

Total serum immunoglobulin E as a marker for missed antigens on in vitro allergy screening

Jeanne L. Hatcher, MD¹, Samuel D. Cohen, MD² and J. Whit Mims, MD¹

Background: The diagnosis of inhalant allergies involves a medical history, physical exam, and allergen sensitivity testing; allergen sensitivity can be assessed by a specific immunoglobulin E (IgE) screen for inhalant allergens. Some patients with clinical suspicion for inhalant allergies have a negative specific IgE screen, but high total IgE. We theorize that elevated total IgE may indicate a false-negative screen caused by “missed allergens” not initially identified.

Methods: Study patients with a negative allergy screen and elevated IgE (>116 kU/L) were identified (n = 26). Control patients (n = 26) were defined as having a negative screen and an IgE <2.95 kU/L. Both groups were tested with an expanded specific IgE panel and completed a questionnaire about other causes of elevated IgE.

Results: The expanded panel was positive for inhalant allergens in 4 study patients (15%) and 0 control patients (p = 0.037). Within the study patients, 50% had asthma and 76.9% had chronic sinusitis. Only 2 control patients had asthma (11.5%), p = 0.003; 4 (19.2%) reported chronic sinusitis, p < 0.0001. Food allergen sensitivity was identified in 5 study patients and 1 control, p = 0.083.

Conclusion: This pilot study evaluated patients clinically suspected of allergy with a negative inhalant IgE screen. Those with a high total IgE were more likely to have a missed inhalant allergen on expanded testing, as well as asthma and chronic sinusitis, compared to those with a low total IgE. Further investigation of “missed antigen” and the role of chronic respiratory inflammatory disease in patients with elevated total IgE is warranted. © 2013 ARS-AAOA, LLC.

Key Words: immunoglobulin E; radioallergosorbent test; respiratory hypersensitivity; allergic rhinitis; sinusitis; asthma

How to Cite this Article:

Hatcher JL, Cohen SD, Mims JW. Total serum immunoglobulin E as a marker for missed antigens on in vitro allergy screening. *Int Forum Allergy Rhinol*. 2013;00:X-XX.

Affecting approximately 20% of Americans, atopic disease, including allergic rhinitis, is a common complaint brought to the otolaryngologist.¹ Diagnosis is best made with a combination of clinical evaluation and testing. One method of assessment for allergies is measurement of the antibody immunoglobulin E (IgE) in serum. Immunoglobulin E levels include that of allergen-specific

IgE or total IgE.^{2–5} There are hundreds of specific allergens available for testing. Thus, testing for every possible allergen is impractical. A common strategy is to use a screening test to assist in the diagnosis of allergic disease.³ Total IgE is frequently elevated in allergic rhinitis, though the clinical utility of IgE has been brought into question because of its elevation in other conditions such as asthma, atopic dermatitis, and nonallergic rhinitis.^{6–10} As previously investigated by Sinclair and Peters, total IgE and specific IgE usually correlate; with a rising total IgE the quantity of specific IgE is also greater. However some patients clinically suspected of having allergic rhinitis have a negative specific IgE screen and an elevated total IgE.^{3,11} This discordance could be attributed to nonspecificity of the total IgE.^{6–8} Or, another plausible reason is that inhalant allergen sensitization is present but not identified on the screening tests; ie, “missed allergens.”^{3,11}

Reactions to food proteins affect around 5% of children and adults. Adverse reactions are broad, including

¹Department of Otolaryngology, Wake Forest School of Medicine, Winston-Salem, NC; ²Wilson Ear Nose and Throat, Wilson, NC

Correspondence to: Whit Mims, MD, Wake Forest School of Medicine, Department of Otolaryngology, 4th Floor Watlington Hall, Medical Center Blvd, Winston-Salem, NC 27157; e-mail: wmims@wakehealth.edu

Funding sources for the study: American Academy of Otolaryngic Allergy Research in Otolaryngology and Allergy Development (ROAD) Scholarship Program.

Potential conflict of interest: None provided.

Received: 27 September 2012; Revised: 21 May 2013; Accepted: 18 June 2013

DOI: 10.1002/alr.21207

View this article online at wileyonlinelibrary.com.

intolerance, gastrointestinal disorders, and allergy. IgE-mediated food allergy can present with rhinitis in addition to skin or other airway symptoms. Common causal foods are milk, egg, peanuts, and shellfish.¹² In a study of pediatric patients with eosinophilic esophagitis, it was found that an elevated IgE was significantly higher in sensitized patients than nonsensitized, suggesting that an elevated IgE would be associated with sensitization to a specific antigen. Over one-half of these patients were sensitized to a food, milk being most common.¹³ Food sensitization may contribute to an elevated total serum IgE.

In patients identified as allergen sensitive on a screening inhalant panel, a more comprehensive test for local allergens is often performed for education on avoidance as well as desensitization. However if the screening test is negative, usually there is no further testing. We theorize that patients with a negative screen and elevated total IgE may be more likely to have a “missed allergen,” either inhalant or food, than patients with a negative screen and low total IgE.

This study investigates patients clinically suspected by the otolaryngologist of having allergic rhinitis. We compared those with a negative inhalant allergen screen and a low total IgE to those with a negative screen and an elevated total IgE by performing expanded allergy testing to inhalant and food allergens in both groups to assess if there is a significant difference of missed allergen. We addressed the possibility of other conditions associated with elevated total IgE in both groups via questionnaire.

Patients and methods

This was a pilot study approved by the Institution’s Internal Review Board and was composed of 2 parts. First, a retrospective chart review was conducted on adults and children (6 years old and up) referred to the Wake Forest Baptist Health Allergy Reference Laboratory in 2005-2006. The Allergy Laboratory at our institution uses ImmunoCAP® (Thermo Fisher Scientific, Waltham, MA) for antigen-specific IgE testing. All blood samples were initially tested for the allergens listed in Table 1. If any of these were positive, then the sample was further tested for a total of 32 inhalant allergens listed in Table 2. Diagnosis, total IgE levels, specific IgE results, age, and gender were recorded. This data was used to assess the range of total serum IgE in patients with a negative initial allergy screen. The arithmetic mean of the total serum IgE values of this population was calculated and an 80% confidence interval was used to determine 2 comparison groups: the bottom 10% (control group) and the top 10% (study group).

The second part of the study was prospective and identified possible etiologies of elevated total IgE with a negative screening specific IgE by performing an expanded specific IgE panel and administering a questionnaire. Patients were identified in outpatient otolaryngology clinics and met eligibility criteria as defined in Table 3. Group sizes of $n = 26$ resulted in an alpha error of 5% and beta error of 50%. The elevated total IgE group was defined as having a total IgE of

TABLE 1. Allergens tested in initial specific IgE screen

Allergen	ImmunoCAP code	Species
Tree mix	tx2 (t1, box elder; t7, oak; t8, elm; t14, cottonwood; t22, pecan hickory)	<i>Acer negundo</i> , <i>Quercus albus</i> , <i>Ulmus americana</i> , <i>Populus deltoides</i> , <i>Carya pecan</i>
Weed mix	wx1 (w1, common ragweed; w6, mugwort; w9, English plantain, ribwort; w10, goosefoot, Lamb’s quarters; w11, saltwort, Russian thistle)	<i>Ambrosia elatior</i> , <i>Artemisia vulgaris</i> , <i>Plantago lanceolata</i> , <i>Chenopodium album</i> , <i>Salsola kali/pestifer</i>
Mold mix	mx2 (m1, <i>Penicillium chrysogenum</i> ; m2, <i>Cladosporium herbarum</i> ; m3, <i>Aspergillus fumigatus</i> ; m5, <i>Candida albicans</i> ; m6, <i>Alternaria alternata</i> ; m8, <i>Setomelanomma rostrata</i>)	<i>Penicillium chrysogenum</i> , <i>Cladosporium herbarum</i> , <i>Aspergillus fumigatus</i> , <i>Candida albicans</i> , <i>Alternaria alternata</i> , <i>Setomelanomma rostrata</i>
Mountain juniper	t6	<i>Juniperus sabinoides</i>
Timothy grass	g6	<i>Phleum pratense</i>
Cat epithelium and dander	e1	<i>Felis domesticus</i>
Dog epithelium	e2	<i>Canis familiaris</i>
Dust mite 1	d1	<i>Dermatophagoides pteronyssinus</i>
Dust mite 2	d2	<i>Dermatophagoides farinae</i>
German cockroach	i6	<i>Blattella germanica</i>

IgE = immunoglobulin E.

116 and a negative screening specific IgE. The control group had a total IgE less than or equal to 2.95 and a negative screening specific IgE. Subjects were tested by an expanded specific IgE panel for a broader array of allergens. Inhalant allergens are listed in Table 2 and food allergens are in Table 4. Patients also completed a questionnaire, which screened for atopic diseases, parasites, and other disorders associated with elevated total IgE (see Table 5).

This study was conducted at Wake Forest Baptist Medical Center.

Results

During the years 2005–2006, there were 530 patients aged 6 years and older who underwent allergy testing at the Wake Forest University Health Sciences Allergy Reference Laboratory. Of these, there were 287 that had a negative initial specific IgE. We found the middle 80% of the total IgE values in this nonallergic (ie, negative screen) group to be 2.95 to 116 kU/L, with a mean of 48.53 kU/L. The study group was defined as having an IgE corresponding to the

TABLE 2. Allergens tested in expanded inhalant-specific IgE panel

Allergen	ImmunoCAP code	Species
Trees		
Box elder	t1	<i>Acer negundo</i>
Common silver birch	t3	<i>Betula verrucosa</i>
Oak	t7	<i>Quercus albus</i>
Elm	t8	<i>Ulmus americana</i>
Walnut	t10	<i>Juglans californica</i>
Maple leaf sycamore	t11	<i>Platanus acerifolia</i>
Cottonwood	t14	<i>Populus deltoides</i>
White pine	t16	<i>Pinus strobus</i>
White ash	t15	<i>Fraxinus americana</i>
Pecan/hickory	t22	<i>Carya pecan</i>
Mulberry	t70	<i>Morus alba</i>
Grasses		
Bermuda grass	g2	<i>Cynodon dactylon</i>
Johnson grass	g10	<i>Sorghum halepense</i>
Weeds		
Common ragweed	w1	<i>Ambrosia elatior</i>
English plantain, ribwort	w9	<i>Plantago lanceolata</i>
Goosefoot, lamb's quarters	w10	<i>Chenopodium album</i>
Goldenrod	w12	<i>Solidago virgaurea</i>
Rough marshelder	w16	<i>Iva ciliata</i>
Common pigweed	w14	<i>Amaranthus retroflexus</i>
Sheep sorrel	w18	<i>Rumex acetosella</i>
Nettle	w20	<i>Urtica dioica</i>
Molds		
Penicillium chrysogenum	m1	<i>Penicillium chrysogenum</i>
Cladosporium herbarum	m2	<i>Cladosporium herbarum</i>
Aspergillus fumigatus	m3	<i>Aspergillus fumigatus</i>
Candida albicans	m5	<i>Candida albicans</i>
Alternaria alternata	m6	<i>Alternaria alternata</i>
Helmithosporium halodes	m8	<i>Helmithosporium halodes</i>
Fusarium proliferatum	m9	<i>Fusarium proliferatum</i>
Rhizopus nigricans	m11	<i>Rhizopus nigricans</i>
Epicoccum purpurascens	m14	<i>Epicoccum purpurascens</i>
Curvalaria lunata	m16	<i>Curvalaria lunata</i>
Aspergillus niger	m207	<i>Aspergillus niger</i>

IgE = immunoglobulin E.

TABLE 3. Eligibility criteria

Age 6 years and over
Read and understand English
Negative screening specific IgE panel
Total serum IgE ≤ 2.95 for control group
Total serum IgE ≥ 116 for study group

IgE = immunoglobulin E.

TABLE 4. Allergens tested in food-specific IgE panel

Egg white	f1
Milk	f2
Fish (cod)	f3
Wheat	f4
Peanut	f13
Soy bean	f14
Maize	f8

IgE = immunoglobulin E.

TABLE 5. Disorders thought to cause an elevated total IgE investigated on the questionnaire

Asthma
Eczema
Parasitic infection
Chronic sinusitis with or without nasal polyposis
Blood disorder
Cancer
Smoking
Allergic reaction to food, medicine, or insects
Exposure to animals

IgE = immunoglobulin E.

top 10% from the nonallergic group (IgE > 116 kU/L). The control group was defined with an IgE corresponding to the bottom 10% (IgE < 2.95 kU/L).

In the prospective portion of the study, the ages of subjects ranged from 11 to 79 (mean $\pm$ standard deviation [SD], 50.5 ± 3.4) years in the study group and 10 to 74 (43.9 ± 3.3) years in the control group ($p = 0.771$). Each group contained more female subjects than males, though they were statistically similar ($p = 0.337$). The mean total IgE of the study group was 363.32 ± 75.24 kU/L and the total IgE of the control group was 2.22 ± 0.06 kU/L; by non-parametric Mann-Whitney U test, the difference between these values was statistically significant, $p < 0.0001$.

Frequency of Positive Expanded Inhalant Allergen Panel

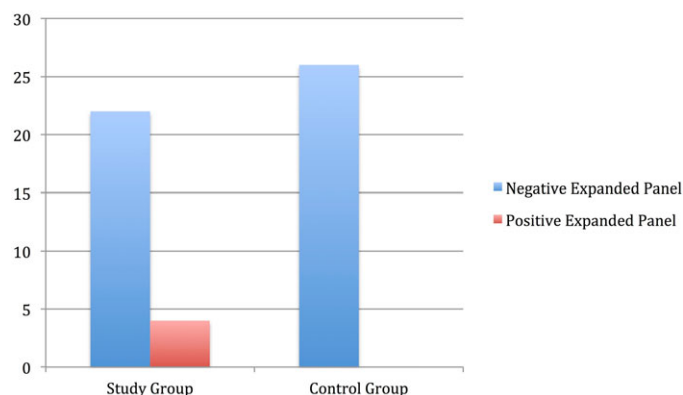


FIGURE 1. Number of subjects with a positive specific IgE result on the expanded inhalant allergen panel in study (total IgE > 116, initial screening specific IgE panel negative) and control (total IgE < 2.95, initial specific IgE panel negative) groups. IgE = immunoglobulin E.

Differences in missed allergens

A missed allergen is defined as the presence of a positive specific IgE result on the expanded panel (all patients had negative specific IgE screens). Goodness-of-fit (chi-square) analyses with Fisher's exact test were performed on the data to determine whether the subjects in the experimental and control groups were significantly different with respect to the presence of a missed allergen on the expanded panel. Results showed a missed allergen in 15.4% of the study group and 0% of the control group ($p = 0.037$); this is shown in Figure 1. Table 6 shows the frequency of response to expanded inhalant allergens for the experimental and control groups. The missed allergens identified on the expanded test (detailed in Table 2) were oak, maple leaf sycamore, *Aspergillus fumigatus*, Johnson grass, *Candida albicans*, *Fusarium proliferatum*, *Rhizopus nigricans*, and *Epicoccum purpurascens*. Three allergens were identified on the expanded test, which were negative on initial screening: oak, *Aspergillus fumigatus*, and *Candida albicans*.

There were a total of 6 positive tests for food allergens, as shown in Figure 2. Five positive tests were in the study group (19.2% of study patients) and 1 in the control group (3.8% of controls). The frequency of response to food allergens is shown in Table 7. Using chi-square analysis, the difference in positive food panel was statistically insignificant, $p = 0.083$. The missed allergens identified on the food panel were wheat, milk, and egg, all of which were a class I response.

TABLE 6. Frequency of response to expanded inhalant allergen panel for subjects in the experimental and control groups

	Negative panel	Positive panel
Study group (n = 26)	n = 22 (84.6%)	n = 4 (15.4%)
Control group (n = 26)	n = 26 (100%)	n = 0 (0%)

Frequency of Positive Food Allergen Panel

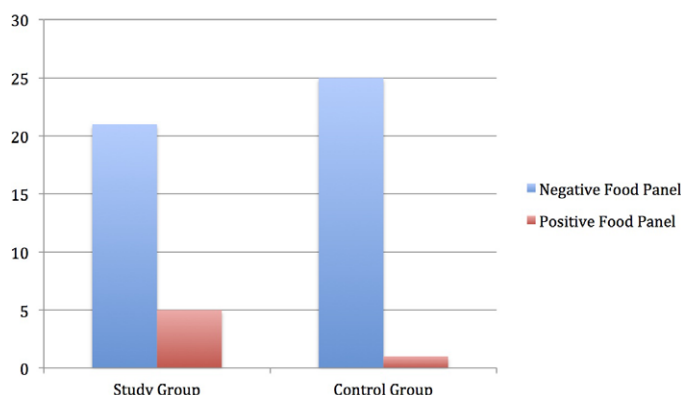


FIGURE 2. Number of subjects with a positive specific IgE result on the expanded food allergen panel in study and control groups. IgE = immunoglobulin E.

TABLE 7. Frequency of response to food allergen panel for subjects in the experimental and control groups

	Negative panel	Positive panel
Study group (n = 26)	n = 21 (80.8%)	n = 5 (19.2%)
Control group (n = 26)	n = 25 (96.2%)	n = 1 (3.8%)

Differences in questionnaire responses

Goodness-of-fit (chi-square) analyses with Fisher's exact test were performed on the inhalant allergen data to determine whether the subjects in the experimental and control groups were significantly different regarding the presence of asthma, chronic sinusitis, nasal polyposis, eczema, parasites, blood disorders, cancer, smoking, pets, and other allergies. Results showed the prevalence of asthma to be 50% of the study group though only 11.5% of the control group ($p = 0.003$), as seen in Figure 3. A significant difference was also found between groups with respect to the presence of sinus disease or nasal polyposis, $p < 0.0001$, as seen in Figure 4. Almost 77% (76.9%) of study subjects reported

Frequency of Asthma

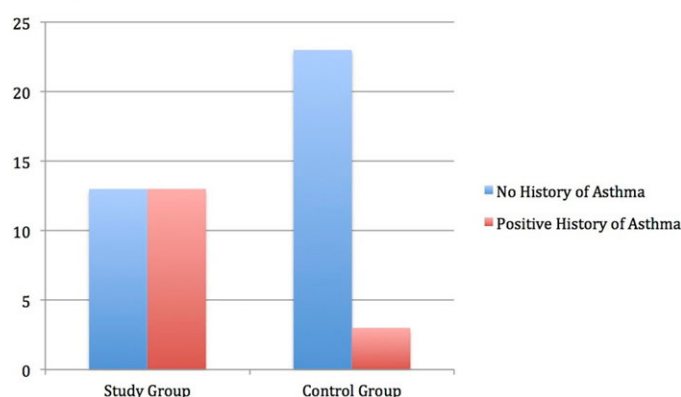


FIGURE 3. Number of subjects with asthma in experimental and control groups.

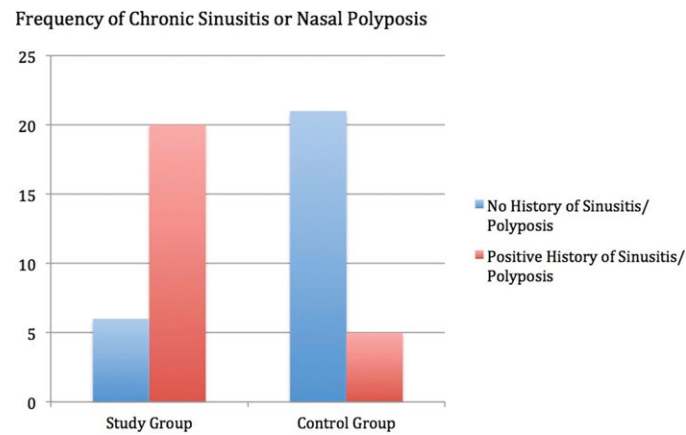


FIGURE 4. Number of subjects with chronic sinusitis or nasal polyposis in experimental and control groups.

having chronic sinusitis or nasal polyposis, whereas only 19.2% of controls reported either diagnosis. There were no significant differences found between groups for history of eczema, parasites, blood disorders, cancer, smoking, history of other allergy, or presence of pets (see Table 8).

Discussion

In this pilot study, patients with clinical suspicion for allergic rhinitis, whose specific IgE screening panel was negative although the total IgE was elevated, were studied. We hypothesized that these patients would be more likely to have an inhalant allergen sensitivity that was not present on the screening panel as compared to a control group with a normal total IgE. We also queried the subjects for other known causes of an elevated total IgE, such as asthma, atopic dermatitis, and allergic rhinitis. Our results showed a small but statistically significant difference in the presence of specific IgE to missed antigens on an inhalant panel. There was no difference in specific IgE to food antigens. The results also showed a significant difference in asthma and chronic sinusitis, known to contribute to an elevated IgE.

Currently, it is controversial to obtain a total IgE when evaluating for specific inhalant allergens because it does not consistently distinguish allergic from nonallergic rhinitis. However, our findings suggest that the presence of

an elevated total IgE when contrasted with a negative inhalant allergen screen may prompt physicians to consider other chronic inflammatory conditions or test for additional allergens.

Clinically, patients suspected of allergic rhinitis with a negative specific IgE inhalant screen would be diagnosed with nonallergic rhinitis. However, other strategies for identifying allergic sensitivities could include additional skin or local allergy testing. The clinical significance of discordant allergen skin and specific IgE testing in the same patient has not been extensively evaluated. Local allergy, or entopy, is defined as identifying specific IgE in nasal samples when skin or serum testing is negative. Local allergy is currently being investigated as a contributor to allergic disease.¹⁴

It was hypothesized that those with elevated total IgE may have increased sensitivities to food allergens. However, IgE-mediated food sensitivity, as assessed by specific IgE testing, was not different between those with a low total IgE and a high total IgE in our study. Interestingly, only 2 patients reported a previous sensitivity to food, 1 that was an unknown reaction type to milk, and 1 that was described as a “breakout” to seafood. Both of these patients were in the control group and had a total IgE of 2.1 kU/L and 2 kU/L, respectively. Both were also not sensitive to milk, shellfish, or any other food allergen tested on our institution’s panel.

There are several factors that make it difficult to extrapolate these results. Normal total IgE range varies by population and geography. The comprehensiveness of any given allergy screen will depend upon region and practice. Another weakness of this study is the relatively small sample size. In this context, statistical significance is not necessarily synonymous with clinical relevance. The decision for expanded allergy testing should be clinically based. The questionnaire administered was based on patient report, which may not represent optimal disease diagnosis. The data from this pilot study is dependent upon clinical patient selection, prevalence of concomitant respiratory inflammatory disease, and the allergen screen used, which may limit the reproducibility of these findings.

The specific IgE allergen screen combined multiple allergens in a single test for weeds, trees, and molds, whereas the expanded panel used individual tests for each of the same allergens that were combined in the screen and any

TABLE 8. Frequency of positive responses to additional factors on the questionnaire

	Eczema	Parasites	Blood disorders	Cancer	Smoke	Other allergy	Pets
Study group (n = 26), percentage of all subjects	3 (5.8%)	0 (0%)	1 (1.9%)	1 (1.9%)	3 (5.8%)	15 (28.8%)	1 (1.9%)
Control group (n = 26), percentage of all subjects	2 (3.8%)	1 (1.9%)	1 (1.9%)	3 (5.8%)	5 (9.6%)	16 (30.8%)	2 (3.8%)
Count and % of all subjects	5 (9.6%)	1 (1.9%)	2 (3.8%)	4 (7.7%)	8 (15.4%)	31(59.6%)	3 (5.7%)
χ ² (1, N = 52)	0.221, p = 0.638	1.02, p = .313	0.00, p = 1.00	1.083, p = 0.298	0.591, p = 0.442	0.081, p = 0.771	0.354, p = 0.552

new allergens. For some allergens, the individual tests on the expanded panel were positive when the multiallergen test on the screen was negative. Of the repeated allergens, 3 were found to be positive on the expanded studies when not found on the initial screening test. The specific IgE levels for these allergens were as follows: 2 class 1; and 1 class 2. The low reactivity of these repeated allergens might represent sensitization without clinical importance.

Our results support that obtaining a total IgE may be helpful in determining which patients would benefit from additional evaluation for chronic respiratory inflammatory conditions or consideration of broader inhalant allergen testing. It remains unclear what mechanisms produce elevated total IgE in these inflammatory processes when a specific inhalant allergen is not identified. Our results also suggest that clinicians seeking to optimize their inhalant allergen screen might consider expanded specific IgE testing of those with an elevated total IgE as a strategy to identify other common local sensitizations. For example, in patients who underwent expanded inhalant allergy testing, over one-half of the allergens identified were molds. If added to future screening studies, this could increase the yield without adding a large increase in cost.

The presence of missed allergens was statistically higher in the elevated total IgE group; however, missed allergens were still uncommon (15.4%). This finding may not clinically support additional allergy testing. There was a significantly higher rate of those with asthma or chronic sinusitis in patients with an elevated total IgE: 22 of 26 (84.6%). This suggests that patients suspected of allergic rhinitis with negative allergy testing and a high total IgE should be evaluated for chronic sinusitis or asthma.

Conclusion

In conclusion, an elevated total IgE in the presence of a negative inhalant-specific IgE screen may suggest the presence of unidentified inhalant allergen sensitization or chronic respiratory inflammatory disease other than allergic rhinitis. Additional study of the clinical utility of an elevated total IgE in evaluating inhalant allergy is warranted. 

Acknowledgments

We acknowledge Barbara Collins, MLT, ASCP, who performed all of the RAST tests and helped to identify eligible patients.

References

1. Ebert CS Jr, Pillsbury HC 3rd. Epidemiology of allergy. *Otolaryngol Clin North Am*. 2011;44:537–548.
2. King WP. Efficacy of a screening radioallergen sorbent test. *Arch Otolaryngol*. 1982;108:781–786.
3. Lehr AJ, Mabry RL, Mabry CS. The screening RAST: is it a valid concept? *Otolaryngol Head Neck Surg*. 1997;117:54–55.
4. Fornadley JA, Corey JP, Osguthorpe LD, et al. Allergic rhinitis: clinical practice guideline. Committee on Practice Standards, American Academy of Otolaryngic Allergy. *Otolaryngol Head Neck Surg*. 1996;115:115–122.
5. Derebery MJ. Otolaryngic allergy. *Otolaryngol Clin North Am*. 1993;26:593–611.
6. Eigenmann PA, Sicherer SH, Borkowski TA, et al. Prevalence of IgE-mediated food allergy among children with atopic dermatitis. *Pediatrics*. 1998;101:E8.
7. Beeh KM, Ksoll M, Buhl R. Elevation of total serum immunoglobulin E is associated with asthma in nonallergic individuals. *Eur Respir J*. 2000;16:609–614.
8. Kang JS, Yoon YD, Ahn JH, et al. B-cell activating factor is a novel diagnosis parameter for asthma. *Int Arch Allergy Immunol*. 2006;141:181–188.
9. Campos A, Reyes J, Blanquer A, et al. Total serum IgE: adult reference values in Valencia (1981–2004). Usefulness in the diagnosis of allergic asthma and rhinitis. *Allergol Immunopathol*. 2005;33:303–306.
10. Sinclair D, Peters SA. The predictive value of total serum IgE for a positive allergen specific IgE result. *J Clin Pathol*. 2004;57:956–959.
11. Redding GJ, Singleton RJ, DeMain J, et al. Relationship between IgE and specific aeroallergen sensitivity in Alaskan native children. *Ann Allergy Asthma Immunol*. 2006;97:209–215.
12. Sicherer SH, Sampson HA. Food allergy. *J Allergy Clin Immunol*. 2006;117:S470–S475.
13. Erwin EA, James HR, Gutenkunst HM, Rossu JM, Kelleher KJ, Platts-Mills TAE. Serum IgE measurement and detection of food allergy in pediatric patients with eosinophilic esophagitis. *Ann Allergy Asthma Immunol*. 2010;104:496–502.
14. Rondon C, Campo P, Togias A, et al. Local allergic rhinitis: concept, pathophysiology, and management. *J Allergy Clin Immunol*. 2012;129:1460–1467.