Allergic fungal airway disease: diagnosis and management

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Abstract

Purpose of the review

Fungal spores are ubiquitously present in indoor and outdoor air. A number can act as aeroallergens in IgE sensitized individuals and some thermotolerant fungi germinate in the lung where they can cause a combined allergic and infective stimulus leading to a number of clinical presentations characterized by evidence of lung damage. We discuss which biomarkers are useful in helping to guide diagnosis, prognosis and treatment of allergic fungal airways disease (AFAD) and briefly review the evidence for a role of anti-fungals in the management of this condition.

Recent findings

Diagnostic biomarkers, such as specific IgEs and fungal culture, for AFAD are limited by sensitivity, although this may be improved with novel agents such as specific IgEs to fungal components and qPCR. Total IgE and hyperoesinophilia are non-specific and do not clearly relate to disease activity. High attenuation mucus and proximal bronchiectasis are specific, albeit insensitive markers of AFAD. Biomarkers that predict prognosis and treatment response are yet to be defined.

Summary

This review summarises the fungi involved and the current debate regarding the diagnostic criteria to define fungal associated airway disease. We advocate the phasing out of the term allergic bronchopulmonary aspergillosis (ABPA) and the use of a more inclusive term such as allergic fungal airway disease (AFAD), together with a more liberal set of criteria based largely on IgE sensitization to thermotolerant fungi, which identifies those patients at risk of developing lung damage.

KEYWORDS

Asthma, ABPA, ABPM, AFAD, thermotolerant fungi.

1.0 Introduction: fungi and the lung

Fungi can act as aeroallergens in those that are IgE sensitised and can cause a range of airway diseases by colonising the lung. Allergen exposure to fungal spores, such as *Alternaria alternata* during the late summer and autumn months [1], has been associated with acute bronchospasm, asthma admissions and deaths [2, 3]. These fungi do not grow at body temperature so cannot germinate in the airway. Adverse events are therefore related to the level of spore exposure. However, thermotolerant fungi such as *Aspergillus fumigatus* can grow at body temperature, enabling them to colonise the lung. Depending on the host airway response, fungal colonisation can exacerbate asthma, or cause chronic sinusitis, fungal bronchitis, chronic necrotizing pneumonia, fungal empyema, allergic alveolitis, or in people with pre-existing lung cavities, the development of a fungal ball (aspergilloma) (Figure 1) [4-9]. Lastly severely immunocompromised individuals can develop systemic infection. This review will focus on the clinical aspects of the involvement of thermotolerant fungi in allergic airway disease.

2.0 What is allergic fungal airway disease (AFAD)?

Thermotolerant fungi are implicated in several different presentations of airways disease and there is no good term that encompasses them all (Figure 2). Allergic bronchopulmonary aspergillosis (ABPA), the most common form of allergic bronchopulmonary mycosis (ABPM), originally described patients with asthma or cystic fibrosis (CF) who were IgE sensitised to *A. fumigatus* and presented with fleeting lung shadows, eosinophilic airway inflammation, and progressive lung damage with lung fibrosis, proximal bronchiectasis and fixed airflow obstruction [10]. *A. fumigatus* was frequently cultured from the sputum, total IgE was high and pathologically bronchocentric granulomatosis was found. A set of criteria based on clinical experience were developed to

try and define this syndrome. These were fleeting lung shadows, eosinophilia, evidence of IgE sensitisation to *A. fumigatus*, total IgE of >417 international units/ml (although 1000IU/ml gives greater specificity), precipitating antibodies to *A. fumigatus* and proximal bronchiectasis. Evidence of colonisation was not part of the major criteria even though it is thought to be the underlying cause of the condition [11]. It is relatively unusual for someone with asthma who is IgE sensitised to *A. fumigatus* to fulfil all these criteria. Some people don't have good evidence of variable airflow obstruction and fleeting shadows are now unusual, due to the widespread use of high dose inhaled steroids reducing large airway inflammation. Total IgE may be below 417 IU/ml and precipitating antibodies to *A. fumigatus* are frequently not raised. Bronchiectasis, where present, is often not proximal and is a common feature of severe asthma without evidence of fungal sensitisation. Therefore only about 10% of people with asthma associated with IgE sensitisation to *Aspergillus* fulfil all the standard criteria for ABPA. ABPA is, thus, considered unusual although sensitisation to fungi, particularly in severe asthma, is common [12, 13]. The diagnostic criteria for ABPA, at present, is unsatisfactory. Recently the International Society of Human and Animal Mycology (ISHAM) proposed a revision to this criteria [14**]. This criteria included 1) the presence of asthma or CF, 2) evidence of specific IgE to *A. fumigatus* and total IgE >1000 IU/ml and 3) at least two of: raised IgG antibodies to *A fumigatus*, abnormal radiology consistent with ABPA and an eosinophil count in steroid naïve patients of $>0.5x10^9$/l. In an accompanying diagnostic algorithm, total IgE was central in distinguishing between ABPA and IgE sensitisation without ABPA. Although less restrictive than the previous criteria there is still no gold standard for the diagnosis of ABPA against which to test the specificity and sensitivity of biomarkers. Ideally the immunological and radiological biomarkers should be tested against a fungal-specific outcome measure, but there are no features of fungal disease which are sufficiently specific

compared to asthma without fungal sensitisation. There is limited evidence that the strength of the Th2 response, denoted by high total IgE and marked eosinophilia, correlates with disease severity in fungal asthma nor that these markers are able to guide management and improve outcomes. A similar problem occurs with the label of severe asthma with fungal sensitisation (SAFS) which is largely separated from ABPA by using a total IgE of <1000 IU/ml as a cut off [15]. A more liberal diagnostic term to describe allergic fungal airway disease that would favour sensitivity over specificity would be more clinically useful than restrictive terms such as these. One solution is to describe anyone with airways disease and IgE sensitisation to colonising fungi as having ABPA, grading them according to the degree of fungal related lung damage and symptom control. However it may not be possible to re-assign such a historically well-established label which relates to the florid end of the spectrum of fungal airway disease. Alternatively a term such as allergic fungal airway disease (AFAD) could be coined, which could be divided into mild, moderate, and severe and the term ABPA phased out or used to describe severe patients. Overall the emphasis should be on determining which biomarkers of AFAD predict prognosis (particularly the risk of lung damage) and treatment response.

3.0 Presentation of allergic fungal airway disease.

Variable airflow obstruction caused by airway smooth muscle (ASM) contraction is pathognomic of asthma [16]. However, this is just one of the five pathophysiological endotypes that occur in asthma as well as other airways diseases, each with distinct pathologies, clinical presentation and response to treatment [17]. Heterogeneity exists within these presentations and these endotypes can coexist within the same patient or occur independently [18-20]. This is also true of fungal associated disease which can be a complication of a number of asthma endotypes and could be regarded as an endotype in its

own right [21], Figure 2. Fungal IgE sensitisation is most commonly seen in 'classical' early onset atopic eosinophilic asthma. It is likely that sensitisation occurs in childhood whereas in adults it is associated with more severe disease [22]. Many patients with adult onset asthma are non-atopic, but a proportion are atopic; a minority of whom are sensitised to thermotolerant fungi. Fixed airflow obstruction can be seen in patients presenting in their 6th decade. This may be due to subclinical fungal colonisation causing progressive loss of lung function. Clinical presentations of AFAD vary, including those presenting with lobar collapse due to mucus impaction, withperipheral blood eosinophilia pointing towards an allergic cause; and fungal pneumonitis caused by a exposure to high levels of fungal spores - often in the context of gardening, leading to acute respiratory illness with pneumonic shadowing [23]. Heavy colonisation with filamentous fungi or yeasts can lead to a fungal bronchitis in patients with underlying airway disease, who present with a cough productive of discoloured sputum, worsening symptoms and lung function. The sputum shows a heavy growth of fungi and the patient has a good and relatively quick response to a course of triazole antifungals. This presentation can occur in the absence of fungal IgE sensitisation [24]. Lastly occult fungal IgE sensitisation is a common cause of a marked eosinophilia and can present in patients with unexplained eosinophilia.

4.0 Which fungi are involved?

The fungal kingdom could contain as many as 1.5 to 3 million species [25, 26]. There are 8-10 phyla within the kingdom [27] and fungal pathogens have evolved independently and repeatedly throughout [28]. Any fungi can be allergenic; however, the most common fungal allergens are those present in high levels either outdoors or in an occupational or residential setting. Most are mesophilic (unable to grow at body temperature, with optimum growth occurring at 18-22 °C) and thrive in temperate climates. Thermophilic

fungi can grow at body temperature, but are unable to grow below 20 °C so are not present in the environment and are rarely associated with human infections. Thermotolerant fungi grow in the environment and at body temperature and are thus associated with human disease, including AFAD.

Studies looking at fungal colonisation of the airways have mainly focused on CF patients [29, 30] with few studies on ABPA or asthma [31]. Many have not looked for fungi other than *A. fumigatus*; an exception being our study looking at 126 subjects with moderate to severe asthma [32]. A good review of non-*Aspergillus* fungi associated with ABPM has recently been published [33]. More than 600 fungal species have been recovered from human infections, with a core 200 seen regularly. In contrast the number associated with the respiratory system is far lower. The two fungi regularly seen are both Ascomycota (*A. fumigatus* and *Candida albicans*), however, fungi from the Ascomycota, Basidiomycota and the group formerly referred to as the Zygomycota have all been implicated (Table 1). Species from 22 fungal genera representing 14 families have been detected so far; and whilst not all of them have been shown to induce allergies, due in part to a lack of reagents with which to test, it is highly probable that most of them would be able to cause AFAD.

Fungal culture is still the main way in which fungal agents are identified in the clinic; however, culture from respiratory samples, particularly sputum, could indicate colonisation of the airways or an upper airway contaminant [34]. Culture is notorious for being insensitive and biased towards the faster growing members of the <10% of the fungal Kingdom able to grow on general growth media. The use of selective media increases the number of fungi that can be detected [35]; however, it is the use of molecular culture-free tests that is likely to result in the greatest leap forward in our understanding of the fungi associated with AFAD [36].

5.0 How important is exposure in causing allergic fungal airway disease?

Epidemiological studies have associated dampness in the home with poor respiratory health and new onset asthma [37]. However whether the microbial content in the home is responsible for this is less clear-cut. Some studies have demonstrated a relationship with fungal exposure, others have not [38]. A case control study found no relationship between increased fungal exposure (as measured by fungal culture or concentrations of ergosterol and (1-3,1-6) beta-D-glucan in dust) and asthma in children, although levels of fungal exposure were relatively low [39]. Furthermore, high levels of yeast exposure in infancy have been shown to be protective for later development of asthma [40].

Increased airborne levels of *A. fumigatus* have been associated with a positive sputum culture, but not IgE sensitisation to *A. fumigatus* in asthma, despite fungal concentrations being within the normal range for non-complaint housing [41]. The reasons for the lack of a consistent message may be due to the complexity of the relationship between fungal exposure and health outcomes. Intervention studies are required to determine if reducing exposure to thermotolerant fungi benefits respiratory health [42].

6.0 Biomarkers for the diagnosis of allergic fungal airway disease (AFAD).

Biomarkers, whether used for diagnosis or prognosis, should be related directly to a disease process or pattern of response to treatment and be useful in predicting, preventing or reversing disease outcomes. In the context of current asthma management, where potent inhaled steroids have reduced the risk of severe exacerbations, the most common abnormalities associated with AFAD are fixed airflow obstruction and bronchiectasis. These develop over many years making direct links to biomarkers difficult. In addition there are few aspects of response to anti-fungal agents which can be used to determine the value of biomarkers in guiding treatment response.

6.1 Specific IgE

The most useful biomarker for the diagnosis of AFAD is the presence of specific IgE to a thermotolerant fungus, particularly *A. fumigatus*. A skin prick test (SPT) or *in vitro* test (such a ImmunoCap) are used to measure sensitisation to fungi, however, discordance exists between the two tests [43]. SPTs are more insensitive with only 60% of subjects with a positive *in vitro* test having a positive SPT, although intradermal testing may increase sensitivity [13]. Cross-reactivity with clinically benign determinants may account for some of the discordance [44**]. In addition, fungal extracts are not standardized and are of variable quality.

Considerable cross-reactivity exists between fungal allergens even between distantly related genera [45]. *Pencillium* species are commonly cultured from sputum. However, positive specific IgE to *P. chrysogenum* without positive IgE to *A. fumigatus* only occurs in around 5% of subjects. About 15% of patients with fungal sensitisation to thermotolerant yeasts (*Candida albicans*, *Malazzesia species* and *Trichophyton spp*) are not sensitised to *A. fumigatus* (personal observation). However the relevance of these fungi to allergic airway disease is uncertain although yeasts, in particular *C. albicans*, are almost invariably cultured from sputum in asthma. A positive specific IgE to *A. fumigatus* is associated with fixed airflow obstruction and bronchiectasis when compared with non-sensitised asthmatics of the same severity and is considered to be clinically relevant in isolation as well as a criterion for ABPA [13, 46, 47]. The use of recombinant proteins of fungal extracts might offer a better correlation with markers of disease activity [48, 49], although at present only Asp F1-6 are commercially available. Differences in the geographical prevalence of fungal sensitisation, the extent to which it is sought and differences in asthma phenotypes may explain the low rates of fungal sensitisation found in Europe (~10% in the ENFUMOSA cohort), the lack of identification of fungal sensitisation in the

USA severe asthma research programme (SARP) [50-52] and the variable rates of *A. fumigatus* sensitisation found between centres found in the UK-based British Thoracic Society severe asthma cohort [53]. For a comprehensive fungal assessment a panel for skin prick testing (where available) and specific IgE consisting of *A. fumigatus, P. chrysogenum, C. albicans, Malassezia* species, *Trichophyton* species, *A. alternata* and *Cladosporium herbarum* should be undertaken.

6.2 Total IgE

Total IgE has been used extensively as a biomarker for AFAD, in particular to define ABPA. A raised total IgE is almost invariably observed in AFAD and is reduced to a modest degree by oral steroids and possibly antifungal agents. However, there is little evidence that precise levels and repeated measurements relate sufficiently closely to be of value in assessing disease severity or activity [54]. A high total IgE is a feature of atopic dermatitis due to sensitiation to *Malassezia spp* and other skin fungi [55] which can confound the interpretation of total IgE in asthma.

6.3 Specific IgG

Current practice measures total *A. fumigatus* IgG rather than precipitating antibodies. This is relatively non-specific, and may represent increased environmental exposure or colonisation. There is no evidence it provides additional information to specific IgE in the diagnosis of AFAD.

6.4 Blood and sputum eosinophil count

The peripheral blood eosinophil count is usually raised in fungal allergy, whichis one of the commoner causes of hypereosinophilia. It is, however, non-specific with blood eosinophilia not correlating wellwith disease activity [56]. A mixed Th2/Th1-17 immune response is suggested by lower values of sputum eosinophils in comparison to higher values of neutrophils found in people with refractory asthma who were sensitised to *A. fumigatus*;

despitecomparable blood eosinophilia [46]. Thus, sputum eosinophil values do not seem of significance in AFAD.

6.5 Detection of fungi in airway secretions.

Fungal colonization (as detected by growth from airway secretion) is thought to be the underlying event that leads to AFAD.

6.5.1 Culture and qPCR

There are no guidelines for processing respiratory samples for fungal detection. Methods using higher concentrations of sputa have yielded higher rates of fungal culture than methods used by the UK National Health Service clinical laboratories [57, 58], with lower rates from healthy subjects suggesting good specificity [14, 28, 32]. This technique has demonstrated a link between impaired lung function and higher culture rates for fungi in IgE-sensitised asthmatics, and shown increased fungal diversity, detecting 27 species of thermotolerant fungi [32]. During a 12 month clinical trial of voriconazole in asthma, 80% of *A. fumigatus* IgE sensitised asthmatics had at least one positive fungal culture [59*]. [59*]. However fungal culture is not very quantitative. Quantitative PCR (qPCR) is an alternative means to detect *Aspergillus spp* which has been used mainly for the diagnosis of invasive aspergillosis. It is very sensitive and potentially more quantitative compared with culture [57, 60, 61], however, assays are not available for all fungi that may be clinically relevant. The extent to which a positive qPCR relates to a clinically relevant outcome of AFAD has not yet been established.

6.5.2 Cell wall components

Galactomannan is a carbohydrate component of the cell wall of *Aspergillus spp* and other fungi and is used as a blood test to aid in the diagnosis of invasive fungal infection in neutropaenic individuals [62]. Galactomannan assays of BAL samples and antibody-based *Aspergillus* assays both provide reasonable sensitivity and specificity for the diagnosis of invasive pulmonary aspergillosis; whereas culture has been found to be insensitive and a

1,3, beta-d-glucan assay non-specific [63, 64*]. There is little information on the value of these assays in AFAD.

6.5.3 Cytology and immunohistology

Fungal spores seen in sputum cytospins often represent contaminants. Fungal elements are rarely seen in bronchial biopsies; even in florid situations of fungal allergic responses, hyphae can be difficult to detect. Other approaches such as measurement of fungal toxins, enzymes or volatile organic compounds in exhaled air have potential as sensitive, specific and quantitative biomarkers for the presence of fungal growth in the lung, but for the moment they remain in the realm of research [65].

7.0 Radiological abnormalities in AFAD

A normal high resolution CT (HRCT) scan is unusual in AFAD and a wide range of radiological abnormalities exist (reviewed in [14**]). No features are absolutely specific, however, high attenuation mucus has been suggested to be characteristic [66], central or proximal bronchiectasis was an original defining characteristic of ABPA and is a specific, albeit insensitive marker of AFAD. Upper lobe fibrosis is a feature which is often apparent on the CXR and is commonly associated with severe fixed airflow obstruction. HRCT scanning has revealed that minor degrees of bronchiectasis are common in severe asthma and COPD. In two series there was about a two fold increase in the rate of bronchiectasis in patients with AFAD compared to asthmatics of matched severity without fungal sensitisation [46, 47]. The extent to which the presence and pattern of bronchiectasis relates to the immunological criteria for AFAD is not clear. Tree in bud shadowing and nodularity are under-appreciated, but are a relatively common feature of AFAD.

8.0 The role of anti-fungals in AFAD

There have been a number of reviews on the management of ABPA, although the evidence base for most interventions is weak and recommendations are coloured by individual experience and prejudice [67*-69]. As colonisation of the airway is thought to be the underlying trigger for AFAD including ABPA it would be expected that an anti-fungal agent would be effective therapy and triazoles, in particular, are widely used. However the evidence base for the use of these agents is limited with four small randomized controlled trials, two conducted in an ABPA population, two in asthmatics with fungal sensitisation and none in CF. The three trials that used itraconazole reported a positive outcome, although these improvements in clinical end points were minor and divergent between the studies, whilst our study which used voriconazole demonstrated no benefit in any of the measured outcomes [59*, 70-72]. Itraconazole markedly increases steroid bioavailability and the improvement seen with this drug may well be due to a steroid enhancing effect rather than its anti-fungal properties [73]. Voriconazole did not eliminate the growth of *A. fumigatus* from the sputum and rates of sputum positivity returned to pre-treatment levels within a few months of stopping the drug. It is possible therefore that the lack of efficacy was due in part to a failure to clear the fungus from the lung. Although our study found no overall benefit, anecdotally there were some people, particularly those with a heavy growth of *A. fumigatus*, who cleared their sputum and had improved control which was maintained for a prolonged period. It may be that people with heavy colonisation of their airways are suffering from a primarily infective process and have an immediate response to antifungals whereas in those with low levels of colonisation the problem is primarily allergic which requires only minor amounts of non-viable fungal elements to cause problems. These patients are more likely to respond to anti-inflammatory drugs or immunotherapy than attempts at fungal eradication. In this regard omalizumab would be expected to be beneficial although its use is often limited by the high levels of IgE seen in

AFAD [74]. The modest if any benefit demonstrated with triazoles in clinical trials suggests that these drugs should be used cautiously in patients with AFAD, although we need better antifungal agents before a definitive view can be taken on the role of fungal colonisation in short-term disease control. These studies do not however inform about the role of fungal colonisation in causing lung damage which would require much longer studies.

9.0 Conclusion

Fungi can cause lung disease either by acting as simple aeroallergens in IgE-sensitised individuals or by colonising the lung, a property largely restricted to yeasts and *Aspergillus* and *Penicillium spp,* particularly *A. fumigatus*. Colonisation with filamentous fungi is associated with a number of distinct patterns of airway disease and the focus on trying to define ABPA, which represents the florid end of what is a spectrum of lung disease, has in our opinion outlived its usefulness. We recommend a more liberal definition of allergic fungal airway disease (AFAD), based solely on the presence of IgE sensitisation to fungi and evidence of fungal-related lung damage. Management of AFAD is similar to that of asthma without fungal sensitisation and depends on the individual pattern of presentation. The emphasis should be on detecting and preventing long-term lung damage which is the most characteristic feature of AFAD. Antifungals may have a place in selected individuals, especially those with heavy colonisation (fungal bronchitis). A priority for research is to standardise and improve the methods for detection of non-invasive fungal growth in the lung and to determine the natural history of AFAD to ascertain whether fungal colonisation and sensitisation are truly causal or a by-product of lung damage.

Summary Points

1. Fungi cause lung disease by acting as aeroallergens or by colonising the lung.

2. A more liberal term is needed to encompass the spectrum of lung disease caused by fungi.
3. Antifungals are of limited effectiveness, but may be effective in those with a fungal bronchitis.
4. The emphasis should be on detecting and preventing long-term lung damage.

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