healthy controls ($n = 37$) demonstrated similar, normal baseline spirometry. AHR occurred in 33% of children with EoE and 11% of healthy controls ($P = 0.04$; 95% confidence intervals [19%, 52%] and [4%, 26%], respectively). The majority of EoE subjects with AHR had no prior diagnosis of asthma. Overall, 69.7% of EoE subjects had either asthma or AHR. For EoE subjects, total serum IgE was the only biomarker associated with a greater risk of AHR (OR = 9.643, 95%CI 1.633, 56.925). EoE subjects with and without asthma were similar to healthy controls in mean levels of serum cytokines (IL-5, IL-9, EGF, FGF-2, eotaxin). In exploratory analyses, the subgroup with EoE and asthma without asthma controller therapy had higher mean FGF-2 than EoE subjects without asthma (110 pg/ml vs. 65 pg/ml, $P = 0.0426$). Conclusions: Asthma and AHR may be more prevalent than previous estimates in children with EoE. For subjects with EoE, elevation in serum IgE was associated with a greater risk of AHR. *Pediatr Pulmonol.* 2015; 9999:1–8 © 2015 Wiley Periodicals, Inc.

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INTRODUCTION

Eosinophilic esophagitis (EoE) is an inflammatory condition of the esophagus driven in large part by allergic mediators. Children with EoE are likely to have other forms of atopic disease, such as asthma, eczema, and allergic rhinitis, although not all children with EoE will manifest each of these conditions. Asthma prevalence in children with EoE has previously been estimated at 24–42%, although all prior data regarding asthma prevalence in children with EoE has been based on family reported history.^{1,2} The diagnosis of asthma is often made with the aid of spirometry, and may utilize bronchoprovocation by methacholine challenge testing. Methacholine challenge testing (MCT) allows one to confirm the presence of airway hyperresponsiveness (AHR), a finding that offers support to a diagnosis of asthma, and that can be detected in asymptomatic children with subclinical asthma. The presence of airway hyperresponsiveness has been associated not only with current asthma, but with an increased risk for future development of asthma.³ Thus it is possible that children with EoE will

have rates of AHR that are higher than previous estimates of asthma prevalence, which would raise the possibility of

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undiagnosed current asthma, or an increased risk for future asthma in this population.

Children with EoE frequently, but inconsistently, demonstrate elevations in serum markers of atopy, including serum IgE levels, peripheral eosinophil counts, and Th2-related cytokines (IL-4, IL-5, IL-13).^{1,2} In fact, there are many patients with eosinophilic esophagitis and other atopic conditions diagnosed in the absence of cellular or molecular indicators of atopy. Other biomarkers of atopy linked to the pathogenesis of EoE and asthma include eotaxin, epidermal growth factor (EGF) and fibroblastic growth factor 2 (FGF-2).^{4–11} Based on currently available literature, it is not clear whether serum markers of atopy may have clinical utility in identifying patients with EoE that may be at greater risk for AHR, since prior studies have only examined atopic markers in the setting of one disease state at a time.

The primary aim of this study is to compare pulmonary function and AHR for children with EoE as compared to healthy controls. The secondary aims are: (1) to determine whether certain clinical factors (e.g., prior diagnosis of asthma, use of EoE, or asthma medications) affect the prevalence of AHR; (2) investigate whether biomarkers such as Th2 cytokines or eotaxin show greater elevations in children with both EoE and asthma compared to children with only EoE, and whether those biomarkers are predictive of AHR. While peripheral blood testing for specific IgE has had very limited clinical utility in EoE or asthma alone in prior studies, this study examines whether subjects with both AHR and EoE may be a subgroup with a higher rate of aeroallergen sensitization as measured by specific IgE. The authors hypothesized that children with EoE have rates of AHR that are higher than previous estimates of asthma prevalence in this population, and that children with both asthma and EoE would be more likely than those with EoE only to have serum elevations of atopic markers.

METHODS

This study was approved by the Indiana University IRB prior to initiation. All subjects provided signed assent, and at least one parent provided informed consent.

Study Population

Subjects with an existing diagnosis of EoE were recruited through the James Whitcomb Riley Hospital for Children Gastroenterology clinic, using an existing eosinophilic esophagitis database. Subjects were eligible if they were age 7–18 years, had an esophageal biopsy with >15 eos/hpf within the last year, were on stable medications for at least 4 weeks (including no systemic antibiotics or systemic corticosteroids in the 4 weeks prior to enrollment), and had no forms of lung disease other than asthma or allergies. Subjects were stratified

based on presence or absence of physician diagnosed asthma, and whether the subject was currently using asthma controller medications (including inhaled corticosteroid, leukotriene receptor antagonist, long acting beta agonist).

Concurrently, healthy control (HC) subjects were recruited from the Indianapolis area, age 7–18 years, with no prior history of any of the following: eosinophilic esophagitis, asthma, any other form of lung disease, wheezing, or use of inhalers or nebulizer treatments. Patients with symptoms of allergic rhinitis, food allergy, or eczema within the last 12 months were also excluded. HC subjects were recruited through local print and electronic advertising, from the general population. Subject recruitment for HC and EoE subjects occurred between November 2009 and June 2012.

Study Procedures

A cross-sectional study design was used, with prospective pulmonary function testing and blood analysis, and retrospective chart review of existing medical records.

At enrollment, demographic data, medical history, including prior diagnosis of atopic diseases, other medical conditions, current medications, and family history of atopic diseases, and physical examination were performed for all subjects. For EoE subjects, the following were also obtained by history and examination of existing medical records: date of diagnosis of EoE, prior proton pump inhibitor (PPI) treatment for EoE group, results of prior allergy testing, date of most recent esophageal biopsy, number of eosinophils per high power field (eos/hpf) on most recent esophageal biopsy, current EoE treatments.

To estimate presence and severity of comorbid atopic diseases, tools that estimate severity of asthma and eczema were employed for all subjects at the time of enrollment. A standardized skin examination tool for atopic dermatitis, SCORing Atopic Dermatitis (SCORAD) was performed, and the Asthma Control Test was administered to all subjects, including healthy controls.¹² Because the maximum ACT score for children aged 4–11 years was 27, and the maximum score for children aged 12 and older was 25, ACT scores were expressed as % of maximum.

Pulmonary Function Testing

Exhaled nitric oxide (eNO) was measured using a chemiluminescence analyzer (NiOX; Aerocrine, Stockholm, Sweden) prior to any forced expiratory maneuvers according to current guidelines at a flow rate of 50 ml/sec.¹³

Subjects were instructed to refrain from using long acting beta agonist for 24 hr prior to bronchoprovocation, and to refrain from short acting beta agonist use for 8 hr prior to bronchoprovocation. Spirometry was measured

by standard method, standing and wearing nose plugs, and reference data from the Global Lung Initiative were used.¹⁴ Each child performed at least three maneuvers. When two maneuvers were obtained that were consistent within 5% of each other, the best effort was recorded. If FEV₁ (forced expiratory volume at 1 sec) was greater than 70% of predicted volume, then bronchial provocation by methacholine challenge was performed per ATS Guidelines with abbreviated protocol, using doses of 0.0625, 0.25, 1, 4, and 16 mg/ml of nebulized, inhaled methacholine.¹⁵ One treatment dose of 2.5 mg of nebulized albuterol sulfate was administered either after a decrease in FEV₁ of 20%, or at the conclusion of the bronchoprovocation study. Provocative concentration necessary to effect a decrease in FEV₁ of 20% (PC₂₀) was calculated by linear extrapolation using log methacholine concentrations before and after 20% change in FEV₁. Airway hyperresponsiveness (AHR) was defined as a PC₂₀ less than 8 mg/ml. For subjects who did not demonstrate a 20% decrease in FEV₁ at 16 mg/ml of methacholine, a value of 32 mg/ml was assigned for analysis.

Peripheral Blood Analysis

Peripheral blood was drawn by venipuncture at the time of enrollment, on the same day as pulmonary function testing was performed, and analyzed for the following: complete blood count (CBC) with white blood cell differential, total serum IgE, serum cytokines, and a specific IgE panel. Complete blood count (CBC) with white blood cell differential and total serum IgE level were measured by the Indiana University Hospital Pathology Lab. Upper limit of normal for serum IgE was 114 kU/L, and for peripheral eosinophilia was 0.3 k/cumm.

Serum cytokine levels (IL-4, IL-5, IL-13, eotaxin, EGF, and FGF-2) were analyzed by Multiplex, according to published methodology.⁴

Specific IgE testing to the following aeroallergens were performed by ELISA as part of a standard, commercially available panel (ImmuneTech, Inc., CA): house dust mite, ragweed, *Alternaria*, timothy grass, Bermuda grass, cedar, and cat. Specific IgE levels were classified as positive if > 0.70 IU/ml ($\geq$ class 2).

Statistical Analysis

The two cohorts, EoE subjects and healthy controls, were compared in terms of anthropomorphic measures, PC₂₀, AHR, and measures of pulmonary function and atopy via tabulation of descriptive statistics and simple hypothesis test procedures of size 0.05. For EoE subjects, medical history in terms of allergy, asthma, eos/hpf biopsy, and medication histories were summarized by tabulation of descriptive statistics.

Descriptive tabulations, Fisher's exact test procedure and multivariable logistic regression models for the prevalence

of AHR were used to investigate the relationships of airway hyper-reactivity to other patient characteristics (EoE, measures of atopic status, eNO) and to generate hypotheses. To evaluate the robustness of the main results to perturbations of the statistical methods and assumptions, similar analyses were performed using multivariable Tobit regression models for PC₂₀.

Exploratory sub-group analyses using analysis of variance models for serum cytokines (EGF, FGF-2, IL-5, IL-9, eotaxin) were used to compare four groups: healthy controls, non-asthmatic EoE subjects, and asthmatic EoE subjects with and without medication control. These results were used to generate hypotheses.

Logistic regression analyses were performed to determine if PC₂₀, as dichotomized into AHR outcomes, was associated with serum and aeroallergen variables. Variables that were statistically significant in the univariate regression models were then used in a multivariable regression model to determine which remained independently significant.

Diagnostic methods were used to investigate whether the data were consistent with analytic assumptions made. Data transformations were performed where variables did not fit the Normal distribution. Non-parametric tests used if necessary. All analyses were performed using SAS v9.3 (SAS Institute, NC).

RESULTS

A total of 37 HC and 33 EoE subjects were evaluated. Anthropomorphic data is shown in Table 1. The two cohorts were similar in gender and race. Means (+/- SE) for age, height, weight, and BMI age-for-weight z-score were smaller for the EoE cohort by 0.7 years (+/- 0.7), 6.5 cm (+/- 3.9), 10.6 kg (+/- 3.8), and 0.8 (+/- 0.2), respectively. These differences are consistent with prior studies.

EoE diagnostic criteria had evolved over the time period in which the study subjects were initially diagnosed. Therefore, medical records were examined to determine whether the EoE subjects had failed prior treatment with a PPI, as per current EoE consensus

TABLE 1—Anthropomorphic Data

	EoE (n = 33)	Healthy (n = 37)	P
Age	12.5 (3.2)	13.2 (2.6)	
Male	28 (84.9%)	25 (67.6%)	0.105
Caucasian	32 (97.0%)	31 (83.8%)	0.207
Height (cm)	151.7 (17.4)	158.2 (15.2)	0.097
Weight (kg)	44.7 (15.3)	55.3 (16.4)	0.007
BMI	18.8 (3.5)	21.6 (3.7)	0.002
BMI z-score	-0.05 (1.0)	0.72 (0.78)	<0.001

Values are mean (standard deviation) for continuous variables and frequency (percent) for categorical variables; *P*-values come from Student's *t*-test for continuous variables and Fisher's Exact tests for categorical variables.

criteria. Within the EoE group, 26 had failed prior treatment with PPI; of the remaining 7, 1 had been treated with ranitidine, 5 were diagnosed with EoE at time of therapeutic endoscopy for food impaction and a trial of PPI was foregone, and for one subject data on prior PPI usage was unavailable.

Subjects in the EoE group had a higher frequency of allergic rhinitis, atopic dermatitis, physician diagnosed asthma, food allergy, and prior wheeze than healthy controls as is expected (Table 2). Also as anticipated, the EoE group also had higher SCORAD, total serum IgE, peripheral eosinophilia, and more frequent sensitization to at least one aeroallergen compared to HC (Table 3). Of the specific allergens tested, only IgE specific for *Alternaria* and cat were statistically significantly elevated in EoE subjects compared to healthy controls. Within the HC group, there was one outlier with a serum IgE > 10 times the upper limit of normal (1152 kU/L); analysis performed both including and excluding this outlier yielded similar results.

The two cohorts were similar and within normal limits on baseline spirometry (Table 4). No subjects in either group had an FEV₁ < 70% predicted, and all subjects successfully completed bronchoprovocation testing with methacholine. EoE subjects had lower PC₂₀ values than HC subjects, indicating greater airway reactivity. Subjects with EoE also had higher eNO compared to HC subjects, suggestive of increased lower airways atopic inflammation, and higher SCORAD scores, indicative of greater degree of atopic skin findings. Asthma Control Test (ACT) results were lower for EoE subjects versus healthy controls indicating more frequent respiratory

TABLE 2—Allergy, Respiratory, and Medication History

	EoE (n = 33)	HC (n = 37)
Medical history		
Physician diagnosed asthma	15 (45.5%)	
Allergic rhinitis	18 (54.6%)	
Atopic dermatitis	17 (51.5%)	1 (2.7%)
Food allergy	27 (81.8%)	1 (2.7%)
Ever wheezed	22 (66.7%)	
Current medications		
Inhaled corticosteroid	5 (15.2%)	
Long acting beta agonist	3 (9.1%)	
Leukotriene receptor antagonist	5 (15.2%)	
Swallowed corticosteroids	20 (60.6%)	
Reslizumab	3 (9.1%)	
Eos/hpf on last biopsy	35 eos/hpf (32)	
Time elapsed since last positive (≥15 eos/hpf) EGD biopsy (months)	5.28 (4.44)	
History of systemic corticosteroids for EoE	0	

Values are mean (standard deviation) for continuous variables and frequency (percent) for categorical variables.

TABLE 3—Atopic Status

	EoE	Healthy controls	P
SCORAD	1.50 (0–22)	0 (0–10)	0.006
IgE (kU/L)	97 (5–1232)	30 (3–1152)	<0.001
peripheral % eosinophils	5 (0–12)	2 (0–19)	0.010
peripheral absolute eosinophil count (k/cumm)	0.2 (0–0.9)	0.2 (0–1.3)	0.034
Specific IgE to aeroallergens			
<i>Alternaria</i>	0 (0–23.21)	0 (0–0.54)	<0.001
Cat	0.22 (0–1.98)	0 (0–0.79)	0.017
Dust mite	0.30 (0–1.40)	0.27 (0–2.34)	0.438
Bermuda grass	0 (0–2.61)	0 (0–1.84)	0.588
Timothy grass	0 (0–19.15)	0 (0–1.31)	0.158
Ragweed	0 (0–0.55)	0 (0–0.66)	0.029
Cedar	0 (0–1.09)	0 (0–1.40)	0.765
# of aeroallergen sensitizations	2 (0–7)	1 (0–5)	0.027
# of aeroallergens ≥0.70 IU/ml	1 (0–4)	0 (0–3)	0.001

Values are median (minimum–maximum) and *P*-values are from Wilcoxon rank-sum test, due to the skewness of the variables.

symptoms. Relative activity of esophageal disease (as measured by number of eos/hpf on most recent biopsy) and SCORAD were not statistically significantly associated, nor with even a moderate effect size, with PC₂₀. Results were similar with the Tobit models.

The distribution of PC₂₀ is (by design) right-censored, and the imputation of a PC₂₀ value of 32 mg/ml unavoidably biased the analysis. Therefore the presence of AHR (PC₂₀ < 8 mg/ml) was also examined. The frequency of AHR was statistically significantly greater in the EoE than HC subjects (OR = 4.13, 95%CI: 1.16, 14.62; *P* = 0.0281) (Fig. 1), as well as EoE without asthma (OR = 6.60, 95%CI: 1.64, 26.58; *P* = 0.0079). The highest frequency of AHR for the EoE group was seen in those subjects without a previous diagnosis of asthma (44.4%, *n* = 8/18). Subjects with AHR and a history of wheezing are likely to meet criteria for a clinical diagnosis of asthma. If taken as one group, those with a known diagnosis of asthma plus those with a likely diagnosis of

TABLE 4—Pulmonary Function Testing

	EoE	Healthy controls	P ¹
FEV ₁ % pred ²	99 (83–141)	100 (79–131)	0.690
FEV ₁ /FVC % ²	82 (66–99)	84 (71–98)	0.184
PC ₂₀	32 (1–32)	32 (2–32)	0.003
eNO	15.5 (3.5–60.4)	10.1 (2.2–30.9)	0.006
ACT (% max)	92 (64–100)	100 (92–100)	<0.001

¹Values are median (minimum–maximum); *P*-values calculated using Wilcoxon rank-sum test.

²Reference values for FEV₁ and FEV₁/FVC were obtained from Global Lung Initiative data.

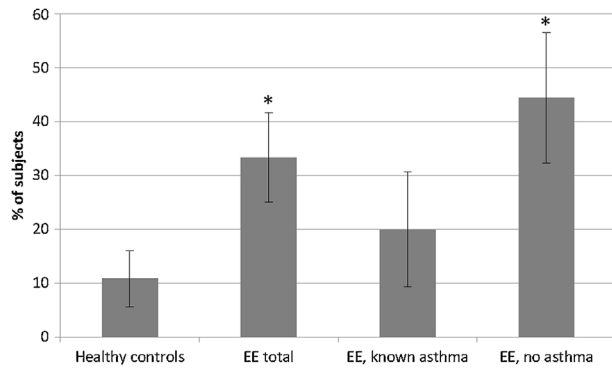


Fig. 1. Rates of AHR (PC20 < 8 mg/ml) among EoE subjects and healthy controls. (* $P < 0.05$, compared to HC). Error bars indicate standard error.

asthma (AHR with history of wheezing) correspond to 66.7% of the EoE group ($n = 22/33$).

To determine if AHR within the EoE population was associated with serum atopic markers and aeroallergen data, the dichotomous AHR variable was used with logistic regression models. For the EoE group, an elevation in total serum IgE above the upper limit of normal (114 kU/L) was associated with higher odds of demonstrating AHR (OR = 9.643; 95%CI [1.633, 56.925]; $P = 0.0124$). eNO and specific IgE elevations were not, however, associated with increased odds of AHR for EoE subjects.

The EoE and HC cohorts were similar in median levels of serum IL-5, IL-9, eotaxin, EGF, and FGF-2. Subjects with and without AHR were similar in median levels of serum IL-5, IL-9, eotaxin, EGF, and FGF-2.

The effect of asthma controller therapy (daily inhaled corticosteroids, and/or leukotriene receptor antagonist) and anti-inflammatory EoE treatments (swallowed corticosteroids and reslizumab) on serum cytokines (EGF, FGF-2, IL-5, IL-9, eotaxin) were explored through exploratory subgroup analysis. The data suggested one association: that non-asthmatic EoE subjects may have lower FGF-2 values than asthmatic EoE subjects lacking asthma controller therapy (107 pg/ml vs. 55.1 pg/ml) (Fig. 2). The use of EoE anti-inflammatory medications had no correlation to EGF, FGF-2, IL-5, IL-9, or eotaxin in our study population.

DISCUSSION

EoE subjects demonstrated similar rates of asthma, allergic rhinitis, food allergy, and atopic dermatitis by history as has been seen in other studies, including an asthma prevalence of 45.5%, comparable to the higher estimates in prior studies. As hypothesized, EoE subjects had higher rates of AHR than healthy controls, but the frequency of likely asthma (diagnosis of asthma, plus those with AHR, and history of wheezing) was higher at 66.7%. This suggests that asthma may be underdiagnosed in children with EoE.

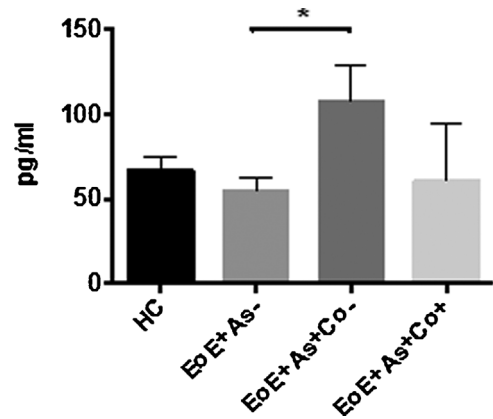


Fig. 2. Serum FGF-2 values by multiplex analysis in healthy controls, EoE with no asthma (EE+As-), EoE and asthma but no asthma controller (EE+As+Co-) and EoE and asthma with asthma controller (EE+As+Co+), mean values with error bars indicating standard error of the mean. * $P = 0.0426$.

Of note, EoE subjects with a known diagnosis of asthma had a lower rate of AHR than subjects with EoE who did not have a known diagnosis of asthma. The 15 EoE subjects with a known diagnosis of asthma had a lower prevalence rate of AHR than the 18 non-asthmatic EoE subjects (20% vs. 44%). However, 53.3% (8/15) of known asthmatics were on controller therapy for asthma, which could suppress AHR. Additionally, known asthmatics likely have closer monitoring of respiratory symptoms compared to those not diagnosed with asthma, which would allow for titration of medications more readily.

In EoE subjects, PC₂₀ was correlated with total serum IgE ($r = -0.33 \pm 0.21$), but not with the number of eos/hpf on most recent biopsy ($r = 0.20 \pm 0.33$). One possibility is that the timing of esophageal biopsy was widely variable and could have been obtained as long as 12 months prior to enrollment. Therefore, treatment may have changed during that time, including medication changes or dietary modifications, and as such the EoE disease activity at the time of enrollment may not be accurately reflected by the last esophageal biopsy. However, an alternate reason for lack of correlation to biopsy results could be that elevations in serum IgE could represent a more systemic manifestation of atopy, and therefore could occur in subjects with multiple organs affected. Conversely, subjects with more localized and isolated disease may be more likely to have normal systemic serum IgE. However, the predictive value of serum IgE in relation to AHR was poor, and so its clinical utility remains limited in this population.

FGF-2 has been shown to be elevated in subjects with asthma¹⁰ but the role of FGF-2 in atopic disease and the effect of medications such as corticosteroids on FGF-2

expression remains poorly understood. Our exploratory sub-group analyses suggested that FGF-2 may be elevated in asthmatic EoE subjects not on asthma controller therapy. Those EoE subjects with asthma who were on daily controller therapy had FGF-2 Levels similar to nonasthmatics and healthy controls. This finding is suggestive of a role of inhaled corticosteroids in ameliorating FGF-2 elevations in asthmatic children, although further study is warranted.

Study limitations include the fact that our measurements were cross-sectional in nature, and therefore it was impossible to determine whether AHR or serum biomarkers may vary over time in relation to esophageal disease activity. Because EoE diagnostic data were obtained retrospectively, there is potential for heterogeneity within the EoE group particularly in that not all patients received a PPI trial to distinguish EoE from PPI-responsive esophageal eosinophilia. However, the large majority of subjects in the EoE group did receive a trial of PPI prior to esophageal biopsy, which is consistent with current EoE diagnostic consensus. An additional limitation is that there was not a comparison group of children with atopic disease without EoE, which limits the ability of this study to distinguish between AHR that is associated specifically with the presence of EoE. This study included only children, and therefore these results may not be generalizable to adult patients with EoE. There was a potential for selection bias, in that parents, particularly those in the EoE group, may have been more likely to enroll their child if they had a concern for asthma. Aeroallergen sensitization was performed using a convenience panel that included a limited number of aeroallergens, as extensive skin prick testing to aeroallergens was outside the feasible scope of this study. Further study may be warranted to investigate further any potential relationship between aeroallergen skin prick testing and AHR.

Future study is warranted to determine how AHR may change over time in children with EoE, particularly with anti-inflammatory therapy. It is conceivable that as EoE disease, atopic dermatitis, or allergic rhinitis become better controlled, or as serum IgE decreases, AHR may improve. Additionally, further investigation is needed into the mechanism of corticosteroid effect on FGF-2.

In summary, providers caring for children with EoE should strongly consider evaluation by an asthma specialist, given the high rate of asthma and AHR in this population. If the child is undergoing allergy evaluation, the allergist may also want to consider specific evaluation for signs or symptoms of asthma, including pulmonary function testing and possibly with methacholine challenge. However, our study suggests that tests for aeroallergen sensitization by specific IgE, or other serum markers of atopy may not be reliable predictors of AHR.

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