Severe asthma and fungi: Current evidence

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Original Article

Severe asthma and fungi: current evidence

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Bronchial asthma is an inflammatory disease of the airways which may be worsened due to numerous extrinsic factors. The most common trigger is continuous exposure to allergens of which fungal agents are important factors. There is overwhelming evidence for the presence of fungal sensitization in patients with asthma. The diagnosis of fungal sensitization can be made either with skin testing with antigens derived from fungi or measuring specific IgE levels. There is also a strong association between fungal sensitization and severity of asthma. Whether this relationship is causal or just casual remains to be investigated. A variety of fungi are known to cause sensitization in asthmatics, but the most important fungal agent(s) causing severe asthma with fungal sensitization (SAFS) are currently unknown. *Aspergillus* species seem to be the strongest candidates as only with *Aspergillus* spp. does one encounter two extreme immunologic phenomena, i.e., the *Aspergillus*-sensitive asthma and allergic bronchopulmonary aspergillosis. The initial clinical management of SAFS should be the same as asthmatics without fungal sensitization. There is some evidence of the role of itraconazole in the management of SAFS but its routine use in SAFS requires further evaluation. This review summarizes the current evidence on the link between fungi and severe asthma.

Keywords severe asthma with fungal sensitization, asthma, *Aspergillus*, allergic bronchopulmonary aspergillosis, ABPA, azoles

Introduction

Asthma is an inflammatory disease of the airways characterized by airway hyperresponsiveness and airflow limitation with resultant characteristic symptoms such as chest tightness, dyspnea, cough and wheeze [1]. It is a serious public health problem affecting people of all ages, throughout the world. When uncontrolled, asthma can lead to severe limitation on activities of daily life and can even be fatal. Severe asthma generally affects only a small percentage (5–10%) of the total asthma population [2]. However, it remains a frustrating disease for both patients and the clinicians treating them. These patients are not only difficult to treat and prone to recurrent exacerbations, but also contribute disproportionately to the overall costs of asthma [3]. Although difficult to define, severe asthma generally encompasses three major domains; (a) physiological severity (measured by pulmonary function tests and assessment of symptom scores), (b) functional severity (which is the impact of the disease on an individual's ability to perform age-appropriate activities), and (c) burden of illness (represents the emotional, social, and financial impact of asthma on the individual, the family and society as a whole) [4].

The severity of asthma varies from patient to patient and even in an individual patient, and the reasons for this are not fully understood. However, it is known that two-thirds of asthmatics are atopic to common allergens [5–7] and individuals with severe asthma may have a greater degree of atopy than other asthmatic patients [8]. Numerous extrinsic factors are known to worsen asthma control, and the most common factor is the continuing exposure to a triggering allergen. The most common allergens implicated are house dust mite (HDM), animal dander from pets, and

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environmental fungi [1,9]. In adults, the putative role of house dust mite (HDM) as the dominant exogenous agent for asthma has been questioned by the recently published cohort mite avoidance studies [10].

The link between fungi and asthma has been known for centuries [11], but the role of fungal allergens as the primary extrinsic factor for asthma severity has been incompletely explored, possibly because the exposure is universal. This review summarizes the current evidence on the link between fungi (both *Aspergillus* and non-*Aspergillus* species) and severe asthma.

Non-*Aspergillus*-related fungi and severe asthma

Fungi can be associated with severe asthma patients in a number of ways, i.e., (a) through inhalation of fungal spores, (b) through fungal sensitization, which is defined as the presence of immediate cutaneous hyperreactivity to fungal antigen or increase in specific IgE antibodies to a particular fungus; fungal sensitization may or may not be associated with severe asthma (Table 1), and (c) through causation of allergic bronchopulmonary mycosis, a form of severe fungal sensitization with resultant irreversible bronchopulmonary damage.

Inhalation of environmental fungal spores

Exposure to environmental fungal spores has been associated with worsening asthma symptoms, lung function, hospital admissions and asthma-related deaths. Neas *et al*. examined the impact of fungal spore concentrations on daily variations in symptoms and peak expiratory flow (PEF) in 108 children [12]. The authors recorded the asthma symptoms, PEF, and the time spent outdoors and correlated it with the 24-hour average fungus spore concentrations. They found that a 10,000 spore per cubic meter increment in *Cladosporium* spore concentrations was associated with a deficit in morning PEF and a 60 spore per cubic meter increment in *Epicoccum* spore concentrations was connected with increased incidence of morning cough and a deficit in morning PEF [12]. In another study involving 22 asthmatics, the authors found that an increase of nearly 4,000 spores per cubic meter worsened asthma symptom scores, increased inhaler use and decreased the evening PEF [13].

Studies have shown that the rates of hospital admission for asthma tend to be exceptionally high on days with high total mold spore counts, but no specific taxa have been consistently implicated [14,15]. The environmental spore levels have also been connected with asthma-related deaths [16,17]. In one study the odds of a death caused by asthma were significantly higher on days with mold spore counts greater than 1000 spores per cubic meter, and remained significant even on multivariate logistic regression analysis with mold spore counts measured as a continuous variable and adjusted for other confounders (tree, grass, or ragweed pollen levels) [17].

Fungal sensitization and severe asthma

Worsening asthma control with inhalation of increased fungal spores is intuitive as a foreign particle is being inhaled into the lung. However, the relationship between severe asthma and fungal sensitization is not easily understood. The first evidence of the link between non-*Aspergillus* molds-related fungal sensitization and asthma was published in 1991 when *Alternaria* sensitivity was associated with an increased risk of respiratory arrest in 11 asthmatics [18]. Subsequently studies have suggested

Table 1 Differences between fungal sensitization, severe asthma with fungal sensitization and allergic bronchopulmonary mycosis.

	Fungal sensitization	Severe asthma with fungal sensitization	Allergic bronchopulmonary mycosis
Skin testing to fungal antigen or increase in fungus specific IgE antibodies	Present	Present	Present
Severe asthma	May be seen	By definition, asthma is severe	Severe asthma is seen in majority
Brownish black mucus plugs	Absent	Absent	Seen in up to 20% of the patients
Total IgE levels	Generally within normal limits	Mildly elevated (usually less than 500 IU/ml)	Grossly elevated (usually more than 1000 IU/ml)
Fungal precipitins	Usually not seen	Usually not seen	Commonly present
Eosinophil counts	Usually within normal limits	Mildly raised (generally less than 500 cells/μl)	Usually elevated (more than 500 cells/μl)
Fleeting pulmonary opacities on chest radiograph	Absent	Absent	Seen in majority of the patients
Central bronchiectasis	Generally absent	Generally absent; if present it usually involves less than three lobes	Seen in almost 80% of patients; usually affects three or more lobes with associated centrilobular nodules, and mucoid impaction

a connection between *Alternaria* sensitivity and bronchial hyperreactivity [19,20] and severe asthma [21].

Studies have also shown a link between sensitization to fungi other than *Alternaria* and asthma severity. A retrospective case-control study which compared asthmatic children requiring 'seasonal' admissions versus those with 'random' month admissions, found that the former group was a distinct sub-population of severe asthma. This group had patients with a family history of fatal asthma, were less likely to 'outgrow' asthma in childhood, were more likely to require maintenance steroid therapy for asthma management, and significantly more often had positive radioallergosorbent tests to *Aspergillus* and *Cladosporium* species [22]. Similarly, a higher prevalence of sensitization to one or more allergens (*Alternaria*, *Cladosporium*, *Epicoccum*, *Helminthosporium*) was associated with intensive care unit (ICU) admission for severe acute asthma in 37 patients compared to 50 asthmatics not requiring ICU admission [23]. The European Community Respiratory Health Survey which investigated 1132 adult asthmatics found that asthma severity (measured in terms of lung function, exacerbations, hospitalizations, oral steroid requirement) was associated with *Alternaria* or *Cladosporium* sensitivity [24]. In another study involving 181 asthmatics, sensitivity to at least one mold (*Aspergillus*, *Alternaria*, *Cladosporium*, *Penicillium*, or *Candida*) was seen in 71% of the 46 severe asthmatics requiring multiple hospital admissions versus 16–19% sensitization in others [25]. Studies have also reported an association between fungi other than *Alternaria* or *Cladosporium*. Niedoszytko *et al*. demonstrated that *Aureobasidium* or *Helminthosporium* sensitivity but not sensitivity to other fungi (including *Aspergillus*, *Alternaria*, *Cladosporium* and others) was connected with asthma severity and asthma hospitalizations in 105 asthmatics [26]. Another recent study involving 258 asthmatics found that *Trichophyton* sensitivity but not sensitivity to other molds was associated with moderate to severe asthma [27].

However, there are several drawbacks with the aforementioned studies on the relationship between fungal sensitization and asthma. Not only have the studies adopted different methodologies and definitions but also the sensitivity to various fungi across the studies are inconsistent. Moreover, no study has included multivariate regression analysis to exclude the effect of one fungus with the others. Finally, most studies have not looked for *Aspergillus* sensitivity because the best model for mold sensitization is *Aspergillus*-related allergic phenomenon. Only with *Aspergillus* spp. does one encounter two extreme spectra of immunologic phenomena, i.e., the *Aspergillus*-sensitive asthma and allergic bronchopulmonary aspergillosis (ABPA). Although other fungi can occasionally cause ABPA-like syndrome, the frequency is far less when compared to ABPA and is generally documented as isolated case reports [28–43].

It was also proposed that demonstration of the efficacy of antifungal therapy for asthma could directly implicate fungal exposure in the pathogenesis of asthma. Two studies have used azoles in patients with severe asthma with fungal sensitization (SAFS). In one investigation, the authors used fluconazole (100 milligrams once daily for five months) in 11*Trichophyton*-sensitive severe asthmatics and found that the use of fluconazole was associated with decreased bronchial hyperresponsiveness to inhaled *Trichophyton*, decreased steroid requirement and increase in PEF [44]. In another recent publication, Denning *et al*. used itraconazole in patients with SAFS, and found that it was linked with modest improvements in quality of life, rhinitis score, PEF and total IgE [45]. One important caveat is that azoles themselves have direct and profound immunologic effects [46–52]. Hence, it is not definite whether it was the anti-fungal action of azole or the anti-inflammatory property of the drug that led to improvement in patients with SAFS.

Aspergillus and severe asthma

Aspergillus can be linked to asthma in a number of ways. Most commonly, patients with bronchial asthma can be sensitized to *Aspergillus* spp., i.e., *Aspergillus* hypersensitivity (AH) [53]. Patients with asthma can also develop ABPA which is known to worsen asthma control [54–59]. The prevalence of AH in asthma is variably quoted in different studies [60]. In a systematic review, we demonstrated a pooled prevalence of AH in bronchial asthma (20 studies, 5092 subjects) of 28 (95% confidence intervals [CI], 24–34%) [61]. The only limitation of this study was that all the primary investigations were performed in Chest or Asthma clinics and may not be representative of the whole asthma population. The evidence of the connection between *Aspergillus*-related fungal sensitization and asthma (in fact, the first paper to suggest a link between fungal sensitization and asthma severity) was published in 1978. In this report, AH was related to the severity of airways obstruction in 193 asthmatics (93 Cleveland, 100 London) [62]. Another study involving 105 asthmatics found that AH (28.5%) was associated with increased steroid usage, higher eosinophil count and IgE levels but the patients' lung function was not different from controls [63]. In a study published from our center, the prevalence of AH was 50.9% in patients with severe acute asthma admitted to an ICU compared to 38.5% in the stable outpatient bronchial asthma group [64]. In a recently study involving more than 400 outpatient chronic asthmatics, we found that patients with

asthma and AH had frequent nocturnal symptoms and poorer lung function compared to those not sensitized to *Aspergillus* species (Unpublished data).

Asthma as a protective response to fungal infection

A recent controversial but interesting paper by Porter *et al*. suggested that *Aspergillus* per se can cause asthma [65]. In an experimental model they analyzed dust from homes of asthmatic children for the presence of active proteinases, and found that many were derived from fungi, especially *Aspergillus niger*. Proteinase-active dust extracts alone were insufficient to initiate asthma-like disease in mice. However, the conidia of *A. niger* in the presence of proteinases, readily established robust allergic inflammation and disease resembling allergic asthma [65]. This suggests that asthma does not primarily result from hypersensitivity to fungal products but occurs secondarily due to the protective response against active fungal infections of the airways by Th2 lymphocytes as a means of containing the fungal infections. Fungal proteinases are therefore crucial factors for allergic lung disease induced by fungal airway infection. In fact, it is probable that the degree of associated proteinase exposure or concomitant fungal infection may dictate distinct lung syndromes of allergic asthma and hypersensitivity pneumonitis respectively [65,66]. It may well be hypothesized that SAFS occurs due to immune response against larger amounts of fungal proteinases, and smaller quantity of fungal conidia. However, more data is required in order to gain a better understanding of this new hypothesis.

Clinical implications of fungal sensitization in asthma

Diagnosis of fungal sensitization - skin testing or specific IgE levels

As noted earlier, the diagnosis of fungal sensitization can be made either with skin testing with antigens derived from fungi or measuring specific IgE levels by the fluorescent enzyme immunoassay systems. Skin tests are believed to be more sensitive but less specific than fungus-specific serum IgE tests in determining fungal sensitization. Two studies have compared skin testing with specific IgE levels (Table 2). One investigation found that measuring specific IgE levels was better than the skin prick test (SPT), whereas the other study found that intradermal skin testing was superior to measurement of specific IgE levels [67,68]. In a meta-analysis we had observed that the prevalence of AH in bronchial asthma was higher with an intradermal test versus SPT (28.7% vs. 24.8%). Although theoretically both the intradermal and SPTs should perform in a similar manner, it has been shown that intradermal tests are generally more sensitive than SPTs [69,70]. The intradermal test is also believed to be associated with a higher complication rate than SPT. However, in our experience of more than 5000 intradermal tests, we have not encountered any complication [54,55,58,59,64,71,72]. Ideally both intradermal testing and specific IgE levels should be performed to obtain the diagnosis of fungal sensitization. However, between the two, the intradermal test is preferable to specific IgE levels.

Another important issue with diagnosis of fungal sensitization is the accuracy of the diagnosis. As there is no gold standard for the diagnosis of fungal sensitization, there is no method to assess the accuracy of diagnosis. Most centers, including ours, use crude antigen to assess fungal sensitization. These antigens lack reproducibility and consistency and frequently cross react with other antigens [73]. It may be possible that these antigens may over- or under- diagnose the prevalence of fungal sensitization in asthma. For example, we currently use a crude antigen prepared from three species of *Aspergillus* viz. *A. fumigatus*, *A. niger* and *A. flavus*. It may be possible that this antigen mix may lead to overdiagnosis of *Aspergillus* sensitization in asthmatics. However, this situation is desirable because we primarily use this antigen as a screening test for ABPA and a positive test is followed by other diagnostic procedures for ABPA [59]. On the other hand, it may also be possible that these crude antigens lead to underdiagnosis of fungal sensitization, and thus in severe asthmatics it is recommended that fungus specific serum IgE levels be routinely measured in addition to skin testing.

The discovery of recombinant specific fungal proteins is the only new prospect which seems to be on the horizon for the diagnosis of fungal sensitization. The advances in molecular techniques have enabled detection of almost 20 specific *Aspergillus* antigens with diverse biochemical nature and function. In fact, a number of allergens from *A. fumigatus* have been cloned. These antigens have also been evaluated for the diagnosis of

Table 2 Studies evaluating skin testing vs. specific IgE levels for diagnosis of fungal sensitization.

	O'Driscoll *et al*. [68] (121 severe asthmatics)		Liang *et al*. [67] (75 allergic rhinitis patients)	
	Skin prick test	Specific IgE levels	Intradermal test	Specific IgE levels
Candida	7%	10%	56.0%	9.3%
Alternaria	3%	7%	22.7%	1.3%
Aspergillus	8%	12%	16.0%	9.3%
Cladosporium	7%	9%	14.7%	1.3%
Penicillium	8%	12%	32.0%	8.0%

ABPA both in patients with asthma and cystic fibrosis. The recombinant allergens Asp f1, Asp f2, Asp f3, Asp f4, and Asp f6 have been evaluated for their diagnostic performance in serological studies in asthmatic patients [74–77] and in patients with CF [76,78–80]. Preliminary data suggest a promising role of these antigens. However, more data is required before their routine use in clinical practice.

Treatment implications of fungal sensitization in asthma

The important clinical question is whether patients with SAFS should be managed differently from other patients with severe asthma without fungal sensitization. The initial management of asthma with fungal sensitization should be on the same basis as those without fungal sensitization. The combination of long-acting β2 agonists (LABA) and inhaled corticosteroids (ICS) when administered through the novel SMART approach (use of a single inhaler of formoterol-budesonide for both maintenance and reliever therapy) achieves better control of asthma than high dose of ICS or ICS-LABA combination in poorly controlled asthmatics [81,82]. If the asthma still remains uncontrolled, then other therapies such as theophylline and montelukast should be added. Omalizumab and itraconazole should be considered when the response is partial or lacking. Under real-life conditions, omalizumab has been shown to be effective add-on therapy in the treatment of patients with severe persistent allergic asthma [83].

Itraconazole has also been investigated for its use in SAFS in the recently published Fungal Asthma Sensitization Trial (FAST) study. This was a randomized, placebo-controlled, investigation assessing the effects of 32-week therapy with itraconazole, 200 mg twice daily, in subjects with SAFS. SAFS was defined on the following criteria; (a) patients taking high-dose ICS (1000 μg/d or more of beclomethasone equivalent dose), or (b) continuous oral steroids (≥5 mg/day of prednisolone or its equivalent for at least 6 mo), or (c) at least four or six courses of systemic steroids over the last 1 or 2 years and in whom fungal sensitization to various fungal strains such as *Aspergillus*, *Cladosporium*, *Penicillium* or *Candida* was documented with skin-prick or specific IgE levels. Patients with IgE levels more than 1000 IU/ml, *Aspergillus* IgG precipitins, left ventricular dysfunction, current bacterial lung infections, itraconazole therapy during the last eight months, pregnancy, evidence of liver function abnormalities (> three times the upper limit of normal), those with documented allergy to azoles, patients taking mandatory therapies potentially interacting with azoles and other immunosuppressive drugs were excluded. The primary end point was change in the asthma quality of life questionnaire (AQLQ) score, with rhinitis score, total IgE, and respiratory function as secondary end points. At 32 weeks, there were modest improvements in all the primary and secondary end-points. However, the 95% confidence intervals for AQLQ and rhinitis scores overlap at 32 weeks, which does not allow a definitive conclusion regarding the role of azole in SAFS. Not only the mechanism regarding the efficacy of azole in SAFS is unclear but also the long-term control of asthma with 32 weeks of itraconazole remains unknown. Although the authors have used itraconazole for 32 weeks, the optimal dose and duration of azole therapy in SAFS remains unknown. Itraconazole also has numerous adverse effects and in a study of 189 patients treated with this antifungal (average, 400 milligrams per day), adverse effects occurred in almost 39% of patients [84]. There are also several drug interactions with the use of itraconazole, the most important of which is that it may inhibit the hepatic metabolism of terfenadine, astemizole, and cisapride, prolonging the electrocardiographic QT interval and thus increasing the risk for cardiac arrhythmia. Itraconazole inhibits the metabolism of methylprednisolone (but not prednisolone) and can lead to increased frequency of side-effects of steroids including profound adrenal insufficiency [85]. Adrenal suppression has also been reported with the concomitant use of itraconazole and inhaled budesonide [86,87]. Thus, itraconazole should be judiciously used in patients with SAFS.

Conclusions

There is definite evidence of fungal sensitization in asthma. There is also a strong association between fungal sensitization and severity of asthma. Whether this relationship is causal or just casual remains to be investigated. A variety of fungi are known to cause sensitization in asthmatics. However, the most important fungal agent causing sensitization and leading to severe asthma is not clear. *Aspergillus* species seem to be the strongest candidates. Patients with SAFS should be initially managed on the same lines as those without fungal sensitization. The role of itraconazole in the management of SAFS requires further evaluation.

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