



Fungal infections and treatment in cystic fibrosis

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Purpose of review

This review summarizes some of the important recent findings concerning fungal airway infections in patients with cystic fibrosis (CF). For many years, both researchers and clinicians have focused on the problems in CF caused by chronic bacterial airway infection with organisms such as *Haemophilus*, *Staphylococcus* and *Pseudomonas*. However, until recently, the lack of sensitive culture techniques to isolate fungi in sputum, bronchoalveolar lavage fluid and other respiratory tract samples has limited the recognition of fungal species and their possible role in CF airway infections.

Recent findings

Recent studies using fungal-selective culture media and molecular techniques have shown a plethora of different fungal species in the sputum expectorated from CF patients. Cross-sectional studies have shown associations between *Aspergillus* and *Candida* in the sputum and worse lung function. The presence of allergic bronchopulmonary aspergillosis is likely to be a negative prognostic factor, but whether simple fungal colonization itself indicates future problems is not clear. Current research is now examining these epidemiological associations to try to determine the clinical implications. This will help determine whether fungal colonization/infection is associated with worse outcome in CF patients.

Summary

At present, there is no conclusive evidence that fungal organisms cause respiratory decline. Recent studies of antifungal therapy in CF patients have reported differing results, so further investigations in this area are needed.

Keywords

cystic fibrosis, epidemiology, fungal, treatment

INTRODUCTION

Cystic fibrosis (CF) is the most common lethal inherited disorder affecting caucasian people. Despite significant improvements in the management of patients with CF, the median survival remains about 40 years. The majority of the morbidity and mortality from CF is due to pulmonary disease caused by chronic suppurative airway infections leading to airway inflammation, scarring and ultimately respiratory failure. It is well recognized that specific types of bacteria, particularly *Haemophilus influenzae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Burkholderia cepacia*, are important causes of airway infections in CF. Epidemiological studies have shown that the isolation of these bacteria is associated with worse outcomes, and recent intervention studies to eradicate these organisms have shown clinical benefit, proving that bacterial infections are important in CF airway disease. But the situation for fungal colonization/infection of the CF airways is much less clear. In recent years, many studies have recognized the presence of

Aspergillus and other moulds together with yeasts such as *Candida* in the airway of CF patients. This review will focus on the complexity of fungal isolation/colonization/infection in the CF airways in the context of increasingly sensitive methods to detect these organisms and increasing recognition of their pathogenic potential.

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KEY POINTS

- Previous dismissal of fungi in the airway secretions of CF patients as 'oral contamination' may have been inappropriate, as recent studies are finding correlations between the presence of fungi in CF airway secretions and worse clinical outcomes.
- Newer fungal-specific culture media and molecular techniques show much higher prevalence of fungi than simple standard cultures.
- Further studies as part of the airway microbiome project will provide more insight into the epidemiology of fungi in the CF airway.
- Longitudinal studies are needed to assist in the determination of the effect of fungal colonization/infection on lung function decline in CF patients.
- Intervention studies to examine the effect of antifungal agents are currently planned to determine whether suppression or eradication of these fungi improves clinical outcomes.

FUNGI

Fungal cells are generally nonmotile and have a rigid cell wall. Overall, fungal species can be divided into yeasts and moulds. Yeasts are typically round or oval and reproduce by budding. The predominant yeast are *Candida* spp., which are often found in the oropharynx. In CF, the incidence of oropharyngeal 'thrush' is increased through the frequent use of oral, intravenous and nebulized antibiotics together with the use of inhaled and oral corticosteroids. Moulds, also called filamentous fungi, develop tubular structures, termed hyphae, and grow by branching and longitudinal extension. However, this differentiation is not absolute, as some fungi, for example *Exophiala dermatitidis*, will grow as a black yeast at 37°C and as a filamentous fungus at room temperature.

Increasing recognition of fungi through advances in culture and identification techniques

For many years, expectorated sputum samples containing fungi were discounted as 'oral contamination'. But recently, fungi have been increasingly recovered from the airways of CF patients, though the pathogenicity of these different fungi, and the involvement of these fungi in the progression of CF lung disease, remains poorly understood. Often, the reporting laboratory did not consider *Aspergillus* or *Candida* as 'pathogens', so these species were often omitted from the final report. At present, the clinical significance of fungal colonization of the airways is not clear, but a number of authors have suggested

that these organisms should now be the subject of appropriate studies to determine whether or not they exhibit pathogenic significance.

One of the difficulties in performing studies of expectorated sputum in CF patients is that standard culture techniques can grow various fungi, but yields can be variable from laboratory to laboratory. This likely reflects the laboratory processing systems, especially the choice of media and the duration of culture, as often fungi are relatively slower growing than the typical CF bacteria. As reviewed by Pihet *et al.* [1], many fungal species are increasingly being recognized, especially when laboratory processes specifically target fungi, for example cultures for 7 days to allow slower-growing fungi such as *Exophiala dermatitidis* to be isolated.

Over the last 5 years, fungal-selective culture media and improved culture techniques have been developed to determine the true incidence and prevalence of fungi in the airways of CF patients, and whether these fungi are associated with worse clinical outcomes. By comparing different culture techniques for expectorated sputum samples, we have previously demonstrated that media selective for one or more fungal species (or genera) increased the recognition of these fungi over standard culture techniques [2]. This confirmed previous work [1] that *Aspergillus fumigatus* was the most commonly isolated fungus, present in up to 50% of subjects [2]. However, in our study, a relatively high prevalence of *Scedosporium* spp. (15%), including the then novel species *Scedosporium auranticum*, was identified [2].

In an elegant study, Nagano *et al.* [3] determined in their CF patients ($n=77$) that fungi could be detected in only 18% of sputum samples using conventional nonselective media, yet 78% of sputum samples revealed fungi when specific fungal culture media were used. These media often included antibiotics to selectively kill *Pseudomonas*, *Staphylococcus* and *Burkholderia* [3].

Polymerase chain reaction (PCR)-based techniques were even more sensitive to the presence of fungi in the expectorated sputum. Such techniques are part of the rapidly advancing field of culture-independent molecular microbiology. For the detection of fungal species and diversity they exploit the presence of a highly preserved 18S ribosomal RNA subunit of fungus. In the study by Nagano *et al.*, total genomic DNA (including fungal genomic DNA) was extracted directly from individual CF sputum samples and the DNA was amplified with internal transcribed spacer (ITS)1 and ITS4 PCR primers. These target the region in the fungus which contains the genetic regions 18S, ITS1, 5.8S and ITS2. For those samples (<10%) that were negative by this first analysis, a nested PCR was performed

with ITS3 and ITS4 PCR primers, amplifying the ITS2 region. This sequential method was positive in all 77 samples tested [3]. To determine the presence of another major filamentous fungus, our group developed a specific multiplex PCR assay to detect and identify the three major human pathogenic species of *Scedosporium* encountered in CF – *S. prolificans*, *S. apiospermum* and *S. auranticum* [4].

The various fungi isolated by Nagano *et al.* [3] (Table 1) differed from those isolated by Blyth *et al.* [2] (Table 2). These differences may reflect subtle differences in techniques used, the different patients studied or environmental factors, but highlight the need for different centers around the world to examine the effect of fungal species on CF lung disease. Less frequently recovered fungi from CF airways include members of the dematiaceous fungi, the Zygomycetes, *Trichosporon* spp. and rare moulds [1]. With the development of next-generation sequencing to detect fungal biodiversity in the human lung microbiome [5], it is increasingly clear that the prevalence of fungal species in the expectorated sputum of CF patients has been underestimated for a number of years.

CLINICAL SIGNIFICANCE OF FUNGAL ISOLATION

One of the most well recognized clinical syndromes of fungal colonization of the airways is allergic bronchopulmonary aspergillosis (ABPA), caused by *A. fumigatus*, though rarely a similar syndrome may be found with other fungi. Classically, ABPA reflects an exuberant immune response to the presence of small numbers of *Aspergillus* spp. in the airways, with elevated *Aspergillus*-specific IgE, positive *Aspergillus* radioallergosorbent and specific precipitins. ABPA is well known to be associated with worse lung function and more rapid lung function decline [6]. Treatment of ABPA involves the use of inhaled and systemic corticosteroids, with many reports outlining clinical trials of azole therapy, often itraconazole, since the initial reports more than 20 years ago [7]. However, despite widespread use of itraconazole as a steroid-sparing agent in ABPA, no randomized double-blind controlled studies of itraconazole in ABPA in CF patients have been performed, despite most clinicians using itraconazole in this situation.

Similarly, the effect of *A. fumigatus* in the airways of patients with CF in the absence of ABPA

Table 1. Prevalence of yeasts and filamentous fungi (%) from 77 cultures in 77 CF adult patients in Belfast (n = 77 patients)

	Conventional culture	Fungal-specific culture	PCR	Overall from any method
Yeasts				
<i>Candida</i> spp.	8/77 (10%)	56/77 (73%)	71/77 (92%)	75/77 (97%)
<i>Candida albicans</i>	2/77 (2.6%)	34/77 (44%)	40/77 (52%)	45/77 (58%)
<i>Candida dubliniensis</i>	0	21/77 (27%)	27/77 (35%)	30/77 (39%)
<i>Candida parapsilosis</i>	0	3/77 (3.9%)	5/77 (6.5%)	7/77 (9.1%)
<i>Candida glabrata</i>	0	1/77 (1.3%)	3/77 (3.9%)	4/77 (5.2%)
<i>Malassezia</i> spp.	0	0	2/77 (2.6%)	2/77 (2.6%)
<i>Rhodotorula</i> spp.	0	1/77 (1.3%)	0	1/77 (1.3%)
<i>Saccharomyces cerevisiae</i>	0	0	5/77 (6.5%)	5/77 (6.5%)
<i>Trichosporon</i> spp.	1/77 (1.3%)	1/77 (1.3%)	2/77 (2.6%)	2/77 (2.6%)
Filamentous fungi				
<i>Aspergillus versicolor</i>	5/77 (6.5%)	5/77 (6.5%)	0	7/77 (9.1%)
<i>Aspergillus fumigatus</i>	1/77 (1.3%)	4/77 (5.2%)	0	4/77 (5.2%)
<i>Exophiala dermatitidis</i>	0	3/77 (3.9%)	1/77 (1.3%)	3/77 (3.9%)
<i>Penicillium citreonigrum</i>	0	4/77 (5.2%)	4/77 (5.2%)	7/77 (9.1%)
<i>Scedosporium apiospermum</i>	0	2/77 (2.6%)	3/77 (3.9%)	3/77 (3.9%)
<i>Aureobasidium pullulans</i>	0	1/77 (1.3%)	0	1/77 (1.3%)
<i>Fuscoptoria ferrea</i>	0	0	1/77 (1.3%)	1/77 (1.3%)
<i>Fusarium culmorum</i>	0	0	1/77 (1.3%)	1/77 (1.3%)
<i>Acremonium strictum</i>	0	0	1/77 (1.3%)	1/77 (1.3%)
<i>Thanatephorus cucumeris</i>	0	0	1/77 (1.3%)	1/77 (1.3%)
<i>Cladosporium</i> spp.	0	0	1/77 (1.3%)	1/77 (1.3%)

Adapted from Nagano *et al.* [3]. Most CF patients (46/77, 60%) had only one fungal species detected in their sputum, 18 patients (23%) had two fungal species, 12 (16%) had three and one patient had five fungal species present. CF, cystic fibrosis; PCR, polymerase chain reaction.

Table 2. Frequency of isolation of filamentous fungi in 218 sputum specimens from 66 CF patients in Sydney

Organism	No. of specimens (%)
<i>Aspergillus</i> spp.	110/218 (50.5%)
<i>A. fumigatus</i>	99/218 (45.4%)
<i>A. flavus</i>	19/218 (8.7%)
Other <i>Aspergillus</i> spp.	9/218 (4.1%)
<i>Scedosporium</i> spp.	32/218 (14.7%)
<i>S. aurantiacum</i>	17/218 (7.8%)
<i>S. prolificans</i>	11/218 (5.0%)
<i>S. apiospermum</i>	4/218 (1.8%)
<i>Penicillium</i> spp.	18/218 (8.3%)
<i>Paecilomyces</i> spp.	7/218 (3.2%)
Other hyaline hyphomycetes	9/218 (4.1%)
<i>Cladosporium</i> spp.	4/218 (1.8%)
<i>Curvularia</i> spp.	3/218 (1.4%)
<i>Alternaria</i> spp.	3/218 (1.4%)
Other dematiaceous fungi	4/218 (1.8%)
<i>Rhizopus</i> spp.	2/218 (0.9%)
Other fungi	3/218 (1.4%)

Adapted from Blyth *et al.* [2]. No sample had multiple species of *Scedosporium* identified, but 60 of 218 (27.5%) sputa contained two or more fungi. *Aspergillus* spp. were more often present in sputa that contained *Scedosporium* spp. and some specimens contained multiple species of *Aspergillus*. CF, cystic fibrosis.

remains unknown. This syndrome, sometimes referred to as *Aspergillus* bronchitis, reflects chronic colonization with *Aspergillus* yet without the classical allergic findings. A recent study has shown that isolation of *A. fumigatus* from the airways of children with CF, in the absence of ABPA, is also associated with a lower forced expiratory volume in 1 second (FEV₁), and an increased risk of pulmonary exacerbations requiring hospitalization [8]. However, it could be argued that those patients with more pulmonary exacerbations receive more antibiotics and thus have more secondary colonization with fungal species, as adjustment for these variables weakened the epidemiological association [8]. This supports a previous subgroup in the Kraemer *et al.* [6] study, in which children with sensitization to *A. fumigatus* but without ABPA had intermediate outcomes between the noninfected and those with ABPA.

Our group has previously examined the clinical associations with *Scedosporium* spp. [9]. In a cross-sectional analysis, there was no association between the presence of *Scedosporium* spp. and worse clinical measures (e.g. FEV₁), but interestingly there was an inverse relationship between the presence of mucoid *P. aeruginosa* and *Scedosporium* spp. We have now also found that long-term colonization (>2 years) with *Scedosporium* spp. is relatively stable

such that those subjects with *Scedosporium* colonization on one culture are more likely to exhibit the same organism in subsequent samples.

Similar studies have now been undertaken to examine yeasts such as various species of *Candida* in the airways of CF patients. A prospective observational study in 86 CF patients showed that acquisition of *Candida* spp. was associated with reduced FEV₁, greater number of pulmonary exacerbations and more rapid decline in FEV₁ [10]. Colonization with *Candida* was very common (49%) and was associated with worse clinical status (e.g. osteopenia, pancreatic insufficiency) and also with co-colonization with *P. aeruginosa* and *A. fumigatus*. Use of corticosteroids was not studied in this report, as they were 'infrequently prescribed' in this clinic (data not shown). Although the epidemiological association suggests that *Candida* colonization of the sputum is also associated with worse outcomes, the possibility remains that colonization is secondary to worse infection and/or the associated antibiotic treatments. Importantly, many of these studies are cross-sectional, and thus do not necessarily indicate cause and effect. Longitudinal studies may provide some useful information, but one of the best ways to determine the impact of these fungi is to try to suppress or eradicate them.

FUNGAL TREATMENT OF THE CYSTIC FIBROSIS LUNG

Since the early case series by Denning *et al.* [7], physicians have been interested in the use of anti-fungal agents, and in particular, agents to eradicate or suppress *A. fumigatus*. In asthma, randomized trials of itraconazole (200 mg b.d.) have been shown to improve lung function and exercise tolerance [11], but similar trials have not specifically studied the use of itraconazole in CF patients. Itraconazole is often a difficult drug to use, as it has variable absorption following oral dosing, with particular need for acidic gastric pH. Given the interactions with food, for example increased absorption following ingestion with cola-containing beverages, itraconazole levels may be useful. Although itraconazole has been used clinically for many years, recently azole resistance has been recognized, especially in those who have been exposed to itraconazole [12]. Voriconazole, a newer azole agent with greater activity against *A. fumigatus*, has recently been associated with photosensitivity in CF, affecting 18 of 31 children in one clinic [13].

A number of recent pilot studies have examined the effect of long-term antifungal treatment for CF patients with *A. fumigatus* in their sputum. Aaron *et al.* [14] performed a randomized double-blind

multicenter study to examine the effect of itraconazole 5 mg/kg/day in CF patients chronically colonized with *A. fumigatus*, who had no evidence of ABPA. Paradoxically, the 18 subjects prescribed antifungal treatment for 24 weeks exhibited a trend toward earlier exacerbations (median time: itraconazole, 77 days; placebo, 134 days) and more frequent exacerbations (itraconazole, 77%; placebo, 44%), though these differences were not statistically significant [14[•]]. Although the sample size was small and the dose of itraconazole subtherapeutic in 43% of patients, the disturbing trend was to worsen the clinical situation.

In contrast, a single-center open-label study in 13 subjects suggested improvements in quality of life and reduced exacerbations when itraconazole (400 mg daily for 6 weeks) was given [15^{••}]. This study also demonstrated important interactions between *A. fumigatus* and the expression of the vitamin D receptor (VDR). VDR expression in CF cell lines was downregulated by the addition of *A. fumigatus* culture filtrates [15^{••}]. Heat treatment and filtration (3 kD exclusion) removed much of the VDR inhibitory factors suggesting the involvement of the fungal metabolite, gliotoxin. Gliotoxin can induce experimental airway inflammation and tissue damage. Finally, the effect was replicated by addition of purified gliotoxin [15^{••}]. Bronchoalveolar lavage fluid from patients with *A. fumigatus* exhibited higher levels of gliotoxin, and increased bronchoalveolar fluid levels of interleukin-5 and interleukin-13. Itraconazole-treated patients exhibited increased VDR expression, and reduced airway gliotoxin levels, together with improvements in CT scans, reduced exacerbations and improved quality of life [15^{••}].

Interactions between fungal colonization and vitamin D levels *in vivo* have previously been demonstrated. Kreindler *et al.* [16] compared CF patients with ABPA and CF patients with *A. fumigatus* in their sputum but no signs of ABPA. Those with ABPA had significantly higher Th2 responses, and these responses were inversely correlated with serum vitamin D levels. Interestingly, addition of vitamin D *in vitro* to dendritic cells reduced expression of pro-inflammatory cytokines, suggesting that exogenous vitamin D may be useful in the treatment of ABPA [16].

Finally, an interesting study by Baxter *et al.* [17[•]] outlined a significant decrease in *Aspergillus* colonization following a course of intravenous antibiotics, which were presumably taken together with nebulized bronchodilators at home, and increased physiotherapy and exercise to remove excess sputum and thus reduce recovery of *Aspergillus* in the sputum.

CONCLUSION

An increasing diversity of fungi is now being recovered from the airways of CF patients. Although the clinical ramifications of these organisms are unknown, they have been demonstrated in CF patients in different clinics internationally, and have been reported to be associated with worse lung function and worse quality of life. Recent intervention studies examining the effects of itraconazole in CF patients with *A. fumigatus* have shown varying results. Further studies are currently underway to better understand the epidemiological associations of fungal colonization/infection in CF lung disease and to determine the optimal management of fungi in CF lung disease.

Acknowledgements

None.

Conflicts of interest

There are no conflicts of interest.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

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This is an important observation suggesting possible interactions between bacteria and fungi in CF airways.