

Characteristics of pathogenic fungi and antifungal therapy in cystic fibrosis

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A defective mucociliary clearance facilitates colonization with bacteria and fungal spores in cystic fibrosis patients. Yeasts and molds are cultured from the cystic fibrosis respiratory tract and often their clinical relevance is unknown. *Candida* spp. are the most commonly isolated yeasts, whereas *Aspergillus* spp., *Scedosporium apiospermum*, as well as *Exophiala dermatitidis* in some countries, are the most frequent molds recovered from respiratory specimens. Molecular biotyping studies have revealed that some fungal genotypes are capable of chronically colonizing the airways. Persistent *Aspergillus fumigatus* infection is associated with an increased risk of pulmonary exacerbations requiring hospitalization. The prevalence of non-*Aspergillus* molds may be underestimated due to overgrowth of *Pseudomonas* and *Aspergillus* spp. on routine media. Allergic bronchopulmonary aspergillosis is usually treated by oral steroids and an antifungal azole drug. Interactions with the co-medication have to be considered. A small number of antifungal pharmacokinetic studies indicate a high inter-subject variability for itraconazole, voriconazole and posaconazole, and therefore therapeutic drug monitoring is recommended.

KEYWORDS: antifungal drug resistance • antifungal therapy • *Aspergillus* • *Candida* • chronic persistence • drug monitoring • filamentous fungi • *Pneumocystis* • *Scedosporium* • yeast

Cystic fibrosis (CF) is an autosomal recessive disorder with an incidence of one in 2500 live births in the Caucasian population. It results from mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*), which is located on chromosome 7. It encodes a chloride channel involved in electrolyte exchanges through the plasma membrane of numerous epithelial cell types and leads to a dysfunction of the exocrine glands. Mutations in *CFTR* genes result in a defective mucociliary clearance with production of viscous and sticky bronchial mucus that facilitates colonization with airborne bacteria and fungal spores [1].

Early in life, CF patients become infected with a limited spectrum of bacteria, mostly *Haemophilus influenzae* and *Staphylococcus aureus*. As the disease progresses, *Pseudomonas aeruginosa* becomes the most common pathogen. However, other bacteria, including anaerobes, viruses, as well as yeasts and molds are cultured from respiratory specimens of CF patients and often their clinical relevance is not clear. Among clinically significant fungi, *Candida* spp. are the most common yeasts, whereas *Aspergillus* spp., *Scedosporium apiospermum*, as well as *Exophiala dermatitidis* in some countries, are the

most frequent molds recovered from respiratory secretions of CF patients, but their isolation rate varies greatly according to different studies [2,3]. Moreover, Nagano *et al.* reported an increasing prevalence of fungi, from 6% in 1995 to 13% in 2005, being isolated from sputum samples of CF patients in the USA [3]. The variation of isolation rates between studies is due to differences between age groups (fungi are more frequently isolated in adolescent and adult patients), different respiratory samples (swabs, sputum and bronchoalveolar lavage), different culture techniques (e.g., selective media for *Scedosporium* spp.) and the use of different molecular tools. In addition, over the past decade, some cases of infection or chronic colonization have been associated with species that have only rarely or never previously been reported in human cases, such as *Penicillium emersonii* and *Acrophialophora fusispora* [4].

Yeasts

Candida spp.

The frequent use of broad-spectrum antibiotics, and oral and inhaled steroids predisposes CF patients to colonization of the upper and lower airways with *Candida* spp. *Candida albicans* is the

most frequent member of the genus isolated from the CF respiratory tract, but its clinical relevance is still unknown. In addition, it is still controversial whether *Candida* spp. are transient or persistent colonizers of the airways or more simply an oral carriage. *C. albicans* was isolated from the respiratory tract in 93% of CF patients, whereas other *Candida* spp. were recovered from 20% of these individuals [5]. An increase in the emergence of other *Candida* spp. has been reported in the literature, which could be the result of an enhanced ability to differentiate members of this genus and, perhaps, their ability to develop antifungal drug resistance [6]. In a study by Doern *et al.*, the non-*albicans* *Candida* species accounted for 15% of all *Candida* isolates. The most frequent of these found were *Candida tropicalis* (6%), *Candida glabrata* (5%) and *Candida parapsilosis* (4%) [7]. In a prospective study of 56 CF patients conducted over a 30-month period, the distribution and persistence of different *Candida* spp. was assessed [5]. *C. tropicalis* was only found in 1% of the isolates from the patient specimens. The prevalence of *C. parapsilosis* (4%) and *C. glabrata* (7%) was similar to the recovery reported by Doern *et al.* [5,7]. Peltroche-Llacsahuanga *et al.* investigated the prevalence of *Candida dubliniensis* in CF patients and reported a colonization rate of 11% [8]. This yeast-like fungus was recovered from the sputa of seven of our patients (12.5%) and in four of them, *C. dubliniensis* was cultured repeatedly over a longer period of time (average 12 months) [5]. The reason for the high colonization rate of *C. dubliniensis* in CF is still unknown. Hydrophobicity could play an important role in virulence because of the resulting difficulties in phagocytosis and the dehydrated sputum in the respiratory tract. The mean persistence of the most frequent *Candida* spp. was 9 months. In patients from whom more than ten isolates were recovered, it was noted that at least 30% were genetically related and transmission of *C. albicans* in siblings was observed. The majority of all isolates were susceptible to all antifungals tested [5]. The relevance of *C. dubliniensis* and whether long-term colonization with *Candida* strains can contribute to the disease course and clinical impact as well as to the chronic infection and inflammation in the CF lungs requires further investigation.

Filamentous fungi

The percentage of CF patients growing filamentous fungi from respiratory tract specimens has significantly increased over time. In 1997, only 2.0% of John Hopkins University (MD, USA) CF patients grew filamentous fungi; by 2007, the incidence had increased to 28.7% [9]. Overall, at least one filamentous fungus was recovered from respiratory culture from 254 (41.3%) members of the study population from 1997 to 2007. Only four fungal isolates (0.004%) were recovered from individuals previously receiving a lung transplant. *Aspergillus fumigatus* was isolated from 223 (36.3%) individuals and was the most prevalent filamentous fungal species (TABLE 1).

Aspergillus fumigatus

Aspergillus fumigatus is by far the most isolated filamentous fungus from respiratory secretions of CF patients, with a prevalence rate ranging from 16 to 56.7%, and up to 57% of CF patients are chronically colonized with this fungus [3]. Interestingly,

colonization of the airways by *A. fumigatus* is uncommon in young children and usually follows bacterial respiratory infections. In an epidemiological study conducted in collaboration between the two CF centers in Angers and Giens (France), the mean age of the patients at date of first isolation of *A. fumigatus* and *S. apiospermum* from respiratory secretions was approximately 12.3 and 14.1 years [4]. By contrast, the first isolation occurred at 5.4 and 8.1 years for *S. aureus* and *P. aeruginosa*, respectively [10]. Similar to bacteria, *A. fumigatus* can directly damage the respiratory mucosa [10]. Indeed, *A. fumigatus* produces some secondary metabolites (e.g., gliotoxins) and proteolytic enzymes (e.g., serine protease) that are able to impair the mucociliary clearance or inhibit phagocytosis [11]. *A. fumigatus* can cause aspergilloma in pre-existing pulmonary cavities (bronchiectasis in CF), and in chronically obstructed paranasal sinuses. Other diseases are IgE-mediated, such as allergic rhinitis and asthma, hypersensitivity pneumonitis, chronic necrotizing pneumonia and allergic bronchopulmonary aspergillosis (ABPA). ABPA is a pulmonary disorder in CF patients with a prevalence of approximately 1–15%, arising from an allergic response to multiple antigens expressed by *A. fumigatus* colonizing the bronchial mucous [12,13]. In CF patients undergoing liver or lung transplantation, it can lead to invasive pulmonary aspergillosis with multiple organ dissemination [14,15]. However, ABPA in CF will not be discussed in more detail in this article because a number of recent comprehensive reviews are available [13,16–19].

Pneumocystis jirovecii

Asymptomatic carriers of *Pneumocystis jirovecii* with CF have been described previously. However, the molecular epidemiology of *P. jirovecii* in CF patients has not yet been clarified. A study by Montes-Cano *et al.* identified the distribution and dynamic evolution of *P. jirovecii* genotypes based on the mitochondrial large-subunit (mt-LSU) rRNA gene. The mt-LSU rRNA genotypes of *P. jirovecii* isolates in 33 respiratory samples from CF patients were investigated using nested PCR and direct sequencing. Three different genotypes were detected: 36.3% genotype 1 (85C/248C); 15.1% genotype 2 (85A/248C); 42.4% genotype 3 (85T/248C); and 6% mixed genotypes. Patients studied during a 1-year follow-up period showed a continuous colonization/clearance cycle involving *P. jirovecii* and an accumulative tendency to be colonized with genotype 3 [20].

Chronic persistence of *A. fumigatus* in the respiratory tract of CF patients

A total of 256 isolates recovered from 89 respiratory samples, obtained from eight CF patients from Belgium and France, were genotyped using a highly discriminant microsatellite-based typing method [21]. Although different genotypes were identified in 88.3% of the patients, recurrent genotypes for seven out of the eight CF patients were isolated on several occasions more than 5 months apart. In another molecular typing study, the *A. fumigatus* colonization patterns from respiratory specimens of 36 CF patients were investigated over a time period of 12 years by typing 204 isolates with the STRAfast assay. From 196 *A. fumigatus* isolates, a genotype

was obtained; the remaining isolates were mixed genotypes. In general, four different colonization patterns can be identified. First, continuous colonization patterns were found. The patients yielded identical or related genotypes for all isolates over a prolonged period of time, indicating that they were not able to clear the *A. fumigatus* isolate. In this collection, 17% of the patients showed colonization with only one genotype. The second colonization pattern consisted of a predominant genotype, meaning that most of the isolates were identical. This group of patients were also not able to clear the *A. fumigatus* isolate and were occasionally co-colonized with a second genotype. This was the case in 19% of the patients. The third colonization pattern, found in 28% of the patients, contained genotypes that succeeded each other. These patients were eventually able to clear the *A. fumigatus* isolate, but were easily recolonized. The remaining 36% of the patients yielded colonization only with unique *A. fumigatus* isolates, indicating that these patients were continuously able to clear the fungus from the respiratory tract [22]. These findings indicate that some genotypes are capable of chronically colonizing the airways. Only recently, it was demonstrated that *A. fumigatus* can grow as a biofilm and produce an extracellular matrix on CF bronchial epithelia [23] and in aspergilloma [24]. Future studies are required in CF in particular to characterize the regulation of *in vivo* growth of *A. fumigatus* under biofilm in patients.

Clinical characteristics of chronic *A. fumigatus* lung infection

In a retrospective cohort study of CF patients at The Hospital for Sick Children (Toronto, Canada) from 1999 to 2006, the effect of chronic *A. fumigatus* infection on lung function and hospitalization was assessed [25]. Persistent *A. fumigatus* infection was defined as the presence of two or more positive sputum or bronchoalveolar lavage cultures for *A. fumigatus* in a given year. The primary outcome measure was the annual number of hospitalizations for pulmonary exacerbations. A total of 230 patients with CF were included in the analysis. The forced expiratory volume in 1 s (FEV₁) of patients persistently infected with *A. fumigatus* was 3.61% ($p \leq 0.0001$) lower during the study period compared with uninfected patients. There was a significant interaction between *A. fumigatus* and *P. aeruginosa* on lung function ($p = 0.0006$). Patients not infected with either organism had the highest pulmonary function. Persistent *A. fumigatus* infection (relative risk [RR]: 1.94; $p = 0.0002$) was associated with an increased risk of pulmonary exacerbations requiring hospitalization, whereas there was no increased risk of pulmonary exacerbations among patients with allergic bronchopulmonary aspergillosis (RR: 1.02; $p = 0.94$). When adjusted for baseline pulmonary function, none of these variables were associated with a significantly increased risk of pulmonary exacerbations, with only chronic *A. fumigatus* infection trending toward significance (RR: 1.40; $p = 0.065$) [25].

Table 1. Prevalence of filamentous fungal and yeast species recovered from respiratory specimens of pediatric and adult cystic fibrosis patients at John Hopkins University (MD, USA) (1997–2007).

Filamentous fungi and yeasts	Number of individuals (%) n = 614	Number of isolates (%) n = 14,288
<i>Aspergillus fumigatus</i>	223 (36.3)	885 (6.0)
<i>Aspergillus</i> (non- <i>fumigatus</i>)	160 (26.1)	107 (0.7)
<i>Trichosporon</i> spp.	13 (2.1)	44 (0.3)
<i>Scedosporium</i> spp.	14 (2.3)	21 (0.1)
Other filamentous fungi†	31 (5.0)	41 (0.5)
Yeast‡	175 (28.5)	324 (2.2)

†*Alternaria*, *Curvularia*, *Fusarium*, *Paecilomyces*, *Penicillium* and *Exophiala* species.

‡*Candida albicans*, *Candida glabrata* and undifferentiated yeast species.

Adapted with permission from [60].

Mold infections in lung transplant recipients

The incidence or significance of mold infections in the explanted lungs of lung transplant recipients was assessed at the University of Pittsburgh Medical Center (PA, USA) between 2005 and 2007 [26]. Invasive mold infections were present in the explanted lungs of 5% (14 out of 304) of patients, including chronic necrotizing pneumonias ($n = 7$), mycetomas ($n = 4$) and invasive fungal pneumonias ($n = 3$). Only 21% (three out of 14) received immunosuppressive therapy within 1 year before lung transplantation, suggesting that lung damage itself predisposed patients to mold infections. The risk of mold infection was higher in patients with CF (11%; four out of 35) than other underlying lung diseases (4%; ten out of 269). Pulmonary mold infections were not diagnosed or suspected in 57% (eight out of 14) of patients. Despite secondary voriconazole prophylaxis, fungal infections developed in 43% (six out of 14) of patients with mold infections of the explanted lungs compared with 14% (42 out of 290) of patients without mold infections ($p < 0.01$). Three patients developed invasive fungal infections while on voriconazole prophylaxis and three developed fungal infections more than 8 months after the discontinuation of voriconazole. The mortality attributable to invasive fungal infections among patients with mold infections of the explanted lungs was 29% (four out of 14) [26].

Non-*fumigatus* *Aspergillus* spp.

Other *Aspergillus* species, such as *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus nidulans*, are transient colonizers of the respiratory tract. Therefore, a new concept of allergic bronchopulmonary mycoses has evolved as not only *A. fumigatus*, but also other molds, can cause IgE-mediated allergic sensitization in CF. For example *Aspergillus terreus* is usually responsible for the chronic colonization of the airways, but was in some cases associated with ABPA in CF [4]. In the study conducted at John Hopkins University between 1997 and 2007, non-*A. fumigatus* species, including *A. flavus*, *A. niger* and *A. terreus*, were recovered from 160 (26.1%) individuals, while *Scedosporium* spp. and *Trichosporon* spp. (more yeast-like, however, in this case acts like a filamentous fungi) were isolated in 2.3% ($n = 14$) and 2.1% ($n = 13$), respectively.

Other filamentous fungal species were recovered from 31 (5.0%) of individuals and included *Alternaria*, *Curvularia*, *Fusarium*, *Paecilomyces*, *Penicillium* and *Exophiala* species [9].

Scedosporium apiospermum

The prevalence of non-*Aspergillus* molds may be underestimated due to overgrowth of *Pseudomonas* and *Aspergillus* spp. on routine media [27]. As a result of traumatic or iatrogenic inoculation of its telluric conidia, *S. apiospermum* can cause various fungal infections [28]. Respiratory diseases, probably due to the inhalation of airborne conidia, can occur in a susceptible host. The diseases associated with this fungus are sinusitis, lung fungus ball and necrotizing pneumonia, as well as disseminated infections in immunocompromised patients [28]. However, all these clinical entities are rare, contrasting with the relatively high colonization rate in CF patients. Recent taxonomic changes have occurred regarding this fungus, which is now recognized as a species complex comprising at least five distinct species [29]. If two of these fungal species have not been described in humans as yet, the three others may be responsible for colonization of the airways in CF patients. *S. apiospermum* is ranked the second most common filamentous fungus associated with CF, with a prevalence rate of 8.6% [27]. This high occurrence in CF cases was later confirmed in two distinct surveys performed in Australia and Austria, in which prevalence rates of 10 [30] and 6.5% [31] were reported. The recovery of *S. apiospermum* from respiratory secretions was usually found to follow the colonization of the airways by *A. fumigatus*. One of the most important questions for physicians involved in the follow-up of CF patients remains the clinical significance of the isolation of this fungus from respiratory secretions. As demonstrated in a genotype study performed on multiple and sequential isolates recovered from long-term colonized patients, *S. apiospermum* is usually responsible for chronic colonization of the airways. Each patient was colonized by a unique genotype different from one patient to another, and conserved over time despite antifungal treatments [32]. Usually, this chronic colonization of the airways is not associated with clinical symptoms. However, Cimon *et al.* showed that *S. apiospermum* may cause allergic bronchopulmonary mycoses similar to the well-known ABPA [27]. The study by Blyth *et al.* demonstrated that a substantial proportion of *Scedosporium* species were recovered only on selective media [33]. In addition, differentiation of species within the *S. apiospermum* complex may be important for optimal monitoring of CF patients, since these species differ in their antifungal susceptibility pattern, the most resistant being *Scedosporium aurantiacum*. The likely underestimation of *Scedosporium* colonization in patients with CF highlights the unanswered role of *Scedosporium* spp. in progressive lung disease and the validity of using *Scedosporium* colonization as a criterion to argue against lung transplantation.

Other filamentous fungi

The respiratory tract of CF patients may also be colonized transiently by other filamentous fungi such as *Alternaria* spp., *Cladosporium* spp., *Penicillium* spp. or *Paecilomyces variotii* [3,10]. *Scedosporium prolificans* and *E. dermatitidis* have also been isolated, sometimes with

a high frequency, but strikingly these species seem to be restricted to some geographic areas. In addition, two thermophilic species, *P. emersonii* (the anamorph state of *Talaromyces emersonii*) and *A. fusispora*, have been isolated in humans, almost exclusively in the context of CF [4].

Scedosporium prolificans

In contrast to *S. apiospermum*, *S. prolificans* is principally known as a causative agent of disseminated infections in patients with hematological malignancies [34]. Only a few reports, mainly from Australia, Spain and the USA, address its isolation from respiratory secretions of CF patients [30,35,36]. Moreover, its pathogenic role in this context remains questionable, since only one case of endophthalmitis after lung transplantation has been reported to date in CF [37].

Exophiala dermatitidis

Although distributed worldwide, *E. dermatitidis*, which grows as a black yeast at 37°C, and as a filamentous fungus at room temperature, has been described in the context of CF, mainly from German patients [38–42]. Its prevalence rate in Germany and Belgium varies from 4.8 to 15.7% [38–43], which contrasts with its very low occurrence in other countries such as France and North America. In the same way as has been suggested for the colonization of the airways by *P. aeruginosa* [44], genetic factors may account for this discrepancy. However, it may also result from differences in lifestyle between the countries, for example, the use of sauna facilities, since this fungus can survive for many months in hot and moist conditions [45]. Nevertheless, this discrepancy may also reflect the lack of standardization of mycological examination of respiratory secretions from CF patients, since detection of this fungus may be difficult with routine isolation procedures. A prolonged incubation time (up to 4 weeks) seems to be necessary for isolation of this usually slow-growing fungus [38–40]. Moreover, due to its frequent association with yeasts and other filamentous fungi, as well as with bacteria such as *P. aeruginosa*, the use of a selective medium such as erythritol-chloramphenicol agar is highly recommended for its isolation [40]. Recently Nagano *et al.* reported the detection of *E. dermatitidis* from sputum samples by PCR [46]. In the context of CF, *E. dermatitidis* may be responsible for a transient or chronic colonization of the airways, but usually without any clinical signs. However, this fungus may also cause true respiratory infections, or invasive fungal infections [38,41]. In addition, *E. dermatitidis*, as well as species of the genus *Aspergillus* and *Scedosporium*, isolated from respiratory secretions of patients with bronchiectasis, should be considered as a biomarker of a CFTR-related disease [47].

Characteristics of antifungal therapy in CF

Antifungal treatment in CF is currently used to treat candidemia, invasive pulmonary aspergillosis, aspergilloma, and proven or suspected ABPA. In the past, oral itraconazole (ITZ) solution was the first drug of choice. However, new antifungal drugs with a different mode of action have been introduced for the treatment of fungal infections in the CF population.

Oral corticosteroid therapy (including methylprednisolone) remains the first therapy for ABPA in CF, and it is still unknown when or how long antifungal drugs should be employed for treatment of ABPA or colonization with *Aspergillus* spp. A number of case reports support the benefit of anti-IgE therapy for some patients suffering from ABPA who do not respond to the current treatments [48]. Antifungals are not often used for treatment among CF patients, but the longstanding and significant adverse effects of corticosteroid therapy, the difficulties associated with a definitive diagnosis of ABPA and the clinical deterioration of chronically ill patients may result in an increase in antifungal usage among CF patients [49]. During the last decade, there has been an increase in the reports of ITZ resistance among *A. fumigatus* isolates obtained from patients with invasive fungal infections receiving antifungal drugs [50,51]. Resistance to voriconazole or posaconazole (PSZ) has been more rarely reported. However, these agents are not routinely used in nontransplanted CF patients. Recently, the association of *A. fumigatus* azole resistance in allergic diseases was described, resulting from prior exposure to an antifungal azole agent [52]. In all cases, treatment failure was reported and several *A. fumigatus* strains were identified as resistant to ITZ, voriconazole and/or PSZ.

Itraconazole

The triazole antifungal drug ITZ is an effective steroid-sparing adjunctive treatment for the therapy of ABPA in CF. ITZ is metabolized by side-chain hydroxylation to hydroxy-ITZ. In the steady state, hydroxy-ITZ is found in concentrations nearly twice that of the unaltered drug. Many fungi are susceptible to both the parent drug and the hydroxylated metabolite. The use of ITZ oral solution formulation in patients with CF is preferred as higher ITZ blood concentrations are achieved than with the capsule formulation. The absorption of the solution is more rapid in the fasting state. Furthermore, absorption of the oral solution, in contradistinction to the capsule preparation, is not reduced by proton-pump inhibitors. In an ITZ pharmacokinetics study in CF patients conducted by Conway *et al.*, steady-state concentrations were achieved after maximally 8 days of dosing [53]. On day 14, average peak plasma concentrations were 404 ± 268 ng/ml (<16 years, $n = 5$) and 779 ± 470 ng/ml (≥ 16 years, $n = 11$). A high inter-subject variability in ITZ pharmacokinetics was seen. Intra-subject variability was low. All of the younger patients and 50% of the older patients failed to achieve a plasma steady-state trough concentration of above 250 ng/ml.

Hennig *et al.* conducted a D-optimal designed population pharmacokinetic study of oral ITZ in adult CF patients [54]. For ITZ, the absorption rate constants (between-subject variability) for capsule and solution were 0.0315 h^{-1} (91.9%) and 0.125 h^{-1} (106.3%), respectively, and the relative bioavailability of the capsule was 0.82 (62.3%; 95% CI: 0.36–1.97), compared with the solution. There was no evidence of nonlinearity. Simulations from the final model showed that a dosing schedule of 500 mg twice daily for both formulations provided the highest chance of target success. The relative bioavailability for ITZ capsules was 82% compared with the solution [54].

In a study by Amorim *et al.*, no *A. fumigatus* isolate resistant to the five tested antifungals, azoles or non-azoles was detected (a few fungal isolates were intermediate to amphotericin B or ITZ) [49].

Voriconazole

Voriconazole (VRZ) is a second-generation triazole antifungal with superior oral bioavailability to ITZ. VRZ has a high oral bioavailability exceeding 90% and its absorption is not affected by gastric pH. It has a high potential for drug–drug interactions by inhibition of CYP3A4, and has been associated with a number of adverse effects [55]. In a prospective pediatric study by Hilliard *et al.*, a total of 21 children aged 5–16 years (median 11.3) received VRZ for between 1 and 50 (22) weeks [56]. VRZ was used as monotherapy in two children with recurrent ABPA; significant and sustained improvements in clinical and serological parameters for up to 13 months were observed, without recourse to oral steroids. VRZ was used in combination with an immunomodulatory agent in a further 11 children with ABPA, with significant improvement in pulmonary function and serology. Eight children without ABPA but who had recurrent *A. fumigatus* isolates and increased symptoms also received VRZ; this group did not improve with treatment. Adverse effects occurred in seven children (33%: photosensitivity reaction in three, nausea in two, rise in hepatic enzymes in one and hair loss in one).

In a study by Berge *et al.*, VRZ pharmacokinetics in CF lung transplant patients were assessed [57]. The duration of VRZ treatment ranged from 9 days to 22 months. The recommended standard dose of VRZ (200 mg twice a day, following the loading dose) resulted in significant plasma concentrations (40.5 mg/l) in 20% of CF lung transplant patients. Therapeutic concentrations were obtained using higher doses (average 570 ± 160 mg/day, 143%; $p < 0.01$). Despite adaptation, concentration remained less than 0.5 mg/l (11%), even when the drug was administered intravenously, highlighting the variability of VRZ pharmacokinetics, possibly enhanced by a CYP2C19 polymorphism. The risk of inefficacy during periods of underdosage was overcome by treatment with antifungal drug combinations (caspofungin, $n = 10$). The therapeutic index was limited by neurologic effects (14%) and hepatic abnormalities (30%). VRZ concentrations correlated significantly ($p < 0.01$) with aspartate aminotransferase levels, but not with bilirubin levels. Large changes in azole concentration affected the magnitude of the drug–drug interactions and adjustment requirements. The authors concluded that therapeutic drug monitoring is required because VRZ levels are often undetectable in treated CF lung transplant patients, supporting the use of antifungal drug combinations until achievement of VRZ concentration at a steady state between 1 and 2 mg/l is assessed [57].

Posaconazole

Oral PSZ was recently introduced for use in adult CF patients at a dose of 800 mg/day. PSZ has a similar effect on CYP3A4 metabolism as other azoles (ITZ and VRZ), despite being metabolized by a different pathway, involving glucuronidation via the UGT1A4 system rather than oxidative CYP3A4 metabolism. The use of PSZ together with the immunosuppressant tacrolimus (TRL) was evaluated in 14 lung transplant patients with CF [58]. PSZ inhibited

CYP3A4 TRL metabolism, resulting in a decrease of TRL dose by a factor of three, with tapering to a mean of 2 mg/day. Previous studies with ITZ and VRZ showed that TRL dose could be decreased by factors of five and four, respectively. By closely managing azole–TRL drug interactions in lung transplant patients with CF, Berge *et al.* were able to adapt TRL dose schedules, decreasing the dose of TRL by a factor of three with PSZ, four in the case of VRZ and six with ITZ. The individual pharmacokinetic variability encountered in patients with CF provides an additional argument for such joint monitoring, to demonstrate the achievement of steady state for the overall prescription [58]. However, to date, only very limited data have been published on PSZ in the context of CF, particularly outside its use in the transplanted population. Therefore, further studies in this population are warranted.

Expert commentary & five-year view

In CF, the production of viscous and sticky bronchial mucus, caused by a defective mucociliary clearance, promotes the colonization of the respiratory tract by bacteria and fungal spores. Hopefully the development of new drug targets that are correcting the basic defect in CF will improve or normalize the mucociliary clearance and reduce the risk of bacterial and fungal lung infections. In the meantime, more attention on the increasing number of yeasts and molds that are cultured from the CF respiratory tract is required. *C. albicans* is the most common yeast, whereas *Aspergillus* spp., *S. apiospermum*, as well as *E. dermatitidis* in some countries, are the most frequent molds recovered from respiratory specimens. However, the number of isolated non-*fumigatus* *Aspergillus* spp. as well as other rare molds, is increasing, and often the clinical relevance of these molds is unknown. The prevalence of non-*Aspergillus* molds is underestimated due to overgrowth of *Pseudomonas* and *Aspergillus* spp. on routine media. However, overgrowth of *Aspergillus* is less of an issue than overgrowth of *Pseudomonas* on the microbiology culture. Such overgrowth precludes the identification of fungi routinely and therefore many

so-called *Aspergillus*-positive patients remain sputum negative. The use of semi-selective culture media and molecular tools will improve the diagnosis and identification of rare molds. DNA chips will allow the detection and direct identification of the different fungal species that can be encountered in the context of CF [59].

Molecular biotyping studies revealed that some *C. albicans*, *A. fumigatus* and *A. terreus* strains, as well as genotypes of the *S. apiospermum* complex, are capable of chronically colonizing the airways. CF patients are exposed to the inhalation of a wide variety of bacterial and fungal genotypes, and only some of them are capable of chronically colonizing the airways. It is still unknown what the reasons are for this genotype selection and, in particular, the propensity of some genotypes to produce biofilms. Future studies will further characterize the impact of fungal biofilms on CF lung disease and antifungal treatment failure. Persistent *A. fumigatus* infection is associated with a decline of FEV₁, and an increased risk of pulmonary exacerbations requiring hospitalization. Allergic bronchopulmonary aspergillosis is usually treated by oral steroids and an antifungal azole drug. Interactions with comedication have to be considered. A small number of antifungal pharmacokinetics studies indicate a high inter-subject variability for ITZ, VRZ and PSZ, and therefore therapeutic drug monitoring is recommended. Future studies have to address the optimal dosage finding for azole antifungals in the CF population as well as the rationale for combination therapy to overcome treatment failure due to a low concentration of a single drug or reduced susceptibility to antifungal drugs due to the extracellular matrix produced in a fungal biofilm.

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Key issues

- In cystic fibrosis (CF), mutations in *CFTR* genes result in a defective mucociliary clearance with production of viscous and sticky bronchial mucus that facilitates colonization with fungal spores.
- Yeasts and molds are cultured from respiratory specimens of CF patients and often their clinical relevance is not clear.
- Among clinically significant fungi, *Candida* spp. are the most common yeasts, whereas *Aspergillus* spp., *Scedosporium apiospermum*, as well as *Exophiala dermatitidis* in some countries, are the most frequent molds recovered from respiratory secretions of CF patients.
- *Candida albicans* is the most frequent member of the genus isolated from the CF respiratory tract, but it is still controversial as to whether *Candida* spp. are transient or persistent colonizers of the airways or simply an oral carriage.
- *Aspergillus fumigatus* can cause aspergilloma in pre-existing pulmonary cavities and in chronically obstructed paranasal sinuses. Other diseases are IgE-mediated allergic rhinitis and asthma, hypersensitivity pneumonitis, chronic necrotizing pneumonia and allergic bronchopulmonary aspergillosis.
- Molecular biotyping studies have revealed that some *C. albicans* and *A. fumigatus* genotypes are capable of chronically colonizing the airways.
- Persistent *A. fumigatus* infection is associated with an increased risk of pulmonary exacerbations requiring hospitalization.
- The prevalence of non-*Aspergillus* molds may be underestimated due to overgrowth of *Pseudomonas* and *Aspergillus* spp. on routine media, and *S. apiospermum* is ranked the second most common filamentous fungus associated with CF.
- Allergic bronchopulmonary aspergillosis is usually treated by systemic steroids and an antifungal azole drug; however, interactions with the comedication have to be considered.
- A small number of antifungal pharmacokinetic studies in the CF population indicate a high inter-subject variability for itraconazole, voriconazole and posaconazole, and therefore therapeutic drug monitoring is recommended.

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