

Hypersensitivity Testing for *Aspergillus fumigatus* IgE Is Significantly More Sensitive Than Testing for *Aspergillus niger* IgE

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Abstract

We sought to determine if sufficient redundancy exists between specific IgE testing for *Aspergillus fumigatus* and *Aspergillus niger* to eliminate one of the assays in determining *Aspergillus* hypersensitivity. We reviewed regional laboratory results comparing *A fumigatus*-specific IgE with *A niger*-specific IgE using the Pharmacia UniCAP system (Pharmacia, Kalamazoo, MI). By using the Fisher exact test as an index of concordance among paired results, we showed a significant difference between 109 paired samples for the presence of specific IgE to *A fumigatus* and *A niger* ($P < .0001$). Of these specimens, 94 were negative for IgE to both species, 10 were positive for *A fumigatus* and negative for *A niger*; no specimen was positive for *A niger* and negative for *A fumigatus*. We conclude that *A fumigatus*-specific IgE is sufficient to detect *Aspergillus* hypersensitivity. The assay for *A niger*-specific IgE is redundant, less sensitive, and unnecessary if the assay for specific IgE for *A fumigatus* is performed.

Specific IgE testing for *Aspergillus* is important for determining allergic sensitivities to environmental mold and is crucial in demonstrating serum sensitivity for the diagnosis of allergic bronchopulmonary aspergillosis (ABPA). We noted that some clinicians order specific IgE testing for both *Aspergillus fumigatus* and *Aspergillus niger* to detect *Aspergillus* hypersensitivity. In fact, clinical practice varies widely, with some clinicians testing for a wide array of fungal sensitivity, others testing for a limited number of mold sensitivities, and others testing for only *A fumigatus* sensitivity.¹ We suspected that the presence of specific IgE to *A fumigatus* was more frequently positive when compared with the presence of specific IgE to *A niger* in the same specimen, and, therefore, the use of both tests for clinical decision making was unnecessary, with the specific IgE testing for *A niger* being of no additional benefit when testing for the specific IgE to *A fumigatus* was performed. We sought to determine if there was sufficient redundancy to allow elimination of one of the assays, thus enhancing clinical efficiency and cost-effectiveness, and if there were specific practice patterns that led to the use of testing for both *A fumigatus*- and *A niger*-specific IgE.

Materials and Methods

We reviewed 5 years of allergy testing (2002-2006) performed at the Special Diagnostic Immunology Laboratory, Hackensack University Medical Center, Hackensack, NJ, comparing specific IgE levels to *A fumigatus* with specific IgE levels to *A niger* using the Pharmacia UniCAP 100 system (Pharmacia, Kalamazoo, MI). This automated system is an enzyme-linked immunoallergosorbent-based assay calibrated

against the World Health Organization standard for IgE and expressed in World Health Organization units of kilounits/liter (kU_A/L), with U_A indicating an allergen-specific unit.

A review of *Aspergillus*-specific IgE testing revealed 870 specimens in which *A fumigatus* was tested alone (not paired with *A niger*) as part of a physician-requested regional environmental panel; 109 specimens for which *A fumigatus* and *A niger* were tested, specifically having been ordered as a pair by the requesting physician; and 17 specimens tested for *A fumigatus* alone on the specific request of the ordering physician. Paired testing for *A fumigatus*- and *A niger*-specific IgE represented 10.9% of the requests for *Aspergillus* IgE.

The values of these 109 clinical specimens were evaluated by using the Fisher exact test as an index of concordance comparing the presence of specific IgE to *A fumigatus* with the presence of specific IgE to *A niger* for each specimen. For the 15 paired specimens with detectable specific IgE to one or both *Aspergillus* species, simple means of the respective species-specific IgE levels were calculated with values of less than $0.35 \text{ kU}_A/\text{L}$ set to zero for purposes of the calculation of this mean.

Results

In the clinical patient samples that showed a detectable specific IgE to *A fumigatus* and/or *A niger*, there was a significant difference in the comparison of the presence of specific IgE to *A fumigatus* and *A niger* ($P < .0001$). Ten specimens were positive for specific IgE to *A fumigatus* and negative

for specific IgE to *A niger*. No specimens that were positive for specific IgE to *A niger* were negative for specific IgE to *A fumigatus*. Of the specimens, 95 had the same IgE class (Pharmacia-determined class ranges: class 0, $<0.35 \text{ kU}_A/\text{L}$; class 1, $0.35\text{--}0.69 \text{ kU}_A/\text{L}$; class 2, $0.70\text{--}3.49 \text{ kU}_A/\text{L}$; class 3, $3.50\text{--}17.49 \text{ kU}_A/\text{L}$; class 4, $17.5\text{--}49.9 \text{ kU}_A/\text{L}$; class 5, $50.0\text{--}100 \text{ kU}_A/\text{L}$; class 6, $>100 \text{ kU}_A/\text{L}$) for both species. Of the 109 specimens, 15 (13.8%) were positive to *A fumigatus* and only 5 (4.6%) of 109 specimens were positive to *A niger*. In 13 specimens, the IgE class was higher in *A fumigatus*, differing by 1 class level in 6, 2 class levels in 3, 3 class levels in 3, and 4 class levels in 1 (Table 1). Of 109 specimens, 1 had the same class level for *A fumigatus* and *A niger* (class 2), and 1 of 109 showed a higher class level in *A niger*, differing by 1 class (class 3 vs 4). For the 15 specimens with a detectable specific IgE level to either *Aspergillus* species, the mean specific IgE level to *A fumigatus* was $5.88 \text{ kU}_A/\text{L}$ and the mean specific IgE level to *A niger* was $1.96 \text{ kU}_A/\text{L}$. Only 2 of the 15 specimens showed a higher level of measured specific IgE antibody to *A niger*.

The most common associated diagnosis for which a positive result was obtained was allergic rhinitis ($n = 6$), with sinusitis (acute or chronic) the second most frequent diagnosis ($n = 4$). The latter diagnosis was always associated with a concomitant diagnosis of allergic rhinitis (Table 1). More than half the testing for any *Aspergillus* IgE (about 57%) was ordered by 2 of 106 physicians, both of whom were allergists. The majority of *Aspergillus*-specific IgE testing was for *A fumigatus* alone; 870 of these assays were performed as part of a regional environmental allergy panel, in which

Table 1
Characteristics of the Subset of Serum Samples With Detectable *Aspergillus*-Specific IgE to *Aspergillus fumigatus* and/or *Aspergillus niger*

Sample No.	<i>A fumigatus</i>	<i>A niger</i>	<i>A fumigatus</i> (kU_A/L)	<i>A niger</i> (kU_A/L)	<i>A fumigatus</i> Class*	<i>A niger</i> Class*	Diagnosis
1	+	–	0.38	<0.35	1	0	Aspergillosis, bronchiectasis
2	+	+	14.3	0.45	3	1	Allergic rhinitis, acute sinusitis, chronic pharyngitis, nasal sinus polyp
3	+	–	0.71	<0.35	2	0	Unknown
4	+	–	1.58	<0.35	2	0	Allergic rhinitis, chronic sinusitis
5	+	+	1.02	2.74	2	2	Allergic rhinitis, chronic sinusitis, asthma
6	+	–	27.8	<0.35	4	0	Urticaria, upper respiratory infection
7	+	–	9.75	<0.35	3	0	Sarcoidosis, bronchiectasis
8	+	–	0.47	<0.35	1	0	Allergic rhinitis
9	+	+	4.58	1.39	3	2	Allergic rhinitis
10	+	+	3.93	23.4	3	4	Allergic rhinitis, sinusitis
11	+	–	13.1	<0.35	3	0	Cystic fibrosis
12	+	–	0.4	<0.35	1	0	Chronic pharyngitis, unspecified immune deficiency
13	+	–	5.02	<0.35	3	0	Unspecified respiratory abnormality
14	+	–	0.39	<0.35	1	0	Unknown
15	+	+	4.73	1.45	3	2	Unknown

kU_A/L , kilounits per liter, with U_A indicating an allergen-specific unit; +, present; –, absent.

* The Pharmacia (Kalamazoo, MI)-determined IgE classes are defined as follows: class 0, $<0.35 \text{ kU}_A/\text{L}$; class 1, $0.35\text{--}0.69 \text{ kU}_A/\text{L}$; class 2, $0.70\text{--}3.49 \text{ kU}_A/\text{L}$; class 3, $3.50\text{--}17.49 \text{ kU}_A/\text{L}$; class 4, $17.5\text{--}49.9 \text{ kU}_A/\text{L}$; class 5, $50.0\text{--}100 \text{ kU}_A/\text{L}$; class 6, $>100 \text{ kU}_A/\text{L}$.

A niger-specific IgE was not included. When ordered as individually selected tests, 86.5% of the time, *A fumigatus* and *A niger* were ordered together. Allergists requested 96 (88.1%) of the 109 paired specimens.

Discussion

The determination of the presence of specific IgE to *Aspergillus* species is clinically relevant for detecting sensitivities in perennial allergic rhinitis, allergic asthma, or ABPA. In the present environment of limited resources for medical diagnosis, elimination of redundant testing is essential.

To our knowledge, this is the largest study of *Aspergillus* hypersensitivity comparing the sensitivity of *A fumigatus* with *A niger*. We demonstrated statistically significant greater sensitivity of the specific IgE assay for *A fumigatus* in detecting allergic sensitivity to *Aspergillus* compared with the specific IgE assay for *A niger*. In fact, in this study, the use of specific IgE to *A niger* alone would have missed two thirds of the *Aspergillus* sensitivity detected by the specific IgE assay to *A fumigatus* if a single assay had been used. Furthermore, when both assays are used, we have shown that there is no case in which sensitivity to *A fumigatus* is not detected when a sensitivity to *A niger* is detected.

We do not believe this is strictly a geographic regional effect, although *A niger* germination is greatly affected by humidity, with a failure to germinate at a humidity of less than 76%. We believe this difference in sensitivity is more than likely a physiologic effect, with colonization of *A niger* in the lung being more difficult to establish than *A fumigatus*. This is likely related to exquisite sensitivity to pH, with *A niger* requiring a pH of 4.5 for optimal germination and germ tube length. Also, structural differences between *Aspergillus* species such as conidial size make alveolar space penetration more difficult for *A niger*.^{2,3} There are only rare reports of aspergillosis in which *A niger* is the pathogen, and these usually involve an immunocompromised state, such as in patients with bone marrow transplantation or lung transplantation.^{4,5}

In addition, *A niger*-associated aspergillosis has been seen in patients with diabetes mellitus, which is thought to create an acidic environment conducive to survival of *A niger* with its affinity for low pH.^{2,6,7} In fact, in our series, the 2 patients with higher specific IgE levels of *A niger* compared with specific IgE levels of *A fumigatus* had a history of diabetes mellitus (Mary A. Michelis, MD, unpublished data, personal communication, January 2011).

Hoshino et al⁸ reported a case of ABPA associated with *A niger* isolated from sputum. In this case, the patient had a positive skin test to *A fumigatus*, likely as part of the clinical criteria for diagnosis of ABPA, thus demonstrating an in vivo cross-sensitivity between *A fumigatus* and *A niger* in this

patient. Lake et al⁹ reported a case of mixed allergic bronchopulmonary fungal disease with *Aspergillus terreus* isolated from bronchoscopy sputum. In this case, *A terreus* induced a positive skin test response (7-mm wheal) with a cross-reacting skin test response to *A fumigatus* (6-mm wheal), the latter of which was twice the size of the cross-reacting skin test reaction to *A niger* (3-mm wheal). This suggests, in this one patient, the generation of a more robust in vivo response of specific IgE to *A fumigatus* compared with *A niger*, resulting in a greater sensitivity of the *A fumigatus* in vivo skin test compared with *A niger*.

Although assays for IgE to *Aspergillus* are classically associated with aspergillosis, only one of the positive samples was ordered with a diagnosis of aspergillosis. As noted, the most common diagnosis associated with the positive *Aspergillus* IgE result in the paired testing in this study was allergic rhinitis (Table 1) and likely reflects that the majority of the physicians requesting this testing were allergists, compared with their colleagues in the specialties of pulmonology, pediatrics, and internal medicine (data on file) who tended to order *A fumigatus* testing alone, when not ordered as part of a panel, but as a specific query for the presence of *Aspergillus* hypersensitivity. This finding suggests the pattern of requesting multiple *Aspergillus* species IgE assays evolved according to specialty and/or local convention.

We conclude, in general, that there is no clinical usefulness or relevance for the *A niger*-specific IgE assay in determining *Aspergillus* sensitivity because the *A fumigatus*-specific IgE assay has a statistically significant superior sensitivity for making this determination. However, certain rare exceptions may exist in specific occupations in which proteolytic enzymes isolated from *A niger* are used, such as phytase, a phosphatase derived from *A niger* used by animal feed additive manufacturers, which is an identified allergen in reported cases of occupational asthma in these workers. Serum of phytase-sensitized workers showed enzyme immunoassay inhibition for IgE specifically to *A niger* but not to other molds.¹⁰ Flaviastase, another proteolytic enzyme isolated from *A niger*, is implicated in inducing occupational asthma in pharmacy workers.¹¹ In these various occupational exposures, the specific evaluation for specific IgE to *A niger* has the potential to contribute to confirming the origin of the occupational exposure, depending on the actual allergen(s) and the ability to detect them with the assay.

In addition, the potential general exposure to *A niger*-derived gene product in certain genetically engineered foods such as soybeans may lead to clinical hypersensitivities to unique allergens, depending on the allergenicity of the product expressed.¹² Since current detection of *A niger* sensitivity in the isolation of enzymes from *A niger* is likely the result of antigen impurities in the isolation, the use of the *A niger*-specific IgE assay in genetically engineered foods or products

may not be relevant as the impurities of the original source of the gene may not be present. For example, the expression of *Aspergillus*-derived α -amylase genes in *Saccharomyces cerevisiae*¹³ may generate sensitivities to α -amylase and/or *S cerevisiae* rather than the *Aspergillus* species of origin. Future considerations regarding genetic engineering need to address certain modes of sensitivity, eg, ingested vs inhaled, leading to different clinical expression of allergic hypersensitivity, and the creation of neoantigens leading to diagnostic conundrums such as the development of clinical allergy hypersensitivity to altered soybeans but with negative specific IgE testing to natural soybeans. These scenarios highlight the importance of a detailed history of exposures when deciding on confirmatory assays to avoid unnecessary testing and the importance of understanding the availability of the testing repertoire and the relevance of the potentially requested assay.

While both *A fumigatus* and *A niger* specific IgE tests are offered with the Pharmacia ImmunoCAP systems and are not considered analyte-specific reagents, difficulties may arise in the context of other *Aspergillus* sensitivities encountered in other specific scenarios, such as exposure of industrial bakers using endoxylanase X24 derived from *Aspergillus nidulans* and α -amylase derived from *Aspergillus oryzae*,¹³ for which these *Aspergillus*-specific IgE tests are not available. However, depending on the history, one could simply obtain a specific IgE test for α -amylase or, as noted, *S cerevisiae*, as applicable, which are available as tests in the current analyzer used in this study and are not considered analyte-specific reagents. However, significant cost considerations remain given the rarity of these testing requests.

As noted, there is little justification for the additional cost of conducting both *A fumigatus*- and *A niger*-specific IgE assays as additional diagnostic information is generally not obtained since the *A fumigatus*-specific IgE assay is the most sensitive test when evaluating for *Aspergillus* sensitivity in most clinical settings. Conversely, in the rare clinical situation in which *A niger*-specific IgE testing is indicated, the addition of *A fumigatus*-specific IgE testing would likely not add direct relevant specific data to the clinical evaluation. Further studies are recommended to assess whether currently entrenched practice patterns lead to duplicate or irrelevant allergy testing in other clinical manifestations of allergy hypersensitivity that can be evaluated for cost savings. This is particularly relevant when selecting the components of allergy testing panels, which themselves can be subject to reduced reimbursement due to caps on the allowable number of tests covered for payment by third-party payers.

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