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Fungi in Cystic Fibrosis: Recent Findings and Unresolved Questions

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Abstract Patients with cystic fibrosis (CF) suffer from chronic airway infection and inflammation. Traditionally, bacteria have been regarded the main CF pathogens while fungi have emerged and more recently warranted greater attention. Fungi are increasingly found to colonize CF airways; however, their precise clinical impact continues to spark controversy. While the clinical relevance of allergic bronchopulmonary aspergillosis (ABPA) in CF has been established, the roles of non-ABPA *Aspergillus fumigatus*, *Candida albicans*, and other more rare emerging fungi remain poorly understood. Here, we summarize and discuss recent findings in this field and refer toward unresolved questions.

Keywords Cystic fibrosis · Fungi · Pathogens

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Fungi in Cystic Fibrosis Lung Disease

Patients with cystic fibrosis (CF) suffer from chronic progressive and infective lung disease, which determines morbidity and mortality [1]. Upon disease progression, CF airways get colonized with characteristic bacteria and fungi, mainly *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus fumigatus*, and *Candida albicans*. While the pathogenic role of bacteria, particularly *P. aeruginosa*, in CF lung disease has been established, the contribution of emerging pathogens [2], such as fungi [3–5], to the clinical course of patients with CF lung disease remains incompletely understood. Among fungi in CF, most evidence exists for *A. fumigatus*. Chronic colonization with *A. fumigatus* can lead to sensitization and, in 1–15 % of CF patients (depending on the region, the patients' age, and the diagnostic criteria), to a severe clinical phenotype termed allergic bronchopulmonary aspergillosis (ABPA). ABPA is a hypersensitivity reaction to *A. fumigatus* characterized by a strong pro-allergic T helper cell type 2 (Th2) immune response (increased total serum IgE levels–typically >1000 IU/ml, skin prick tests positive, *A. fumigatus*-specific IgE and IgG, precipitins to *A. fumigatus*, sometimes eosinophilia) with pulmonary infiltrates (typical: central bronchiectasis) and antibiotic-refractory clinical deterioration, usually treated with antifungals (mostly azols) and corticosteroids [6]. Besides ABPA, however, the significance of non-ABPA *A. fumigatus* colonization and the potential clinical impact of other fungi remain unclear [7]. Fungi are predominantly found in adult CF patients and are more prevalent in patients with preexisting severe pulmonary disease and a long-term course of inhaled antibiotics [3]. Several studies support the concept that fungi and *P. aeruginosa* frequently co-exist in CF airways, supported by a recent study showing that antibacterial treatments targeted at *P. aeruginosa* reduce the *A. fumigatus* load in CF airways [8••]. However, the interaction of fungi and bacteria is

rather complex and involves synergistic as well as antagonistic growth conditions, including biofilm formation and release of toxic metabolites [9, 10]. Despite studies, the overall clinical relevance of *C. albicans* and other more rare but emerging fungi, such as *Scedosporium spp*., remains even more incompletely examined. Faced with the increasing prevalence of fungi in patients with CF lung disease, the challenges toward the future remain to (i) define those fungi causing harm to the host and differentiate them from innocent bystanders, (ii) develop sensitive and specific biomarkers for diagnosis and treatment monitoring, and (iii) evaluate specific antifungal and anti-inflammatory therapeutic regimens and the contexts in which these are applicable. In the chapters below, we summarize and discuss recent findings on fungi in CF, with a particular focus on studies contributing to the pathophysiological understanding and clinical treatment in CF lung disease. For more comprehensive and in-depth discussion of the field, we refer to previously published reviews [3–5, 7, 11, 12].

Recent Findings on Fungi in Cystic Fibrosis Lung Disease

Aspergillus

The airways of CF patients are frequently colonized with *Aspergillus* species, but the disease relevance of this fungal colonization pattern remains poorly understood. This is largely due to the lack of an unified and harmonized immunological classification of *Aspergillus*-associated disease phenotypes in CF lung disease. Such a classification is hampered by the fact that CF patients, beyond the severe but rare ABPA, show a wide range of immunological responses to *Aspergillus*, which require a more precise classification to define them and to assess their clinical relevance. A recent study integrated two novel methods of *Aspergillus* detection in airway fluids (sputum), namely galactomannan (GM) and reverse transcription polymerase chain reaction (RT-PCR) [13••]. Both new methods were more sensitive in detecting *Aspergillus* species in CF sputum samples compared to conventional culture-based methodology. These two new *Aspergillus* markers in combination with *Aspergillus* serology allowed the stratification / classification of CF patients into three distinct *Aspergillus* disease entities: (i) *Serologic ABPA*: patients with positive RT-PCR, positive GM, and elevated total and specific *A. fumigatus* IgE/IgG; (ii) *Aspergillus bronchitis*: patients with positive RT-PCR, positive GM, and elevated *A. fumigatus* IgG (not IgE); and (iii) *Aspergillus sensitized*: patients with or without positive RT-PCR, negative GM, and elevated *A. fumigatus* IgE (not IgG). This novel classification may help to identify and clinically follow-up different *Aspergillus* disease phenotypes in order to develop specific screening and treatment approaches. However, sub-phenotypes probably exist within these broader classifications groups, which will require future studies to define.

Upon CF lung disease progression, both *A. fumigatus* and *P. aeruginosa* are increasingly found in CF airway fluids. However, the interaction between these two microbes in patients with CF lung disease is poorly understood, particularly the potential therapeutic implications if both pathogens are chronically detected in CF sputa. A recent study assessed the effect of short-term antipseudomonal intravenous antibiotics on the presence of *Aspergillus* in CF airways in 26 adult CF patients [8••]. This study showed that intravenous antibiotics significantly decreased the presence of *Aspergillus* in CF airways and improved lung function parameters, suggesting that the presence of *P. aeruginosa* might help *Aspergillus* to survive within the CF airway microenvironment, maybe through mixed biofilm formation, and provides a rationale for intravenous antibiotic therapy in patients with advanced CF lung disease and co-colonization with *P. aeruginosa* and *Aspergillus*.

The more CF patients that undergo lung transplantation, the more the question arises, which bacteria or fungi, found in CF airways prior to transplantation, increase the risk for invasive infections afterwards. This is of particular relevance for *Aspergillus*, as invasive aspergillosis is a critical cause of mortality in patients undergoing lung transplantation. In case of CF, it remains unclear whether the colonization with *Aspergillus* represents a risk factor for invasive aspergillosis post transplantation. A recent study addressed this question by studying 93 CF patients before and after lung transplantation with regard to their *Aspergillus* colonization status using *Aspergillus* sputum culture and bronchoalveolar lavage GM as *Aspergillus* colonization markers [14••]. The study demonstrated that 70 % of CF patients were colonized with *Aspergillus* before undergoing lung transplantation and 22.5 % developed invasive aspergillosis with a mean time of 42 days following transplantation. The related 1-year mortality was 16 %. In search of identifying *Aspergillus*-associated risk and screening factors, the authors further showed that particularly the positive intraoperative detection of *Aspergillus* resulted in a four-fold higher risk of developing invasive aspergillosis, suggesting that this method could be used for early risk assessment in CF patients undergoing lung transplantation. Moreover, the therapeutic implications of these findings for potential prophylactic approaches in CF patients scheduled for lung transplantation should be further discussed.

Traditionally, ABPA is considered rare and mainly found in older CF patients. Up to 15 % of CF patients have been reported with ABPA and an even higher

percentage with subclinical disease. These notions, however, are based on different heterogenous reports and lack broad high-quality epidemiological evidence. Therefore, a recent study performed across countries estimated the different manifestations of aspergillosis in CF patients [15]. Incorporating various international CF registries, this study confirmed the view that ABPA prevalence substantially varies by country, probably due to the current inadequate diagnostic ABPA criteria and genetic influences. Moreover, this study implicates an underdiagnosis of ABPA that occurs in children and teenagers with an estimated rate <1 % under 4 years and increasing throughout childhood and adolescence. When reviewed in combination, this study reinforces the awareness to screen for ABPA and other *Aspergillus*-related disease phenotypes (*Aspergillus* bronchitis and *Aspergillus* sensitization, see above) as early as infancy to prevent disease progression into full-blown ABPA.

Nevertheless, it remained a matter of debate in the CF field whether *Aspergillus* colonization and/or sensitization without ABPA are associated with lung function declines. To address this question, Baxter and coworkers from the National Aspergillosis Centre, University Hospital of South Manchester, UK, performed a 2-year prospective observational cohort study including 55 adult CF patients [16]. In this cohort, 69 % showed airway colonization with *Candida* and 60 % with *Aspergillus* species. The authors did not find an association between the presence of these fungi in CF airways and lung function decline. However, patients sensitized for *Aspergillus* species showed a greater lung function decline and an increase in their need for intravenous antibiotics. Despite these intriguing findings, further studies in larger and particularly younger CF cohorts are required to dissect the relationship between fungal colonization, sensitization and longitudinal lung function declines in patients with CF.

Fungi Other Than Aspergillus

The clinical relevance of fungi other than *Aspergillus*, such as *Candida*, *Scedosporium*, *Pseudallescheria*, *Pneumocystis jirovecii*, and *Penicillium*, remain poorly understood, but there is an increasing number of studies analyzing these fungal species in the context of CF lung disease. Accordingly, a recent study found a high prevalence of a non-albicans *Candida* species, namely *C. dubliniensis*, which was even more prevalent than *C. albicans* in this CF study cohort [17]. *C. dubliniensis* was mainly detected in combination with *P. aeruginosa* and *S. aureus*.

Scedosporium species, mainly the *Scedosporium apiospermum* complex, are emerging and found in airway fluids from older CF patients, but the clinical relevance of this finding is debated. A recent study did not find an association between sensitization against the *S. apiospermum* complex and poorer lung function in patients with CF [18]. Further studies are required to understand the potential role of this fungus in CF lung disease.

Similar to *Scedosporium*, *Exophiala dermatitidis* is a fungus that frequently colonizes the airways of CF patients with an unclear clinical relevance. A recent study demonstrated that *E. dermatitidis* was recovered in 17 % of CF patients, with higher levels of IgG antibodies to *E. dermatitidis* in the positive CF patients [19]. Interestingly, those patients were more often colonized with non-tuberculous mycobacteria, required more intravenous antibiotic treatment, and had a lower lung function, suggesting that this fungus could have a harmful impact on the course of CF lung disease.

A prospective multicenter study in France analyzed the prevalence of *P. jirovecii* in 104 patients with CF lung disease and found that it was present in 12.5 % of them [20]. In contrast to other fungi, the presence of *P. jirovecii* was associated with the absence of *P. aeruginosa*. Detection of *P. jirovecii* was associated with a greater lung function decline, suggesting that this fungus could play an important, but hitherto underestimated, role in patients with CF lung disease. In order to improve the methods of fungal detection, two studies were recently published. Bernhardt and colleagues used multilocus sequence typing (MLST) to characterize *S. apiospermum* and *Pseudallescheria boydii* isolates from CF patients [21]. Collectively, these studies demonstrated that MLST technology is a highly effective tool for the study of fungal colonization at an epidemiological level and to investigate whether fungal isolates are clonal or have undergone recombination. These studies also showed that CF patients are colonized by individual *S. apiospermum* and *P. boydii* strains for up to a year-long period.

Masoud-Landgraf and coworkers compared different mycological culture methods to study fungi in CF sputum [22]. These studies demonstrate that *Candida albicans*, *C. dubliniensis*, and *C. parapsilosis* were the most common yeast species and *Aspergillus fumigatus* the most common filamentous fungus, followed by *S. apiospermum/P. boydii group* and *A. terreus*. Longitudinal analyses also revealed that fungal colonization patterns in CF patients are stable and colonize the airways independent from antifungal treatments. Methodologically, this study also showed that various fungal species, particularly *E. dermatitidis*, *Rasamsonia* (*Geosmithia*) *argillacea*, were isolated only from homogenized sputum samples using mucolytics.

A significant number of CF patients suffer from ABPA or “pre-ABPA” conditions. There is no doubt on the indication to treat ABPA; however, there is still an ongoing debate what treatment regimen should be chosen. Pre-ABPA refers to non-defined clinical conditions of patients not fulfilling all ABPA criteria, but showing disease

deterioration associated with *Aspergillus* colonization and/or sensitization without any further microbial or clinical explanation. In addition, other non-IgE mediated conditions, such as *Aspergillus* bronchitis [23], have been described in CF patients. Due to the fact that *Aspergillus* is the leading pathogen and the trigger of the above-mentioned disease entities, a very interesting approach seems to be the reduction of fungal load in the airways. However, a recent placebo-controlled trial of antifungal treatment in CF patients with *A. fumigatus*-positive sputum reported a non-significant trend toward poorer lung function in the patient group receiving itraconazole compared with placebo [24•]. These results were not only attributed to the small study size (n=35), but also due to difficulties to achieve therapeutic itraconazole levels in more than 40 % of the patients. The latter finding emphasizes the need for further clinical trials. In line with this, other administration routes, e.g., inhalative azole therapy, and alternative treatment options, such as anti-IgE (omalizumab), should be evaluated in multicenter approaches. However, due to the dynamic shift between the different disease entities, it will be difficult to define inclusion criteria for these studies, but usage of enhanced mycological techniques and modern biomarkers may help to accurate clinical stratification into new classification models.

Unresolved Questions

While major efforts have expended over the last decade in understanding the role of fungi within the CF airways, significant unresolved questions remain. Although we have improved our fungal detection and evaluation methodology, we are not able to date to conclusively comment on their clinical relevance [3]. *Aspergillus*, *Candida*, and *Scedosporium* species have been the major focus of most work to date owing to their isolation frequencies [4, 18, 25–27]. In order to determine the clinical effects of any particular fungi, investigations into their virulence, pathogenesis, and signaling pathways are imperative.

- A major challenge for CF clinicians looking toward the literature for insight on the field is the cross-sectional nature of many clinical studies making it hard to establish causality for episodes of clinical deterioration. As a consequence, a major unanswered clinical question is that of treatment: when, why, and how? A genuine need for investment in prospective placebo-controlled trials to evaluate antifungal therapies in a wide range of clinical settings is now therefore justifiable. This would begin to address the key and largest question in the field to date: what is the clinical significance of the fungi detected in CF samples and when and if treatment is indicated. Of course, the outcomes will vary dependent upon the fungi, CF clinical status, and the individual's response to infection.
- When and by what mechanism are fungi pathogenic? The current literature is littered with controversies and debate within this field. Do they establish infection early in infancy or later in disease? Do they cause harm initially or over time? Does synergism exist? What is the relevance of the clinical setting or fungal virulence? Should they be treated and if so what agents and for what duration? All such questions are relevant but lack data to answer effectively at present.
- Significant differences in isolation and identification techniques exist internationally between mycology laboratories resulting in some misidentification of certain fungi [3, 28]. This has been reported in the literature and impacts on our understanding of the "real" incidence of colonization and infection in CF populations. Additionally, it is likely that there is an underestimation of both importance and significance of fungi in CF. The increasing use and focus on molecular-based detection techniques and genotyping for fungi in CF represent a major step forward in standardization, and further important questions remain with regard to the diagnosis and use of biomarkers in ABPA [29]. The new immunological classifications described in this review will continue to be refined allowing the identification of sub-phenotypes of fungal "colonizers" previously unrecognized that are likely at risk of clinical deterioration [13••].
- Host-derived factors, including the state of the immune system, severity of CF disease, effect of inflammation, and the burden of treatment, all play critical roles in determining susceptibility, while fungal type, virulence, genotype, and immunoevasive capabilities all too have their place [30]. It is this combination of host and pathogen-related factors that determines if a particular fungus at a certain point in time will represent a colonizer or pathogen within the CF lung.

Recent findings provide new insights into (i) the relevance of fungal colonization and sensitization in CF beyond ABPA, (ii) the clinical impact of the interaction between fungi and bacteria, and (iii) the potential role of rare fungi for the outcome of CF lung disease. Looking into the future, our focus should remain on both developing methodology to accurately detect and attempting clinical trials to eradicate in an effort to provide an evidence base for treatment as and when it may be required.

Compliance With Ethics Guidelines

Conflict of Interest O Eickmeier, A Hector, A Singh, SH Chotirmal, and D Hartl all declare no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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