Comparison of an Inhalant Allergy Immunoassay With Allergen Specific IgE Determinations in Human Serum

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The diagnosis of inhalant allergy can be elusive, with symptoms resembling viral or bacterial infection, as well as immunologic deficiency. In this study an inhalant allergy immunoassay was investigated as a possible screen to rule in or out respiratory inhalant allergy in patients with allergic-type symptoms. The results of this screen were compared in 192 serum specimens submitted to our laboratory for specific IgE allergy testing and 73 blood bank samples. When the discrepant results of the inhal-

ant allergy immunoassay were resolved by Western blot, a final sensitivity of 94.7% and specificity of 97.5% was calculated. We have found this inhalant allergy immunoassay to be an effective screen for detecting inhalant allergies, and believe it to be a useful tool for the primary care physician or non-allergist trying to differentiate inhalant allergens from chronic sinusitis or other causes of sinopulmonary congestion. J. Clin. Lab. Anal. 12:213–217, 1998. © 1998 Wiley-Liss, Inc.

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INTRODUCTION

Practicing primary care physicians, including family practitioners, internists, and pediatricians, are often faced with patients having recurrent sinopulmonary infections and/or allergic symptomatology. Such patients suffer acute or chronic sinusitis caused by *Streptococcus pneumoniae*, *Hemophilus influenzae*, and staphylococci, which may be precipitated by acute viral respiratory tract infection or inhalant allergy. Additionally, exacerbations of chronic sinusitis may be caused by gram-negative bacilli or anaerobic microorganisms, as well as those mentioned previously. Symptoms of these infections include tender and swollen sinuses, frontal headaches, nasal obstruction, and discharge, as well as general malaise. These same symptoms may be the result of allergic rhinitis. In these patients, there is often a question of whether the upper airway and sinus congestion is due to allergic disease, immunologic deficiency, or structural problems. A simple and sensitive screen to rule in or out respiratory inhalant allergy in patients with these symptoms would be of significant benefit to the nonallergist attempting to evaluate such patients.

Since in vitro-specific IgE testing and skin-prick allergy testing for common inhalant allergies such as grasses, trees, and weeds have generally been shown to be equivalent (1–5), the initial phase of this study focused on the sensitivity and specificity of an inhalant allergy immunoassay compared to specific IgE enzyme immunoassays. We examined serum

specimens submitted to our laboratory for specific IgE allergy testing along with blood bank samples which were tested using two specific antigen IgE systems. Each specimen was tested for total IgE as well as the following 12 specific allergens included in the screen: *Dermatophagoides pteronyssinus* (d1), cat epithelium (e1), dog dander (e5), Bermuda grass (g2), Timothy grass (g6), *Penicillium notatum* (m1), *Alternaria tenuis* (m6), Birch tree (t3), Japanese cedar (t17), Common ragweed (w1), English plantain (w9), and *Parietaria officinalis* (w19). These allergens represent a balanced mixture of relevant allergens most commonly associated with inhalant allergies. The sensitivity and specificity of the inhalant allergy immunoassay was determined by comparison to specific IgE, or in some instances to a Western blot assay.

MATERIALS AND METHODS

Clinical Samples

A total of 192 serum specimens submitted to our laboratory for specific IgE allergy testing along with 73 blood bank samples were included in this study. Of these specimens, 29

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samples were positive for allergens not present in the screen including foods, house dust, feather, latex, and insects. All samples were shipped to the laboratory on ice and then stored at -20°C in a nondefrosting freezer until the inhalant allergy immunoassay, specific IgE, and total IgE, assays could be performed.

Inhalant Allergy Immunoassay

All testing was performed according to the instructions of the manufacturer for the IMMULITE AlaTOP Allergy Screen (Diagnostics Products Corporation (DPC), Los Angeles, CA). The DPC AlaTOP assay is a chemiluminescent enzyme-labeled immunoassay that is described in more detail elsewhere (6). Briefly, 50 μl of patient serum and ligand-labeled allergens are simultaneously introduced into the test unit, which contains an antiligand-coated polystyrene bead, and incubated for approximately 30 minutes at 37°C with intermittent agitation. During this time, allergen-specific IgE in the sample binds to the ligand-labeled allergen, which in turn binds to the antiligand on the bead. Unbound serum is then removed by a centrifugal wash. An alkaline phosphatase-labeled anti-human IgE antibody is added and incubated for another 30 minutes. The test unit is again washed, a substrate is added, and the photon output is measured on a luminometer after another 10-minute incubation. The system is automated and handles sample and reagent additions, incubations, washes, and photon measurement. Results are reported as positive or negative which are calculated from a stored cutoff value determined by an included adjuster.

Total IgE

Quantitative IgE antibodies were measured by the DPC IMMULITE as described above, with the exception of the solid phase polystyrene bead being coated with polyclonal IgE specific antibody. This assay requires 10 μl of patient serum.

Specific IgE

The Pharmacia CAP specific IgE assay (Pharmacia-Upjohn, Uppsala Sweden) has been described in greater detail elsewhere (7). Briefly, 50 μl of patient serum is added to a balanced mixture of the relevant allergens which are covalently coupled to a hydrophilic carrier polymer encased in a capsule. After a 90-minute incubation, the capsule is washed and an enzyme labeled anti-IgE conjugate is added to the capsule. After a 30 minute incubation, the capsule is washed again, and a fluorescence substrate is added. The fluorescence is then read on a luminometer.

The Sanofi Allercoat Microplate System

The Sanofi Allercoat Microplate System (Sanofi Pasteur Diagnostics, Chaska, MN) uses paper discs as the solid phase which are incubated with 50 μl of patient serum for 3 hours.

After washing, an alkaline phosphatase conjugate is added and the plates are incubated for 18–24 hours. The plates are then washed again followed by a 2-hour substrate incubation, and then measured at 405nm.

Western Blot

For Western blot analysis, the DPC AlaBLOT Specific IgE test was performed. The allergen extracts *Dermatophagoides pteronyssinus* (d1), dog dander (e5), Bermuda grass (g2), Timothy grass (g6), and *Alternaria tenuis* (m6), were electrophoresed in 4–20% gradient polyacrylamide gels. The resolved extract components were then electrotransferred to nitrocellulose membranes which are cut into strips. Fifty ml of patient serum and controls were then mixed with 450 ml of the sample diluent, added to a well with the membrane strip, and incubated on a rotator for 2 hours at room temperature. The strips were then washed 3 times with wash solution, and incubated at room temperature for 30 minutes with 500 ml of alkaline phosphatase-labeled anti-IgE conjugate. After another three washes, 500 ml of 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium substrate solution was added, and incubated for 15 minutes or until sufficient color developed. The molecular weights of the bands were then estimated by comparing the relative mobilities of the bands with the relative mobilities of protein markers of known molecular weights.

Statistical Analysis

Since there is no way to establish unequivocally the presence or absence of allergic disease (7), the sensitivity and specificity of the AlaTOP screen was calculated based on the presence or absence of specific IgE antibodies as determined by the Pharmacia CAP and Sanofi Allercoat Microplate systems. The data from all 265 serum samples were then used to calculate agreement, sensitivity, and specificity for the investigated AlaTOP screen compared with the specific IgE allergens. The specific IgE results were expressed in kUA/l and were converted to one of seven classes (Zero through Six) (8). A sample was considered positive for specific IgE antibodies when a modified RAST score of class 2 or higher was obtained for one or more of the 12 allergens. A class-1 score was considered equivocal, and class-0 scores were negative. For calculating agreement, specificity, and sensitivity, the following formulas were used: A = a specimen with a positive result by both allergy screen and specific IgE assays; B = a specimen with a positive result by allergy screen, but negative result by specific IgE; C = a specimen with a negative result by allergy screen but positive result by specific IgE; D = a specimen with negative results by both assays. Agreement was equal to $(A + D)/(A + B + C + D)$, sensitivity was equal to $A/(A + B)$, specificity was equal to $D/(B + D)$.

RESULTS

Comparison of the AlaTOP Allergy Screen to Specific IgE Results

The data from 265 serum samples were used to calculate an agreement of 93.7%, a sensitivity of 90.6%, and a specificity of 97.4% for the investigated AlaTOP screen compared with the specific IgE allergens (Table 1). The final tally yielded 139 positive, 115 negative, and 11 equivocal results. Included in these results are 29 samples which were positive for allergens not present in the AlaTOP screen including foods, house dust, feather, latex, and insects. All 29 of these samples were negative by the AlaTOP. All discrepant sample results were retested, and the 11 equivocal results were not included in the calculations.

Of the 16 discrepant results, there were 13 false negative results (samples with a positive result for specific allergen IgE, and a negative AlaTOP screen result). The remaining 3 discrepant results were identified as being falsely positive, having a negative result for the 12 specific allergens, but having a positive AlaTOP screen result (Table 2). Upon closer examination of the false negative results, 3 samples (2, 4, and 6) had a specific class-2 result for *Dermatophagoides pteronyssinus* (d1). Four other false negative samples (13, 14, 15, and 16) had class-2 results for Timothy grass (g6). Additionally, sample 15 had a class-2 result for Bermuda grass (g2), and sample 16 had class-2 results for Bermuda grass (g2) and Common ragweed (w1). Sample 1 had class-3 results for dog dander (e5), and Bermuda grass (g2), and a class-2 result for *Alternaria tenuis* (m6). The remaining five false positive samples, (3, 7, 10, 11, and 12) had a class-2 result for *Parietaria officinalis* (w19), cat epithelium (e1), dog dander (e5), *Alternaria tenuis* (m6), and Japanese cedar (t17), respectively. The three false positive AlaTOP screen results were negative for the 12 specific allergens.

In an attempt to increase the sensitivity of the assay, the AlaTOP screen was reevaluated by combining it with total IgE results. A specimen was considered positive if it had either a positive screen result or an elevated total IgE level. This increased the sensitivity of the assay to 94.2%, but decreased the agreement and specificity to 88.6% and 81.7%, respectively (Table 3).

Samples where the result was positive for specific allergen IgE but negative by the AlaTOP screen result were then run by Western blot to resolve these discrepancies. Nine of the 13 false negative specimens by the screen had sufficient sample

volume for AlaBLOT Western blot analysis, only three of which showed positive banding patterns. The discrepant sample #1 (Table 1) showed banding patterns for the allergens dog dander (e5), Bermuda grass (g2), and *Alternaria tenuis* (m6), confirming this sample as positive. Sample #4 showed no bands for *Dermatophagoides pteronyssinus* (d1), but a weak band for Timothy grass (g6), which had an equivocal class-1 result by specific IgE. Sample #10 showed bands for dog dander (e5). The remaining discrepant results (#2, 6, 7, 11, 15, and 16) showed no bands at all, suggesting that these were actually negative samples. This increased the sensitivity 94.7% (Table 4).

To further investigate the sensitivity of the assay, 8 samples were serially diluted with a negative serum pool, and tested for IgE to the allergens Timothy grass (g6), English plantain (w9), Bermuda grass (g2), Birch tree (t3), *Dermatophagoides pteronyssinus* (d1), dog dander (e5), Common ragweed (w1), and cat epithelium (e1) (Table 5). For three samples (A, C, and H) the endpoint titers (the highest dilution in which the result remained positive) were identical for the two assays. For the 5 remaining samples, 2 samples (B and E) had a higher endpoint titer on the specific IgE than the AlaTOP, while 3 samples (D, F, and G) had a higher endpoint titer on the AlaTOP than the specific IgE.

To evaluate the effects of high total IgE levels on the AlaTOP screen, two negative samples spiked with 1000 kU/L and 3000 kU/L of myeloma IgE were tested. Both samples tested negative by the AlaTOP screen, indicating that the system is not sensitive to high levels of nonspecific IgE.

DISCUSSION

The AlaTOP Inhalant allergy immunoassay proved to be an excellent screen for individual inhalant allergens having only three false positives, resulting in a specificity of 97.4%. There were 13 false negative results with the screen resulting in a sensitivity of 90.6% (Table 1). Of the 13 discrepant results, the specific allergens were missed in the following frequencies: *Dermatophagoides pteronyssinus* (d1) 3/13 (23.1%), cat epithelium (e1) 1/13 (7.7%), dog dander (e5) 2/13 (15.4%), Bermuda grass (g2) 3/13 (23.1%), Timothy grass (g6) 4/13 (30.8%), Birch tree (t3) 1/13 (7.7%), Japanese cedar (t17) 1/13 (7.7%), *Alternaria tenuis* (m6) 2/13 (15.4%), and Common ragweed (w1) 1/13 (7.7%).

In an attempt to increase the sensitivity of the AlaTOP assay, total serum IgE levels were examined in conjunction with the specific IgE results as suggested by the manufacturer. Most allergies are mediated by the IgE class of immunoglobulin which bind to the surface of mast cells and basophilic granulocytes. When exposed to specific allergens, these cells release histamine and other vasoactive substances which initiate the allergic reaction (9,10). Measurement of total circulating IgE levels can aid in making a diagnosis of allergy (11,12). IgE levels normally show a gradual increase during child-

TABLE 1. Comparison of AlaTOP Inhalant Screen to Specific IgE Results

		Specific IgE Allergen			
		Pos.	Neg.	Equiv.	
DPC AlaTOP	Pos.	126	3	1	Agreement = 93.7% Sensitivity = 90.6% Specificity = 97.4%
	Neg.	13	112	10	

TABLE 2. Discrepant Results of AlaTOP Inhalant Screen and Allergen Specific IgE

TABLE 2. Descriptant Results of AlaTOP Allergen Screen and Allergen Specific IgE															
Sample	Sx/age	DPC		AlaTOP	d1	e1	e5	g2	Specific IgE class						
		Tot-IgE							g6	t3	t17	m1	m6	w1	w9
1	33M	>600	neg			0	3	3					2		
2	70F	137	neg		2	0	0	0	0	0	0	0	0	0	0
3	67F	>600	neg		0	0	0	0	1	1	0	0	0	0	1
4	18F	55.0	neg		2	0	0	0	1	0	0	0	0	0	0
5	1M	47.6	pos		0	0	0	0	0	0	0	0	0	0	0
6	4F	170.0	neg		2	0	0	0	0	0	0	0	0	0	0
7	49M	33.1	neg		0	2	0	0	0	0	0	0	0	0	0
8	1F	55.2	pos		0	0	0	0	0	0	0	0	0	0	0
9	>18	19.7	pos		0	0	0	0	0	0	0	0	0	0	0
10	3F	601.0	neg		0	0	2	0	0	0	0	0	0	0	0
11	29F	18.5	neg		0	0	0	0	0	0	0	2	0	0	0
12	>18	19	neg		0	0	0	0	0	0	2	0	0	0	0
13	>18	77.4	neg		0	0	0	0	2	0	0	0	0	0	0
14	>18	9.3	neg		0	0	0	0	2	2	0	0	0	0	0
15	>18	19.9	neg		0	0	0	2	2	0	0	0	0	0	0
16	>18	12.7	neg		0	0	0	2	2	0	0	0	0	2	0

dl, Dermatophagoides pteronyssinus; e1, cat epithelium; e5, dog dander; g2, Bermuda grass; g6, Timothy grass; m1, Penicillium notatum; m6, Alternaria tenuis; t3, Birch tree; t-17, Japanese cedar; w1, Common ragweed; w9, English plantain; and w19, Parietaria officinalis.

hood, and eventually reach adult levels by approximately twenty years of age (13,14). The total IgE level also increases with the number of allergies which a person has and with the amount of exposure to different allergens (11). Therefore, the AlaTOP Inhalant allergy immunoassay was reevaluated by combining it with the total IgE results as suggested by the manufacturers. A specimen was considered positive if it had either a positive screen result or an elevated total IgE level compared to the age matched controls. Determination of elevated IgE levels was based on the following: 0–1 yrs >9 IU/mL, 1–2 yrs >13 IU/mL, 2–3 yrs >23 IU/mL, 3–9 yrs >72 IU/mL, 9–15 yrs >162 IU/mL, over 15 yrs >93 IU/mL (15). This increased the sensitivity of the AlaTOP screen to 94.2% by reclassifying five of the initial negative screen discrepant (1, 2, 3, 6, and 10) (Table 2) as positive. It also reclassified as positive an additional 18 samples, which were initially negative and had no specific IgE for the 12 screen allergens. This decreased the overall agreement to 88.6% and the specificity to 81.7% (Table 3). The lack of correlation may be due to other causes of increased IgE levels besides inhalant allergy such as gastrointestinal allergy, drug allergy, active stages of certain parasitic infections, or, much less commonly, IgE myeloma or pulmonary aspergillosis (10,11,12). By having to run both the AlaTOP Inhalant allergy immunoassay and the total IgE, the cost increased by 20%. Therefore, the de-

crease in overall agreement and specificity, along with the additional cost, did not justify the inclusion of total IgE testing in our study as suggested by the manufacturer.

When Western blot was used to resolve the discrepancies between the inhalant allergy immunoassay and specific allergen IgE, the overall agreement increased to 96.1%, sensitivity to 94.7%, and specificity to 97.5% (Table 4). When the results of the serial dilutions were examined, the AlaTOP inhalant screen and the individual allergen assays were essentially equivalent in sensitivity. There were 2 samples for each assay where a higher dilution of the sample was determined positive by one assay but not the other (Table 5). This is very encouraging since it indicates that the mixture of allergens in the screen does not significantly decrease the sensitivity in detecting specific IgE to individual antigens. The AlaTOP inhalant screen assay also did not seem to be affected by non-specific high IgE concentrations since the two samples from myeloma patients having extremely elevated IgE levels tested negative by the screen.

The IMMULITE system is very simple to perform and completely automated; the only manual requirement is the addition of patient sample. Test results are reported in as little as 35 minutes for total IgE, and 75 minutes for the AlaTOP screen. The cost per test result for the Alatop including labor, reagents, and supplies is approximately \$5. In comparison,

TABLE 3. Comparison of AlaTOP Inhalant Screen and Total IgE Results to Specific IgE Results

		Specific IgE Allergen			
		Pos.	Neg.	Equiv.	
DPC AlaTOP	Pos.	131	21	1	Agreement = 88.6% Sensitivity = 94.2% Specificity = 81.7%
w/ total IgE	Neg.	8	94	10	

TABLE 4. Comparison of AlaTOP Inhalant Screen to Specific IgE Results With Discrepant Resolved by Western Blot

		Specific IgE Allergen			
		Pos.	Neg.	Equiv.	
DPC AlaTOP	Pos.	126	3	1	Agreement = 96.1% Sensitivity = 94.7% Specificity = 97.5%
w/ western blot	Neg.	7	118	10	

TABLE 5. Sensitivity of AlaTOP Inhalant Screen and Specific IgE by Serial Dilution of Positive Samples

Sample	Allergen	Class	Endpoint Titer	
			AlaTOP	Specific IgE
A	g6	5	1:64	1:64
B	w9	6	1:128	1:256
C	g2	5	1:8	1:8
D	t3	5	1:64	1:32
E	d1	5	1:32	1:64
F	e5	6	1:64	1:32
G	w1	3	1:16	1:8
H	e1	3	1:16	1:16

testing all 12 allergens for specific IgE would cost approximately \$33. We believe that the AlaTOP Inhalant allergy immunoassay is an effective screen for inhalant allergies, detecting up to 94.7% of patients with positive specific IgE levels. The high specificity of 97.5% also makes it a very useful test in excluding inhalant allergy in a patient. This assay should be a useful tool for the primary care physician or nonallergist trying to determine if a patient is suffering from inhalant allergies, chronic sinusitis, or other causes of sinopulmonary congestion. In the current era of managed care with great effort being made to decrease test utilization and subspecialty referral, the use of this AlaTOP Inhalant allergy immunoassay along with highly effective antihistamines, bronchodilators, and nasal steroid preparation should allow the primary care physician or nonallergist to effectively manage an increasing percentage of patients with inhalant allergy.

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