Fungal Sensitization in Childhood Persistent Asthma Is Associated With Disease Severity

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Summary. Rationale: Recent observations, especially in adults, suggest that asthma severity may be associated with fungal sensitization. Other studies suggest that some patients with severe asthma and fungal sensitization may benefit from anti-fungal therapy. Currently, the prevalence of fungal sensitization among children with severe asthma is not well characterized. Methods: We determined prevalence of fungal sensitization among children with moderate to severe persistent asthma and compared clinical characteristics between sensitized and non-sensitized children, including asthma severity, serum immunoglobulin E, and pulmonary function. Results: Of the 64 children enrolled, 25 (39%) had evidence of sensitization to one or more fungi. Nineteen of 25 (76%) children with fungal sensitization were categorized as severe persistent compared to 13 of 39 (33%) children without evidence of fungal sensitization (odds ratio = 6.33, 95% confidence interval 2.04–19.68, $P = 0.0014$). Of 32 severe persistent asthmatics, 19 (59%) demonstrated evidence of fungal sensitization. Serum immunoglobulin E was significantly higher ($P < 0.001$), and pulmonary function (including FEV1, FEV1/FVC, and FEF25–75%) significantly lower in the fungal-sensitized patients ($P = 0.016$, 0.0004, and 0.002, respectively). Bronchial biopsy of sensitized children revealed basement membrane thickening and eosinophil infiltration. Conclusions: Fungal sensitization in children with persistent asthma is associated with disease severity. Almost 60% of our severe persistent asthma patients had evidence of fungal sensitization and, based on our previous studies, may be potential candidates for anti-fungal therapy. **Pediatr Pulmonol.** © 2013 Wiley Periodicals, Inc.

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INTRODUCTION

Asthma is a heterogeneous disease with multiple etiologies resulting in airway obstruction and inflammation. Current therapies for asthma focus on controlling symptoms and include beta agonists, corticosteroids, and other anti-inflammatory agents such as leukotriene receptor antagonists. While most patients with asthma respond to these conventional medications, some continue to have significant symptoms despite escalating therapy. As such, recent investigations have focused on the identification and characterization of specific subtypes of asthma—particularly when disease is not responding to traditional treatment—in an attempt to develop individualized therapy.[1–4]

Fungal-associated asthma may represent one such subtype. Fungi are known to exacerbate respiratory symptoms in individuals with asthma, and sentization has been described as a risk factor for life-threatening disease.[5–7] Although anti-fungal treatment has been reported to improve clinical symptoms for patients with allergic bronchopulmonary aspergillosis (ABPA), a hyper-immune response to fungal colonization that has been described in asthmatics,[8] few children fulfill the recommended criteria for diagnosis—including peripheral eosinophila $> 10\%$, serum immunoglobulin E (IgE) $> 1{,}000$ IU/ml, positive *Aspergillus* skin test, *Aspergillus*-specific IgE, precipitating immunoglobulin G antibodies against *Aspergillus*, recurring radiographic infiltrates and, in the most severe cases, central bronchiectasis. As such, pediatric asthma patients are rarely considered candidates for anti-fungal therapy. However, observations in adults and, to a much lesser extent, children with fungal sensitization in the context of severe persistent asthma suggest benefit from prolonged antifungal therapy even if they do not fully meet criteria for ABPA.[9–11] Thus, the contribution of fungi to poorly controlled asthma in children is likely to be underestimated, as is the number of patients who might benefit from antifungal therapy.

Previously, we described a small series of children with severe persistent asthma and fungal sensitization who also carried a common mutation in the *CHIT1* gene, which results in decreased enzymatic activity of chitotriosidase.[12] Earlier studies have shown that chitotriosidase, a member of the chitinase family that is highly expressed in the human lung, demonstrates antifungal activity in vitro and in vivo.[13–15] For the current manuscript, we recruited patients with moderate to severe persistent asthma in order to estimate the prevalence of fungal sensitization, identify patients who could potentially benefit from anti-fungal therapy and compare clinical characteristics between fungal-sensitized patients and those without fungal sensitization. Portions of this manuscript have been previously reported in abstract form.

ABBREVIATIONS:

ABPA	Allergic bronchopulmonary aspergillosis
FEF25–75	Forced expiratory flow, 25–75%
FEV1	Forced expiratory volume, 1 sec
FVC	Forced vital capacity
IgE	Immunoglobulin E
IRB	Institutional review board
PFT	Pulmonary function test
RAST	Radioallergosorbent test

PATIENTS AND METHODS

Study Design

We conducted a cross-sectional study to investigate fungal sensitization in children with moderate to severe persistent asthma. Subjects were recruited from two academic pediatric pulmonary practices in the greater New York area between November 2010 and June 2012. All children (age 2–21 years) with a diagnosis of asthma who were categorized as having moderate to severe persistent disease by the National Asthma Education and Prevention Program guidelines and were failing Step 4 or greater therapy were approached for enrollment (guidelines available at http://www.nhlbi.nih.gov/guidelines/asthma/asthsumm.pdf). Reversible airway obstruction was demonstrated in all age-appropriate children by pulmonary function tests (PFTs). All patients were followed by pediatric pulmonary sub-specialists and underwent at least quarterly evaluations to determine control of symptoms, adherence to therapy, and optimization of co-morbid conditions including environmental allergies, gastroesophageal reflux, and obstructive sleep apnea.

Informed consent and, when applicable, assent were obtained prior to enrollment. The study was approved by the Institutional Review Boards of Cohen Children's Medical Center, Weill Cornell Medical College and Albert Einstein College of Medicine.

Pulmonary Function Tests

Pulmonary function tests, performed as part of routine care, were reviewed for all patients enrolled in the study. Average values of forced expiratory volume in 1 sec (FEV1), forced expiratory volume in 1 sec/forced vital capacity ratio (FEV1/FVC), and forced expiratory flow 25–75% (FEF25–75) for the year preceding enrollment were recorded.

Serum Collection

Serum was analyzed for total IgE levels and radioallergosorbent tests (RAST) for environmental allergens. Fungal allergens represented in routine RAST panels

for our region include: *Aspergillus spp*, *Alternaria spp*, *Candida spp*, *Cladosporium spp*, *Setomelanomma spp*, *Mucor spp*, and *Penicillium spp*. For the purposes of this manuscript, all RAST responses equal to or greater than 0.35 KU/L (i.e., class 1 or higher) were considered to be indicative of sensitization. At the time of routine serum collection for IgE and RAST, an additional 2–5cc was drawn and stored at -80°C for additional analysis.

Diagnosis of Severe Persistent Asthma

Severe persistent asthma was diagnosed if patients fulfilled one major and at least two minor criteria as recommended by the Severe Asthma Research Program (SARP) guideline.[16] *Major criteria:* (1) treatment with high dose inhaled corticosteroids for age or (2) treatment with oral corticosteroids for >50% of the year. *Minor criteria*: (1) use of an additional controller medication to maintain asthma control; (2) use of short-acting bronchodilators at least 5 of 7 days of the week; (3) baseline FEV1 < 80% predicted; (4) one or more urgent care visits in the previous year; (5) three or more oral corticosteroid bursts in the previous year; (6) a history of prompt deterioration in asthma symptoms with any reduction in corticosteroid therapy; or (7) a near-fatal asthma event requiring intubation in the past.

ABPA Screening

Further screening for ABPA was performed in children who demonstrated sensitization against *Aspergillus* as well as an IgE level > 1,000 IU/ml, including eosinophil counts, chest radiography, serum precipitants, and skin prick testing. Computed tomography was not routinely recommended for these patients.

Flexible Bronchoscopy

Several patients in both the fungal-sensitized and non-fungal-sensitized groups underwent flexible bronchoscopy during the course of their evaluation. For all patients who underwent bronchoscopy, medical records were reviewed and findings were recorded, including results from bronchoalveolar lavage (BAL) and, when applicable, endobronchial biopsies.

Statistical Analysis

Comparisons for age, PFTs and serum IgE levels were performed using the Mann–Whitney Test. Odds ratio (OR) was calculated to identify any associations between severe persistent asthma and fungal sensitization. A *P*-value <0.05 was considered significant. All statistics were done with Graph Pad InStat software (San Diego, CA).

RESULTS

General Characteristics

Sixty-four patients with moderate to severe persistent asthma were recruited into the study, all of whom were maintained on Step 4 or higher therapy. The majority of patients were recruited in the outpatient setting, although two were enrolled while in the pediatric intensive care unit for severe exacerbations. Of the 64 patients enrolled, 25 (39%) demonstrated evidence of fungal sensitization by RAST. All but two children demonstrated class 2 or higher responses to one or more organisms and 56% of children demonstrated class 3 or higher responses. Twenty-five patients (39%) were sensitized to non-fungal allergens (including, but not limited to, dust, cockroach, trees, cat, and dog), and 14 (22%) were non-sensitized.

Twelve patients in the fungal-sensitized group who demonstrated sensitization to *Aspergillus* and a serum IgE level > 1,000 IU/ml were also evaluated for the presence of ABPA, none of whom met diagnostic criteria. Of these patients, one had a positive skin test but negative precipitants, normal eosinophil levels and unremarkable radiologic studies, while two had eosinophil counts >10% within the year prior to enrollment but otherwise negative skin testing, serum precipitants and radiologic studies. ABPA screening was not recommended for one patient who demonstrated *Aspergillus* sensitivity and an IgE level > 1,000 IU/ml; this patient was a 4-year-old boy classified as a moderate persistent asthmatic whose symptoms eventually came under good control following the winter season while continuing his high-dose inhaled corticosteroid and montelukast.

There was no difference in male:female ratio between fungal-sensitized patients and those without fungal sensitization. Fungal-sensitized patients were slightly older than those without fungal sensitization (median values 11 years vs. 9 years, respectively, $P = 0.02$) and demonstrated higher serum IgE levels (1,049 IU/ml vs. 78 IU/ml, respectively, $P < 0.0001$). Fungal-sensitized patients also exhibited worse PFTs, including FEV1 (81.5% predicted vs. 95.5% predicted, respectively, $P = 0.016$), FEV1/FVC (71.5 vs. 83, respectively, $P = 0.0004$), and FEF25–75% (55% predicted and 78.5% predicted, respectively, $P = 0.002$). Differences in serum IgE, FEV1, FEV1/FVC, and FEF25–75% remained significant when comparing fungal-sensitized patients to a subgroup of patients who were sensitized to non-fungal environmental allergens. Results are summarized in Table 1.

The most commonly implicated organisms in fungal-sensitized patients included: *Aspergillus spp* (84%),

TABLE 1— Clinical Characteristics

	Fungal sensitization (n = 25)	No fungal sensitization (n = 39)	*P*-value	Sensitization to non-fungal allergens (n = 25[1])	*P*-value
Male:female	2.6	2.0	0.65 (NS)	1.8	0.55 (NS)
Age (years)	11 (IQR 9.5–14.5)	9 (IQR 6–12)	0.02	9 (IQR 7–15)	0.2 (NS)
IgE (IU/ml)	1,049 (IQR 566–2319)	78 (IQR 21–308)	<0.0001	257 (IQR 80–480)	<0.0001
	Fungal sensitization (n = 22)	No fungal sensitization (n = 26)	*P*-value	Sensitization to non-fungal allergens (n = 19[1])	*P*-value
FEV1 (% predicted)	81.5 (IQR 65–88)	95.5 (IQR 81–101)	0.016	96 (IQR 81–101)	0.034
FEV1/FVC	71.5 (IQR 64–78)	83 (IQR 78–88)	0.0004	82 (IQR 75–89)	0.008
FEF25–75% (% predicted)	55 (IQR 36–61)	78.5 (IQR 60–86)	0.002	74 (IQR 59–85)	0.011

NS, not significant.
Medians and inter-quartile ranges (IQR) are shown (except for male:female ratio).
P-values are for comparison with fungal sensitization cohort.
[1]Subset of "No fungal sensitization" group.

Alternaria spp (72%), *Candida spp* (52%), *Cladosporium spp* (36%), *Mucor spp* (32%), *Setomelanomma spp* (16%), and *Penicillium spp* (16%). Seventy-two percent of fungal-sensitized children demonstrated sensitization to more than one organism.

Fungal Sensitization and Asthma Severity

Among the 25 fungal-sensitized patients, 19 (76%) were characterized as severe persistent, while 6 (24%) were characterized as moderate persistent. In comparison, only 13 of 39 (33%) patients without fungal sensitization were characterized as severe persistent while 26 (67%) were characterized as moderate persistent (Fig. 1). OR for having severe persistent asthma among fungal-sensitized patients compared to those without fungal sensitization was 6.33 (95% confidence interval [CI] 2.04–19.68, $P = 0.0014$), and the association remained significant when comparing fungal-sensitized patients to a sub-group of patients who were sensitized to non-fungal aeroallergens (OR 4.75, 95% CI 1.4–16.1, $P = 0.012$). Of the 32 patients with severe persistent asthma, 19 (59%) demonstrated evidence of fungal sensitization.

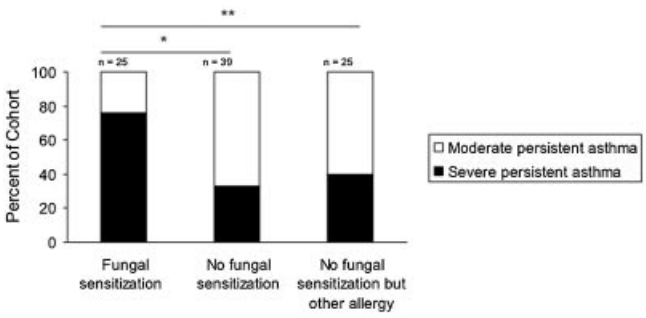

Fig. 1. Asthma severity and fungal sensitization. Presence of severe persistent asthma is associated with fungal sensitization. *Odds ratio = 6.33, 95% confidence interval = 2.04–19.68, $P = 0.0014$ for comparison between patients with and without fungal sensitization. **Odds ratio = 4.75, 95% confidence interval = 1.4–16.1, $P = 0.012$ for comparison with subset of patients without fungal sensitization, but with sensitization to other allergens.

Bronchoscopy Data

Eight patients with fungal sensitization and 12 without underwent flexible bronchoscopy during the course of their evaluation, as dictated by their primary pulmonologist. Routine fungal cultures of BAL fluid were negative for all samples reviewed. Eosinophil (but not neutrophil) counts were elevated in BAL fluid from fungal-sensitized patients compared to those without fungal sensitization (Fig. 2). Endobronchial biopsy was performed in six patients with severe asthma and fungal sensitization. Four (67%) demonstrated significant basement membrane thickening and submucosal eosinophil infiltration.

DISCUSSION

Fungi and asthma have long been linked in the medical literature, but associations between the two are frequently viewed as either coincidental observations (i.e., generalized allergic sensitization contributing to asthma) or uncommon phenomena (i.e., ABPA). Nonetheless, there has been increasing interest in fungal-associated asthma with respect to asthma severity. Most findings supporting this connection have been limited to adults in whom fungal sensitization has been associated with severe asthma requiring admission to the intensive care unit or multiple hospitalizations.[5,7] In a separate cross-sectional study of adults from Europe, mold

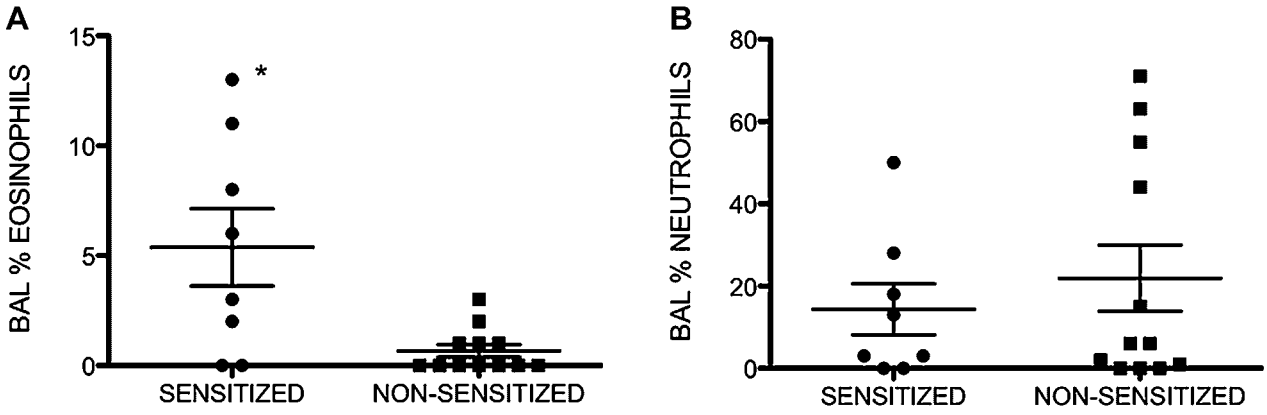

Fig. 2. BAL fluid eosinophil and neutrophil counts among children undergoing bronchoscopy for asthma evaluation. A: Eosinophil percentages are higher in fungal-sensitized patients (n = 8) compared to those without fungal sensitization (n = 12; means of 5.4% vs. 0.7%), respectively, $^{*}P = 0.025$. B: In contrast, there were no differences in neutrophil percentages, among the groups. For both figures, means and SEM are shown.

sensitization positively correlated with asthma severity, which included decreased pulmonary function.[17] Recently, fungal sensitization has also been linked to asthma morbidity in inner city children.[18]

In the current investigation, we found that almost 40% of our pediatric patients with persistent asthma were sensitized to one or more fungi, and that sensitization was associated with severe persistent disease. Consistent with these findings, fungal-sensitized patients demonstrated significantly higher IgE levels and lower PFTs (including FEV1, FEV1/FVC, and FEF25–75). IgE and PFT associations remained significant when comparing fungal-sensitized patients with a sub-group of patients who were sensitized to other environmental allergens, suggesting a more robust generalized asthma response to fungal allergens. Among our patients with severe persistent disease, almost 60% demonstrated evidence of fungal sensitization and elevated IgE levels, and as such could be considered potential candidates for anti-fungal treatment based on previous reports in adults. Importantly, large controlled trials evaluating the effectiveness and safety of itraconazole specifically in asthmatic children have not been performed, and to our knowledge, current pediatric data are limited to our own case reports.[12] Collectively, our results are consistent with previous reports demonstrating fixed airway obstruction and bronchiectasis in adult asthma patients sensitized to *Aspergillus spp*.[19,20] and as such highlight the importance of early detection of fungal sensitization in asthmatic children. However, it remains unclear whether early treatment can prevent or alleviate fixed obstruction in such patients. Moreover, recent reports have found only weak associations between *Aspergillus* sensitization and asthma severity in adults.[21] It seems likely that fungal sensitization represents a continuum of disease which can range from incidental sensitization in patients with intermittent asthma, to more severe disease including severe asthma with fungal sensitization (SAFS) and ABPA with central bronchiectasis, possibly dictated by varying T-helper cell type 2 responses among individuals.[11] Longitudinal studies may help to determine which patients with fungal sensitization in early life progress to develop ABPA as adults.

It is unclear from this study whether fungal sensitization reflects ongoing infection of the lower airway or results from repeated but transient environmental exposure. Unfortunately, fungal culture of sputum and bronchoalveolar lavage fluid has historically demonstrated low sensitivity for infection, even in the face of known invasive lung disease. In our study, we evaluated BAL fluid from a limited number of patients, none of which was positive for fungus. Although our findings of increased eosinophils in BAL fluid from fungal-sensitized patients may suggest a localized response to colonization, our limited data is insufficient to imply causality. Recently, some have questioned the standardized techniques for fungal culture utilized in many hospital settings, demonstrating increased sensitivity with procedure modifications.[22] Clearly, new methods for identifying patients with low-microbial-burden airway infection or colonization are required to determine which patients might truly benefit from anti-fungal therapy, and which might be more appropriately managed with targeted avoidance measures or other environmental modifications. In previous investigations, we and others have analyzed bronchoalveolar lavage fluid for antibodies to fungal antigens as a possible reflection of direct airway exposure or colonization.[23,24] Although these techniques have limited clinical utility at present and require further validation, similar methods may be useful in the

future. Indeed, some investigators have begun to apply a variety of culture-independent tools to profile lower airway microbiomes in chronic respiratory diseases including asthma.[25]

There are several limitations to the current study, including a small sample size. Because we recruited patients with moderate to severe persistent asthma under sub-optimal control, the small sample size was expected. Furthermore, patients were approached for enrollment only after ensuring that ongoing symptoms were not specifically due to poor compliance with or inappropriate use of medications, further limiting sample size. In order to maximize enrollment, patients were recruited independently by four sub-specialty pediatric pulmonary practitioners at two different locations in the greater New York City area (Cohen Children's Medical Center and Weill Cornell Medical College). It should be noted that there are some differences regarding diagnostic criteria of severe persistent asthma as recommended by SARP and those recommended by the National Asthma Education and Prevention Program Expert Panel Report 3 (EPR-3). Because EPR-3 diagnostic criteria include certain subjective measures (i.e., limitation of activity), we chose to simplify our diagnostic criteria for the purposes of this manuscript but acknowledge the challenges in differentiating moderate-persistent and severe-persistent asthma. An additional limitation is that we were unable to account for environmental fungal burden, which is likely to be a critical component in sensitization. Lastly, we may have under-estimated the prevalence of fungal sensitization by not routinely performing skin prick testing and testing for only a limited number of organisms. Indeed, skin prick testing can be positive in the absence of a serologic response, and sensitization against fungi for which we did not test has been described in adults.[7,26]

In summary, we demonstrate that fungal sensitization in childhood persistent asthma is associated with disease severity. As such, analysis of serum IgE and fungal sensitivity may become important in the evaluation and management of children with persistent asthma, although confirmatory studies are certainly indicated. While not currently viewed as standard treatment for patients with fungal-sensitized asthma, itraconazole or other anti-fungal agents could be considered under some circumstances as an alternative to escalation of corticosteroid therapy or addition of cytotoxic agents. At minimum, targeted environmental assessments should be considered and focused avoidance measures recommended for select children. New methods for detecting the presence of fungi in the lower airways are ultimately required to better characterize the significance of low-burden infection or colonization in asthma. Additional studies could focus on long-term sequelae of fungal sensitization in asthma, regional differences in prevalence, optimal treatment protocols and preventive strategies.

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