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

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Key Words: Immunology; Aspergillus species; IgE; Hypersensitivity testing; Allergy panel testing

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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.

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 (kUA/L), with UA indicating an allergen-specific unit; +, present; –, absent.

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 kUA/L set to zero for purposes of the calculations 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 kUA/L; class 1, 0.35-0.69 kUA/L; class 2, 0.70-3.49 kUA/L; class 3, 3.50-17.49 kUA/L; class 4, 17.5-49.9 kUA/L; class 5, 50.0-100 kUA/L; class 6, >100 kUA/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. 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 kUA/L and the mean specific IgE level to *A. niger* was 1.96 kUA/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

<table>
<thead>
<tr>
<th>Sample No.</th>
<th><em>A. fumigatus</em></th>
<th><em>A. niger</em></th>
<th><em>A. fumigatus</em> Class</th>
<th><em>A. niger</em> Class</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>–</td>
<td>&lt;0.35</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>14.3</td>
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<td>3</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>–</td>
<td>0.71</td>
<td>&lt;0.35</td>
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</tr>
<tr>
<td>4</td>
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<td>–</td>
<td>1.58</td>
<td>&lt;0.35</td>
<td>2</td>
</tr>
<tr>
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<td>+</td>
<td>+</td>
<td>1.02</td>
<td>2.74</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>–</td>
<td>27.8</td>
<td>&lt;0.35</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
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<td>9.75</td>
<td>&lt;0.35</td>
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</tr>
<tr>
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<td>–</td>
<td>0.47</td>
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<td>1</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>14.3</td>
<td>1.39</td>
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<td>+</td>
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<td>23.4</td>
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<td>–</td>
<td>13.1</td>
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<tr>
<td>12</td>
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<td>0.4</td>
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<td>1</td>
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<tr>
<td>13</td>
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<td>–</td>
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<td>&lt;0.35</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
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<td>–</td>
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<td>&lt;0.35</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>+</td>
<td>4.73</td>
<td>1.45</td>
<td>3</td>
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</tbody>
</table>

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

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

References