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What is the importance of classifying Aspergillus disease in cystic fibrosis patients?


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Aspergillus species are commonly isolated from lower respiratory tract samples of patients with cystic fibrosis (CF) and markers of immunological sensation to *Aspergillus* are frequently encountered in this group of patients; however, the contribution of *Aspergillus* to CF lung disease outside of the typical complications of ABPA and aspergilloma formation remains largely unclear. Patients with CF show discretely different responses to *Aspergillus*, though the underlying reasons for this variation are unknown. Recent work has begun to allow us to categorize patient responses to *Aspergillus* based upon molecular markers of infection and immune sensitization. *Aspergillus* sensitization and/or airway infection is associated with worse FEV1, in CF and other patients (asthma, chronic obstructive pulmonary disease, bronchiectasis). Classification of different clinical phenotypes of *Aspergillus* will enable future studies to determine the natural history of different manifestations of *Aspergillus* disease and evaluate the effects of intervention with antifungal therapy.

Cystic fibrosis (CF) is an inherited multisystem disorder associated with a reduced life expectancy. Pulmonary disease is almost universal and progressive lung damage leading to respiratory failure causes over 90% of premature deaths. *Aspergillus* species are ubiquitous, filamentous, spore-forming fungi and potential opportunistic pathogens. The relationship between *Aspergillus* and CF lung disease is complex and the differentiation of potential *Aspergillus*-related disease from other complications of CF is a major challenge. It is recognized that patients with CF are susceptible to the development of allergic bronchopulmonary aspergillosis (ABPA), while, much less commonly, other classical *Aspergillus*-related complications can be encountered, including the formation of aspergillomas and invasive aspergillosis in immunocompromised CF patients post lung transplantation. *Aspergillus* species are however frequently isolated from the lower respiratory tract of patients with CF who do not have evidence of invasive infection or aspergilloma formation, and many other patients display markers of immunological sensitization without fulfilling the consensus criteria for ABPA [1].

The clinical significance of the culture of specific bacterial pathogens, such as *Staphylococcus aureus*, *Haemophilus influenzae*, *Pseudomonas aeruginosa* and *Burkholderia cenocepacia*, from CF sputum samples is well established. *Aspergillus* is also frequently isolated from the lower respiratory tract secretions of patients with CF, but in contrast to known bacterial pathogens, the relevance of this finding is often unclear. Until recently, as no firm correlations had previously been found between the culture of *Aspergillus* in CF sputa and the development of ABPA, most CF physicians left its presence untreated. This assumption was challenged when Shoseyov and colleagues reported a beneficial clinical response to antifungal treatment in six CF patients who were failing to respond to conventional antibiotic therapy but who had persistent growth of *A. fumigatus* in their sputum samples [2]. These

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patients had either no or minimally elevated IgE serological markers of sensitization to *Aspergillus*, and hence a separate clinical entity of ‘*Aspergillus* bronchitis’ was proposed. Recent studies have identified associations between isolation of *Aspergillus* in sputum samples and risk of pulmonary exacerbations [3], hospitalizations [4,5] and radiological abnormalities [6], but others have not confirmed these associations [7]; an association between sensitization to *Aspergillus* and increased requirement for intravenous antibiotics and greater lung function decline have also been reported [8]. Alongside these observations in CF are emerging data in asthma, bronchiectasis and chronic obstructive pulmonary disease, indicative of a similar set of differing responses to *Aspergillus* [8-10]. These findings have highlighted the need to establish a new classification for *Aspergillus*-related conditions in CF, to enable clinicians to phenotype patients to determine the risk factors for and natural history of *Aspergillus*-related complications, identify those patients who may benefit from antifungal therapy and evaluate treatment responses.

**Methods of detection of Aspergillus**

The current methods of assessing the presence of *Aspergillus* and associated host response may underestimate the contribution of these fungi to lung disease in CF. There is a clear need to improve and standardize methods both to detect the presence of *Aspergillus* in the lower airways and to identify markers of immunological sensitization. The prevalence of *Aspergillus* in CF sputum samples varies widely between published studies and sampling techniques, and culture conditions are important in determining the yield [11,12]. *Aspergillus* colonies can be overgrown *in vitro* by colonizing bacteria leading to false negatives, conversely false positives can arise from airborne contamination of culture plates. Similarly, the results of commercially available tests for *Aspergillus* IgG antibodies can vary when applied to the same patient [13]. Molecular methods such as real-time PCR-based tests are much more sensitive than culture for the detection of *Aspergillus* in CF sputum samples and can allow a direct assessment of antifungal resistance mutations; however, *Aspergillus* can be embedded within mucoid biofilms in CF sputum, and optimizing detection rates requires homogenization of sputum to extract *Aspergillus* DNA. A recent study utilizing a combination of dithiothreitol and sonication to homogenize sputum demonstrated significantly increased culture and PCR yields in comparison to standard processing of CF sputum samples [14]. PCR tests for *Aspergillus* are not yet routinely used in clinical practice and their exquisite sensitivity may detect all *Aspergillus* DNA, not just growing organisms. The addition of other tests, such as antigen detection tests for galactomannan, which is released during hyphal growth, can help confirm active organisms, although may be falsely positive because of antibiotic therapy. Other potential biomarkers such as basophil CD203c and thymus activation and regulated cytokine may also have roles in evaluating *Aspergillus* disease in CF [15].

**Current therapeutic options**

*Aspergillus* infection is treated with antifungal agents, while the treatment of acute exacerbations of ABPA usually relies on corticosteroid therapy (although the use of anti-IgE omalizumab has been reported in a small case series), often with the addition of antifungal therapy to prevent exacerbations. Treatment options are limited by the relatively small number of antifungal agents that are currently available and lack of prospective intervention studies. A recent Cochrane review concluded that there are no randomized controlled trials to evaluate the use of antifungal therapies for the treatment of ABPA in people with CF [16]. Triazole compounds such as itraconazole, voriconazole and posaconazole form the major class of therapeutic agents against *Aspergillus*. However, antifungal therapy with triazoles is expensive and is hampered by variable bioavailability (with itraconazole), drug resistance and the frequent occurrence of side effects in patients with CF. Previous attempts to evaluate Aspergillus treatment have been hampered by small numbers of subjects and mainly use of retrospective data in published studies. A small (n = 34), prospective, randomized, placebo-controlled study of itraconazole therapy for 24 weeks in sputum *Aspergillus*-positive non-ABPA patients with CF found no difference between the active therapy and placebo groups in the primary outcome of rate of exacerbations requiring intravenous therapy [17]. However, the study had many limitations, most notably underrecruitment and failure to achieve therapeutic drug levels in 43% of patients. Treatment with itraconazole in non-ABPA CF patients has also been shown to result in reduced levels of the fungal metabolite gliotoxin, enhanced vitamin D receptor expression and decreased IL-5 and IL-13, in conjunction with improvements in both patient symptoms and mosaic perfusion patterns seen on CT scans [18]. Interestingly, one study found a fall in *Aspergillus* levels in the sputum of CF patients with intravenous antibiotic therapy [19]. Classifying different clinical phenotypes of *Aspergillus* disease will provide the necessary platform to enable larger prospective studies to investigate the potential clinical benefits of triazole therapy balanced against the potential side effect profiles of these agents.

**Immunological & genetic predisposition**

The division of patients into classes of infection and immunological response will allow future studies of genetic predisposition and proinflammatory cytokine responses in relation to different phenotypes of *Aspergillus* disease in CF. This work began a number of years ago in non-CF patients with ABPA, where links have been made between HLA-DR restrictions, single nucleotide polymorphisms in IL-4 receptors, sensitivity to IL-4 as observed by upregulation of CD23 receptors on B cells, IL-10 polymorphisms, surfactant A2 polymorphisms and toll-like receptor polymorphisms [20]. *In vitro* work with human and animal cell models has provided interesting observations on inflammatory response to *Aspergillus* in CFTR-deficient cells. The fungal metabolite gliotoxin has been shown to downregulate vitamin D receptor expression in CF macrophages and airway epithelial cells and increase the levels of IL-5 and IL-13 [18]. CFTR-deficient lymphocytes display an aberrant Th2 cell immune response, leading to an enhanced IgE response to *Aspergillus* [21].
A new classification for Aspergillus disease in CF

Recently, a new classification for aspergillosis in CF has been proposed that integrates two new methods of Aspergillus detection, sputum galactomannan and real-time Aspergillus PCR, alongside established serological markers. 146 adult patients with CF were investigated by ImmunoCap total IgE, specific A. fumigatus IgE, specific A. fumigatus IgG, sputum galactomannan antigen detection and sputum real-time Aspergillus PCR. Latent class analysis of triazole-naive patients (n = 130) identified a nondiseased group and three distinct classes of aspergillosis in CF patients: class 1 (no disease) (n = 49, 37.7%) represented CF patients with or without a positive PCR but no immunological response to A. fumigatus and a negative galactomannan; class 2 (ABPA) (n = 23, 17.7%) represented CF patients with a positive PCR, elevated total and specific A. fumigatus IgE/IgG and a positive galactomannan; class 3 (Aspergillus sensitized) (n = 19, 14.6%) represented patients with or without a positive PCR, elevated A. fumigatus IgE (not IgG) and a negative sputum galactomannan; and class 4 (Aspergillus bronchitis) (n = 39, 30%) represented patients with a positive PCR, elevated A. fumigatus IgG and a positive sputum galactomannan. This novel classification provides criteria to recognize Aspergillus bronchitis from simple colonisation and to differentiate Aspergillus sensitization from ABPA. However, 17% of the subset of patients evaluated longitudinally moved between classes over a 9-month period of follow-up. All those with Aspergillus disease or sensitization had reduced FEV1 at 2 years compared with other patients. The classification still needs to be reproduced in other studies in both adult and pediatric populations, ideally alongside other potential biomarkers (e.g., thymus activation and regulated cytokine). A new classification will facilitate improved phenotyping of patients and begin to allow researchers to determine the underlying predisposing factors and natural history of aspergillosis in CF, and evaluate the management of different disease manifestations.

Future implications

The development of a new classification of Aspergillus disease in CF allows the beginning of a new era for our understanding of the role of this commonly encountered fungus in CF lung disease. Combined with immunological and molecular research into the mechanisms of the disease, there will be for the first time an opportunity to describe the development of Aspergillus infection and host response in CF. It is likely that many factors, such as concurrent pulmonary disease, nonpulmonary diseases such as CF-related diabetes mellitus, and external factors of genetics and environment are likely to influence class grouping. The CF lung is now seen as a complex biome of microorganisms, including bacteria, viruses, fungi and yeasts, which compete for survival. Research examining these interactions and their potential impact in CF is in its infancy. Microbiomic tools driven by Next Gen sequencing will start to enable an increased understanding of the interactions between Aspergillus and host factors in the CF airway, and provide insights into factors that determine the behaviour of Aspergillus as a pathogen, sensitizer or colonizer. Longitudinal studies are now required to further evaluate the recently proposed classification of aspergillosis in CF, to assess how and why patients may move between different classes, and establish the associations between different clinical phenotypes of aspergillosis with clinical progress; in turn, this will develop the foundations to embark on future multicenter interventional studies with antifungal therapy in CF.

Financial & competing interests disclosure

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