Original Article

IgE Antibody Detection and Component Analysis in Patients with Eosinophilic Esophagitis

Elizabeth A. Erwin, MD[a], Anubha Tripathi, MD[b], Princess U. Ogbogu, MD[c], Scott P. Commins, MD, PhD[b], Maria A. Slack, MD[c], Christine B. Cho, MD[c], Robert G. Hamilton, PhD[d], Lisa J. Workman, BA[b], and Thomas A.E. Platts-Mills, MD, PhD[b] *Columbus, Ohio; Charlottesville, Va; Baltimore, Md*

What is already known about this topic? Dietary avoidance can often successfully treat eosinophilic esophagitis (EoE), suggesting that foods are causal. Although serum IgE antibody assays identify more positive food results than does skin prick testing, neither test consistently identifies the correct foods to avoid.

What does this article add to our knowledge? Assays on diluted sera and component analysis suggest that in patients with EoE, the IgE antibodies to milk are specific for proteins that represent a small proportion of the proteins in the whole extract (α-lactalbumin and β-lactoglobulin).

How does this study impact current management guidelines? Although IgE is not the primary mechanism, molecular allergen and extract-based IgE antibody assays for cow's milk suggest that the quantitatively minor whey components are relevant to EoE. The relevant proteins in wheat have not yet been identified.

BACKGROUND: Although IgE antibodies to cow's milk and wheat are common in patients with eosinophilic esophagitis (EoE), titers are low and responses to diet are not dependent on having IgE antibodies.

OBJECTIVE: To better define specific IgE antibody responses to foods, focusing on those foods that appear to play a role in EoE.

METHODS: Adult (n = 46) and pediatric (n = 51) patients with EoE were recruited for skin prick testing and serum measurement (whole and diluted) of IgE antibodies specific for aeroallergens, food extracts, and component allergens by ImmunoCAP. Immuno Solid-phase Allergen Chip analysis was also used to measure the specificity of IgE antibodies to 112 allergen molecules.

RESULTS: In adults and children, there was a higher prevalence of sensitization to food extracts by ImmunoCAP than by skin prick testing. Using Immuno Solid-phase Allergen Chip to assess the specificity of IgE antibodies to 112 allergen molecules, we found that results for food allergens were mostly negative. In contrast, ImmunoCAP assays for specific milk allergens gave positive IgE antibody results in 31 of 34 sera. The correlations between specific IgE antibody to Bos d 4 or Bos d 5 and milk extract were strong (R = 0.89 and 0.76, respectively; $P < .001$). The evidence that IgE antibodies to foods were directed at minor components of the extracts was further supported by measurements on diluted sera.

CONCLUSIONS: The IgE responses in cow's milk–sensitized patients with EoE are frequently to whey proteins Bos d 4 and Bos d 5, minor components of the extract. These IgE assays may be able to identify the proteins that are relevant to EoE even though IgE is not the primary mechanism. **(J Allergy Clin Immunol Pract 2015;■:■-■)**

Key words: *Eosinophilic esophagitis; Food allergy; Serum IgE measurements; Component-resolved diagnostics*

Eosinophilic esophagitis (EoE) is a chronic disease that affects children and adults. In some patients it starts in childhood and lasts into adulthood.[1,2] In most patients, EoE is associated with food and aeroallergen sensitization.[3-8] Furthermore, the disease typically improves or resolves with food elimination diets.[5,9-12] However, symptoms do not usually occur immediately on ingestion of the problem food(s). Therefore, triggers can be difficult to identify and the contribution of specific antigens to the disease process is not yet understood.

When evaluating patients with EoE, serum IgE and skin prick testing to foods is recommended for consideration of immediate hypersensitivity although the role of these diagnostic modalities in planning dietary treatment is not clear.[3] The relationship between skin prick testing and clinical response to diet has been described with positive predictive values for individual foods

[a]Center for Innovation in Pediatric Practice, Nationwide Children's Hospital, Columbus, Ohio
[b]Division of Allergy and Immunology, University of Virginia, Charlottesville, Va
[c]Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, The Ohio State University Wexner Medical Center, Columbus, Ohio
[d]Dermatology, Allergy and Clinical Immunology Laboratory, Johns Hopkins University School of Medicine, Baltimore, Md

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Corresponding author: Elizabeth A. Erwin, MD, Center for Innovation in Pediatric Practice, Nationwide Children's Hospital, 700 Children's Dr, Columbus, OH 43205. E-mail: eae9m@virginia.edu.

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Abbreviations used
EoE- eosinophilic esophagitis
GM- geometric mean
ISAC- Immuno Solid-phase Allergen Chip
PPI- proton pump inhibitor
PPI-REE- proton pump inhibitor responsive esophageal eosinophilia

ranging from 57% to 96% and negative predictive values ranging from 14% to 65%.[9] As such, in some children, resolution of symptoms has been demonstrated in patients who avoid milk *and* foods that are positive by skin prick and patch testing.[10] However, in other pediatric and adult studies, although food seems to play a causal role, skin testing has not identified the problem food(s).[11,12] We have previously reported in pediatric patients that serum IgE antibody assays detect more allergic sensitization to foods than does skin prick testing.[8] This may also be true for adults.[6]

In Europe, measurements of IgE antibodies specific for purified allergens (components) have suggested that at least some food sensitization in adults with EoE is related to (birch) pollen cross-reactivity.[13] In general, among patients with food allergy other than EoE, sensitization to specific allergenic molecules has provided information about distinct clinical symptoms upon exposure, and the pathway for the development of IgE antibodies.[14-16] In addition, it has been reported that for the same clinical pathways, the prevalence of sensitization to different allergen specificities may vary regionally.[17] Serum IgE antibodies to specific proteins have not been reported in patients with EoE who live in the United States. We report on a cross-sectional study using different testing modalities to detect and delineate IgE antibodies in patients with EoE. The objective of this study was to measure food and aeroallergen sensitization (IgE antibody positivity) in adult and pediatric patients recruited in parallel and to investigate serum IgE antibodies to component allergens for those foods that elimination diets suggest may be relevant to the eosinophilic inflammation.

METHODS

Adult patients (n = 46) who were referred to the Allergy Clinic at the Ohio State University Wexner Medical Center for the evaluation of EoE and had 15 or more eosinophils/hpf documented by esophageal biopsy were recruited between September 2010 and December 2013. Although not all the patients had been treated with proton pump inhibitors (PPIs) for a full 8 weeks before biopsy, more than 90% were taking a PPI at allergy evaluation and had not had resolution of symptoms. We did not exclude patients who had not been fully treated with PPI before the biopsy. This study was approved by the institutional review board of the Ohio State University, and all patients provided written informed consent.

Pediatric patients (n = 51) were recruited from the Allergy Clinic at Nationwide Children's Hospital (Columbus, Ohio) during the same time period. A separate protocol for children was approved by the institutional review board at Nationwide Children's Hospital.

At a single study visit, subjects completed questionnaires detailing symptoms of EoE and treatment for other allergic diseases. In adults, skin prick tests were performed to multiple foods (chicken egg, cow's milk, wheat, soy, peanut, tree nuts, fish, shellfish, legumes, grains, meats, and vegetables) and a range of common aeroallergens. Tests were applied with a Greer Pick (Greer, Lenoir, NC) using standard allergen extracts. In pediatric patients, skin prick tests were performed to a panel of 15 foods (chicken egg, cow's milk, wheat, soy, peanut, cashew, shrimp, oat, beef, chicken, pork, rice, rye, pea, and corn) and a range of common aeroallergens using Sharp-Test Applicators (Panatrex, Placentia, Calif). A skin wheal diameter of at least 3 mm larger than that of the negative diluent control was considered positive.

Total serum IgE and allergen extract–specific IgE antibodies were measured by ImmunoCAP (Thermo Fisher Scientific/Phadia, Uppsala, Sweden). The food and aeroallergen specificities tested included cow's milk, chicken egg, wheat, soy, peanut, cashew, beef, dust mite, cat, dog, mold mix, birch, rye grass, weed mix, and ragweed. Specific IgE antibodies to galactose-α-1,3-galactose, MUXF3 (bromelain), *Candida albicans*, and Staphylococcal enterotoxins A and B were also measured by ImmunoCAP. Specific IgE antibody values of 0.35 IU/mL or more were considered positive. In addition, for patients who had a positive IgE antibody test result to cow's milk, wheat, or peanut, IgE antibodies specific for molecular allergens (ie, components) from these whole food extracts were also measured using ImmunoCAP (see Tables E1 and E2 in this article's Online Repository at www.jaci-inpractice.org). To test for quantitative accuracy, samples with positive results to wheat, milk, or soy and enough remaining serum were reanalyzed at serial dilutions (1:2 through 1:8) by ImmunoCAP (see the Methods

TABLE I. Clinical characteristics, skin prick tests, and endoscopy results of patients with EoE

Clinical feature	Adults (n = 46)	Children (n = 51)	*P* value*
Age (y), median (range)	38 (18-64)	11 (1.4-20)	
Sex: male, n (%)	28 (61)	34 (67)	.6
Allergic sensitization†, n (%)			
None	3 (6.5)	12 (24)	.03‡
Food only	0	3 (5.9)	—‡
Aeroallergen only	13 (28)	16 (31)	.7
Food and aeroallergen	29 (63)	17 (33)	.003
Associated diseases, n (%)			
Asthma ever	15 (33)	25 (49)	.1
Rhinitis	35 (76)	36 (71)	.5
Self-reported food allergy	18 (39)	20 (39)	>0.9
Symptom(s), n (%)			
Dysphagia	38 (83)	26 (51)	.001
Food stuck	43 (93)	28 (55)	<.001
Vomiting	8 (17)	28 (55)	<.001‡
Abdominal pain	16 (35)	28 (55)	.05‡
Reported stricture	15 (33)	0	—
Endoscopy, n (%)			
Furrows	17 (37)	37 (73)	<.001‡
Rings	16 (35)	2 (3.9)	<.001
White plaques	5 (11)	15 (29)	.04‡
Stricture(s)	6 (13)	0	—
Biopsy results (eosinophils/hpf)	15-50§	20-110‖	—
Total IgE, GM (95% CI)	52 (5.4-110)	120 (100-310)	.01

*P value based on χ^2 test comparing adult and pediatric groups except as noted.
†Sensitization in this table is based on having a positive skin prick test result.
‡More common in children.
§Biopsy results for 3 adults were reported as "consistent with EoE."
‖One child's biopsy was reported as more than 100.