

Aspergillus fumigatus in the cystic fibrosis lung: pros and cons of azole therapy

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Abstract: *Aspergillus fumigatus* is the main fungus cultured in the airways of patients with cystic fibrosis (CF). Allergic bronchopulmonary aspergillosis occurs in ~10% of CF patients and is clearly associated with airway damage and lung function decline. The effects of *A. fumigatus* colonization in the absence of allergic bronchopulmonary aspergillosis are less well established. Retrospective clinical studies found associations of *A. fumigatus*-positive cultures with computed tomography scan abnormalities, greater risk of CF exacerbations and hospitalizations, and/or lung function decline. These findings were somewhat variable among studies and provided only circumstantial evidence for a role of *A. fumigatus* colonization in CF lung disease progression. The availability of a growing number of oral antifungal triazole drugs, together with the results of nonrandomized case series suggesting positive effects of azole therapies, makes it tempting to treat CF patients with these antifungal drugs. However, the only randomized controlled trial that has used itraconazole in CF patients showed no significant benefit. Because triazoles may have significant adverse effects and drug interactions, and because their prolonged use has been associated with the emergence of azole-resistant *A. fumigatus* isolates, it remains unclear whether or not CF patients benefit from azole therapy.

Keywords: itraconazole, voriconazole, posaconazole, azole resistance, allergic bronchopulmonary aspergillosis

Introduction

Cystic fibrosis (CF) is an autosomal recessive genetic disease characterized by mutations in the gene encoding for the CFTR protein, which regulates transport of chloride and bicarbonate ions at the epithelium surface. The disease, which was lethal in the first years of life 50 years ago, has benefited from several therapeutic advances, resulting in a major increase in life expectancy with most patients reaching adult age and median survival estimated at least to be 40 years in developed countries.¹ As a result, the number of adult CF patients already outgrows the number of CF children in many countries,² and demographic forecasts predict a marked increase in the number of CF (mostly adult) patients in the next decade.³

Fungi in the lung in CF patients – a focus on *A. fumigatus*

The CF lung is highly vulnerable to respiratory infections that promote airway inflammation and remodeling.^{4,5} Most available data on infection in CF airways are based on sputum bacteriological cultures that identified Gram-negative (eg, *Pseudomonas*

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aeruginosa) and Gram-positive (eg, *Staphylococcus aureus*) bacteria as predominant pathogens.⁶ More recently, it has become clear that other infectious agents (eg, nontuberculous mycobacteria and fungi) may contribute to chronic lung disease in CF patients.⁷ Although several fungi can be cultured in CF airway secretions, *Aspergillus* is the main fungus cultured in CF airways.^{8,9} Recent studies that used mass spectrometry and/or molecular methods confirmed that among *Aspergillus* spp. (ie, *Aspergillus fumigatus*, *Aspergillus terreus*, and *Aspergillus niger*), *A. fumigatus* is by far the most common species isolated in the sputum of CF patients.^{9,10}

Registry data suggest that *A. fumigatus* is cultured in 10%–25% of CF patients,¹¹ and is reported more frequently in adolescents and adults.¹² However, these data likely underestimate the true prevalence of *A. fumigatus*-positive culture in CF airway fluids because young children (who usually produce less sputum) are less likely to be sampled and because sampling practices in older children and adults may vary among different CF centers. Studies that have used systematic sputum sampling in adult CF patients have usually reported higher, although variable, rates of *A. fumigatus*-positive cultures. For example, a recent study in 146 adult CF patients reported positive sputum cultures for *A. fumigatus* in 27% of patients,¹³ and studies at our center reported positive sputum cultures for *A. fumigatus* in up to 57% of adult patients.^{8,14} Variations in the percentage of patients colonized with *A. fumigatus* could be explained by various factors including environmental exposure, interactions with other CF pathogens, and therapeutic interventions. Rocchi et al found marked differences in *A. fumigatus* conidia concentrations in the home of 16 CF patients.¹⁵ Although the latter study was not designed to address the relationship between *A. fumigatus* exposure load and positive culture in sputum, it seems likely that exposure to higher load of *A. fumigatus* could result in higher rates of *A. fumigatus* colonization. Delhaes et al reported that *P. aeruginosa* is less likely to be associated with *A. fumigatus* than *Candida albicans*,¹⁶ and Briard et al found that *P. aeruginosa* products decrease *A. fumigatus* growth in vitro.¹⁷ Further, steroids are well known to promote *A. fumigatus* growth, and Noni et al reported increased risk of *A. fumigatus*-positive cultures in CF subjects treated with inhaled steroids.¹⁸ The chronic use of inhaled antibiotics (eg, tobramycin or colomycin) has also been associated with increased risk of *A. fumigatus* sputum-positive cultures,^{12,19} whereas a recent study reported that a single course of intravenous antibiotics targeting *P. aeruginosa* reduced the presence of *Aspergillus* (as detected by polymerase chain reaction [PCR] and galactomannan; explained in the “Methods for

detecting *A. fumigatus* or immune response to *A. fumigatus* in CF patients” section) in adult CF sputum.²⁰ However, in the latter study, only two of 26 patients had positive sputum cultures for *Aspergillus*.²⁰ Long-term use of azithromycin was also associated with increase in *A. fumigatus*-positive sputum cultures.²¹

Why does *Aspergillus* stay in CF lungs?

Mechanisms leading to frequent *A. fumigatus* presence in CF airways are not fully understood. *A. fumigatus* is present in the environment and regularly enters the airways with inhaled air. The repeated presence of *A. fumigatus* isolates in the CF lungs may result either from chronic colonization with a single (or a predominant) isolate or from recurrent colonization by genotypes that succeed each other.²² Host defenses against inhaled *A. fumigatus* involve both innate immune responses (eg, mucociliary clearance, airway epithelial cells, and phagocytes such as neutrophils and macrophages) and adaptive immune responses via T cell-mediated responses.^{23,24} An autopsy study identified *A. fumigatus* within mucous plugs in CF airways,²⁵ suggesting a role for defective mucociliary clearance in *A. fumigatus* persistence in CF airways. A direct role for CFTR in the clearance of inhaled *A. fumigatus* spores by airway epithelial cells has also been suggested.²⁶ Further, CFTR-deficient T-lymphocytes response to *A. fumigatus* challenge is biased toward Th-2-dependent immune response involving IgE.²⁷ A recent study suggested that the selective proteolysis of the soluble pattern recognition receptor pentraxin 3 (PTX3 may contribute to the persistence of *A. fumigatus* in the lung of CF patients).²⁸ Regardless of the mechanisms, the role of CFTR defect in *A. fumigatus* colonization in CF airways has been confirmed by recent human studies with drugs directly targeting the CFTR dysfunction. Ivacaftor, the first marketed CFTR potentiator, reduced the occurrence of *A. fumigatus*-positive sputum cultures in CF patients with a G551D mutation.²⁹

Methods for detecting *A. fumigatus* or immune response to *A. fumigatus* in CF patients

The most classical method for detecting *A. fumigatus* and other fungi in CF airways is the culture of airway secretion (eg, sputum, bronchoalveolar lavage fluid) on appropriate culture medium (eg, Sabouraud). Investigators have highlighted the technical differences (eg, use of different culture medium, use of different lengths of time for cultures) among

laboratories processing CF samples.³⁰ Use of specific medium or long incubation time may allow for recovering other fungi (eg, *Scedosporium* sp. and *Exophiala dermatitidis*)³¹ but is unlikely to affect the rates of positive culture for *A. fumigatus*. However, culture-based identification of *A. fumigatus* is not sensitive, and investigators have developed culture-independent methods for assessing *A. fumigatus* in CF samples. These techniques include detecting *A. fumigatus* nucleic acids or *A. fumigatus* products (eg, galactomannan).

Baxter et al compared real-time PCR targeting a portion of the 18S rDNA of *Aspergillus* with fungal cultures in homogenized and sonicated CF sputum.³² The authors tested 121 sputum samples obtained from CF patients and found that 33 (30%) were culture and PCR positive, 48 (43%) samples were culture negative, but PCR positive ($P < 0.0001$), and 30 (27%) samples were culture and PCR negative.³² Comparable results have been obtained by another group,³³ suggesting that PCR is more sensitive than culture for detecting *A. fumigatus* in CF sputum. In the near future, high-throughput methods targeting fungal ribosomal RNA may be used to examine fungal diversity in CF airways,^{16,34} but these techniques have not yet reached routine use.

Galactomannan is produced during the logarithmic growth phase of the fungus; thus, its presence is considered to represent active growth of the fungus, associated with infection rather than colonization. Although galactomannan is most likely related to *A. fumigatus* in CF sputum, galactomannan is also a component of other fungal cell walls and could be present in the absence of *A. fumigatus*.^{35,36} Serum galactomannan is often used to detect invasive aspergillosis in immunocompromised patients, but invasive pulmonary aspergillosis is a rare finding in CF patients.³⁷ Warren et al tested sera from 138 pediatric and adult CF patients for the presence of galactomannan: all serum samples were negative for galactomannan, regardless of the presence of positive cultures for *A. fumigatus* in sputum.³⁸ These data suggest that testing for serum galactomannan in stable CF patients is of limited interest. More recently, Baxter et al reported that galactomannan could be assessed with good reproducibility in homogenized sputum.¹³ The authors suggested that negative sputum galactomannan could be useful for the identification of CF patients without significant *A. fumigatus*-related disease.

Immune responses to *A. fumigatus* are complex events,³⁶ which may involve, among other mechanisms, IgG and/or IgE production. The detection of IgG against *A. fumigatus* can be performed using enzyme-linked immunosorbent assays, but immunoprecipitin detection is usually considered

the reference technique.^{39,40} This technique suffers from the lack of standardization, and results may vary across different laboratories. More recently, a Western blot for detecting IgG has been commercialized and may contribute to more standardized detection of IgG against *A. fumigatus*.⁴¹ However, usefulness of this assay in CF patients has not yet been established. The detection of IgE-mediated immune response to *A. fumigatus* may involve *A. fumigatus* skin prick test and/or the detection of total IgE and IgE that recognizes various *A. fumigatus* antigens in serum.⁴²

Clinical consequences of *A. fumigatus* in CF patients

Only rare cases of invasive pulmonary aspergillosis or of aspergilloma have been reported in CF patients, and these consequences of *A. fumigatus* will not be further discussed. By contrast, IgE sensitization defined as positive skin test to *A. fumigatus* and/or high *A. fumigatus*-specific serum IgE concentration is present in up to 40% of CF patients.⁴³ Allergic bronchopulmonary aspergillosis (ABPA) occurs in ~10% of CF patients.⁴⁴ Although no universally accepted definition of ABPA exists, the disease is usually characterized by *A. fumigatus* sensitization, *A. fumigatus* IgG response with clinical symptoms (eg, wheezing), and computed tomography (CT) scan manifestations.⁴⁴ Both IgE sensitization and ABPA have been consistently associated with clinically meaningful outcomes (eg, lung function decline and/or pulmonary exacerbations) in children and adults with CF. Wojnarowski et al reported that 26% of a cohort of 118 CF children were sensitized to *A. fumigatus* (based on the results of total IgE, specific *A. fumigatus* IgE, and skin prick tests).⁴⁵ The authors reported lower lung function in CF children with positive *A. fumigatus* skin prick test and/or specific IgE and elevated total IgE.⁴⁵ In a retrospective study, Kraemer et al analyzed serial lung function tests in 122 CF children.⁴⁶ The authors reported that *A. fumigatus* sensitization (ie, increased specific IgE to recombinant *A. fumigatus* antigens) was associated with increased rates of lung function decline in subjects with ABPA and in subjects without ABPA.⁴⁶ The authors suggested that IgE sensitization to *A. fumigatus* could represent early-onset ABPA.⁴⁶ These data were largely confirmed by reports on mixed populations of children and adults with CF^{47,48} and in a study of CF adults.⁴⁹ Additionally, Baxter et al found that adult CF patients with *A. fumigatus* sensitization ($n=33$) received approximately twice as many days of intravenous antibiotics than those without sensitization ($n=22$) over a 2-year prospective follow-up study,⁴⁹ a finding that was confirmed in a study by Peetermans et al.⁴⁸

Many CF patients without IgE sensitization or ABPA have repeated positive sputum samples when examined for *A. fumigatus* using cultures or PCR, and these patients are considered to exhibit *A. fumigatus* colonization or infection.⁵⁰ Several studies have assessed the association of *A. fumigatus* colonization with clinically relevant outcomes in CF patients, and these studies have reported conflicting results. Ramsey et al performed bronchoalveolar lavage at age 3 months, 1 year, and 2 years in CF children diagnosed by newborn screening and correlated their findings with lung function (as measured by forced expiratory volume in the first three-quarters of a second, FEV_{0.75}) at early school age (4–8 years).⁵¹ Early-life respiratory tract infections with proinflammatory pathogens including *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Aspergillus* sp. were associated with subsequent reductions in lung function (measured by FEV_{0.75}).⁵¹ Fillaux et al reported larger decline in forced expiratory volume in 1 second (FEV₁) in CF patients with persistent *A. fumigatus* carriage (defined by either a persistent *A. fumigatus*-positive culture or positive *Aspergillus* IgG precipitins of *A. fumigatus* compared with the control group).⁴⁷ Amin et al performed a retrospective cohort study in 230 children and adolescents with CF between 1999 and 2006 and reported that 16% of these patients had persistent (≥ 2 /year) positive sputum culture for *A. fumigatus*.⁵⁰ Patients with persistent *A. fumigatus*-positive cultures had lower FEV₁ (79.2% vs 86.1% predicted in the *A. fumigatus*-negative group) at baseline, and their FEV₁ remained 3.61% predicted lower during the study period. However, it remained unclear whether these findings were independently related to *A. fumigatus*.⁵⁰ In the latter study, persistence of *A. fumigatus* in sputum was an independent risk factor (relative risk, 1.94) for pulmonary exacerbations requiring hospital admission.⁵⁰ de Boer et al also found that adult CF patients with frequent CF exacerbations had increased rates of repeated positive cultures for *A. fumigatus* in sputum,⁵² although it remains unclear whether this association was independent of other risk factors for exacerbations. Overall, these studies are consistent with the suggested proinflammatory role of *A. fumigatus* on the airway epithelium: Reihill et al reported that incubation of cultured CF airway epithelial cells with *A. fumigatus*, but not with *C. albicans*, resulted in the production of the proinflammatory cytokines IL-6 and IL-8.⁵³ However, not all studies reported an association between *A. fumigatus*-positive cultures and lung function decline. In a cross-sectional analysis of 259 children and adults with CF, de Vrankrijker et al found that subjects with repeated positive sputum cultures for *A. fumigatus* had lower lung function and higher rates of

hospitalization.¹² However, these associations were no longer significant in multivariate analysis.¹² Most importantly, the authors performed a longitudinal analysis of lung function decline in 163 patients followed for 5 years and reported that the duration of *A. fumigatus* colonization did not affect FEV₁ decline.¹² Baxter et al compared FEV₁ decline over 2 years in 33 CF patients with repeated positive detection of *A. fumigatus* in sputum by PCR with 22 CF patients without repeatedly positive PCR.⁴⁹ The authors found no difference in FEV₁ decline between the two groups.⁴⁹ Finally, McMahon et al found no difference in FEV₁ % predicted when they cross-sectionally compared 16 CF patients with repeated *A. fumigatus*-positive sputum cultures with 16 CF patients without *A. fumigatus*.⁵⁴ The authors further reported greater CT scan abnormalities in *A. fumigatus*-positive patients and suggested that FEV₁ was not sensitive enough to detect changes associated with *A. fumigatus* colonization.⁵⁴ It should be noted that an important limitation of all published studies is that they were designed to examine possible statistical associations between *A. fumigatus* colonization and clinical outcomes. Such data may provide circumstantial evidence but cannot provide causal relationship. We conclude that the relationship between *A. fumigatus* colonization, lung function decline, and/or CF exacerbation remains unclear and that definitive data on the role of *A. fumigatus* in the clinical course of disease in CF patients will require performing randomized controlled trials using oral (eg, azoles) or inhaled (eg, amphotericin B) antifungal agents. These studies should be designed to examine both microbiological end points (eg, fungus eradication or reduction in fungal load) and clinically meaningful end points (eg, lung function, exacerbations, quality of life, and imaging).

A role for azole therapy in CF patients?

The availability of oral antifungal drugs (itraconazole, voriconazole, and posaconazole) active against *A. fumigatus* has provided an opportunity to examine the role of *A. fumigatus* in CF lung disease. Initial reports focused on the possible role of azole therapy in CF patients with ABPA. Hilliard et al reported the results of an uncontrolled, open-label, retrospective review of CF patients treated with voriconazole.⁴³ These authors reported an improvement in lung function and in *A. fumigatus* serology in two children with ABPA treated with voriconazole monotherapy,⁴³ which was also described in eleven CF children with ABPA treated in combination with immunomodulatory agents. Although these data suggested possible effect of voriconazole in CF patients with ABPA,

no randomized controlled trial has evaluated the use of anti-fungal therapies for the treatment of ABPA in CF patients.⁵⁵

In recent years, several uncontrolled open-label case studies reported on the effects of azoles in patients with *A. fumigatus* colonization in the absence of ABPA. Hilliard et al reported that treatment with voriconazole had no effect on FEV₁ in eight CF children who had recurrent *A. fumigatus* isolates and increased symptoms.⁴³ Shoseyov et al used itraconazole or voriconazole in six patients who had *A. fumigatus* growing in sputum cultures, deteriorating respiratory function, and were not responding to antibacterial therapy.⁵⁶ These authors reported improvement in lung function, nutritional status, and serum IgE concentrations in most patients.⁵⁶ Coughlan et al studied 13 CF patients who were colonized with *A. fumigatus* and received 400 mg/day of itraconazole for 6 weeks. The authors reported reduced *A. fumigatus* load in airway secretions, which was associated with improvement in respiratory symptoms and decrease in CT scan abnormalities.³³ No significant change in FEV₁ was observed.³³ Aaron et al performed the only randomized control study with itraconazole in CF patients with *A. fumigatus* colonization.⁵⁷ CF subjects with chronic *A. fumigatus* colonization were randomized to receive itraconazole (5 mg/kg/day) (n=18) or placebo (n=17) and followed for 24 weeks. Although the study was terminated prematurely due to difficulties in patient recruitment, no difference was observed either in the rate of CF exacerbations (primary outcome) or in FEV₁ decline (secondary outcome). An important limitation of this study is that therapeutic itraconazole blood levels were not achieved in 43% of patients randomized to itraconazole, underscoring the absolute requirement for therapeutic drug monitoring when using azole therapy (described in the “Adverse effects of azoles” section). In conclusion, the evidence favoring the use of azoles in CF patients with *A. fumigatus* colonization or with ABPA is exclusively based on case series, and no definitive conclusion on their possible efficacy can be derived from these reports.

Adverse effects of azoles

An important aspect when examining the harm/benefit ratio of azole therapy in CF patients relates to the safety profile of these drugs. Although azole therapies are usually described as being well tolerated, several adverse effects and drug–drug interactions may occur especially during prolonged treatments.^{58–60} A summary of the main adverse effects of commercially available triazoles (ie, itraconazole, voriconazole, and posaconazole) in CF patients is presented in Table 1.

The pharmacokinetics of azole is highly variable among patients and over time in the same patient, underscoring the need for regular therapeutic drug monitoring.⁶¹ The inability to reach therapeutic concentrations could be related to drug–drug interactions. For example, the absorption of oral itraconazole and posaconazole (but not voriconazole) is promoted by acidic pH and is thus decreased by coadministration of proton pump inhibitors^{58,62} that are frequently used for the treatment of gastroesophageal reflux in CF patients. The use of rifampicin, for the eradication of methicillin-resistant *S. aureus* or nontuberculous mycobacteria, accelerates itraconazole metabolism.

Azoles exert their effects on *A. fumigatus* via the inhibition of cytochrome P450 enzymes, which are important for the biosynthesis of sterols that are vital components of fungal cell membranes.⁵⁸ A major drug interaction of azoles exists with systemic and inhaled corticosteroids. Although the interaction with prednisolone is considered minor, itraconazole increases serum concentrations of methylprednisolone and dexamethasone. Most importantly, itraconazole also increases serum concentration of the inhaled corticosteroids budesonide and fluticasone.⁵⁸ These effects could result in clinically significant Cushing syndrome with adrenal suppression,⁶³ sometimes leading to adrenal insufficiency with fatigue and/or growth failure.⁶⁴ A recent study that performed systematic synacthen testing in 12 CF patients receiving itraconazole and inhaled fluticasone found that all patients had abnormal synacthen results.⁶⁵ A recent

Table 1 Summary of main adverse effects of azoles in cystic fibrosis patients

Affected organs	Itraconazole	Voriconazole	Posaconazole
Gastrointestinal	Nausea, vomiting	Nausea, vomiting	Nausea, vomiting
Liver	Elevation of liver enzymes	Elevation of liver enzymes	Elevation of liver enzymes
Skin	None	Skin rash Photosensitivity Skin cancers	None
Cardiovascular	QT/QTc prolongation	QT/QTc prolongation	QT/QTc prolongation
Endocrine	Frequent Cushing syndrome/ adrenal suppression ^a	Cushing syndrome/ adrenal suppression ^a	Cushing syndrome/ adrenal suppression ^a

Note: ^aWhen associated with systemic and/or inhaled steroids.

report also suggested that similar interactions may occur with voriconazole⁶⁶ or posaconazole.⁶⁷ These findings are of particular relevance because ~40% of CF patients are treated with inhaled corticosteroids.⁶⁸

Voriconazole, unlike other azoles, is associated with a significant risk of skin rash and photosensitivity. In a retrospective report, Rondeau et al found that 58% of 31 CF patients treated with voriconazole exhibited photosensitivity despite the use of recommended skin protection.⁶⁹ The authors suggested that for unknown reasons, CF patients appeared more photosensitive to voriconazole than non-CF patients.⁶⁹ These findings are of particular concerns in view of the emerging description of increased risk of skin cancers associated with prolonged voriconazole treatment.⁷⁰

Finally, the use of azoles could be associated with the emergence of azole-resistant *A. fumigatus* isolates.

Emergence of azole resistance in *A. fumigatus* isolates from CF patients

During the past 15 years, azole resistance has emerged in *A. fumigatus* isolates⁷¹ and has been associated with treatment failure in subjects with aspergillosis.^{72–74} This increase in azole-resistant *A. fumigatus* isolates was suggested to occur as a consequence of prolonged therapeutic exposure to azoles

in patients treated for chronic cavitary aspergillosis⁷² or as a consequence of environmental exposure to fungicides, which are used in agriculture and have a molecular structure very similar to that of the medical triazoles.⁷⁵ Several methods for assessing resistance of *A. fumigatus* to triazoles have been developed, including culture-based methods and culture-independent methods that usually detect CYP51 mutations.⁷⁶

Until recently, the prevalence of azole resistance has remained unknown because most clinical laboratories do not routinely perform susceptibility testing of clinical *A. fumigatus* isolates in CF patients. In the past 5 years, several studies from various European countries have systematically examined the prevalence of azole-resistant isolates in CF sputum (Table 2). Although there was some variability among studies, most studies reported that ~5%–8% of *A. fumigatus* isolates obtained in CF patients were resistant to azoles. In most isolates, mutations in the CYP51A gene were observed. The most prevalent mutation was TR34/L98H, previously reported in azole-naïve patients, and ascribed to exposure of *A. fumigatus* to fungicides used in agriculture. Because many studies were performed in *A. fumigatus* isolates stored in the microbiology laboratories and contained only limited amount of clinical data, few data are available on the relationship between previous therapeutic exposure to azole and the prevalence of azole-resistant

Table 2 Summary of studies that examined azole resistance in respiratory samples from cystic fibrosis patients

Reference	Country and study type	Patients, n	Technique for assessing azole resistance	Azole-resistant strains	CYP51A mutations
Amorim et al ⁸²	Portugal Single center (Porto)	11	CLSI	0/159 isolates ^a	N/A
Bader et al ⁸³	Germany Multicenter	N/A	Etest® EUCAST CYP51A sequencing	9/163 (5.2%) isolates	TR34/L98H (n=3) M220I F219C
Burgel et al ¹⁴	France Single center (Paris)	131	Etest CLSI CYP51A PCR	6/131 (4.6%) patients 5/25 (20%) patients exposed to itraconazole within the previous 3 years	TR34/L98H (n=2) M220I M220R G54E
Fischer et al ⁹	Germany Single center (Cologne)	221	EUCAST CYP51A sequencing	6/573 (1.0%) isolates	TR34/L98H (n=4) M220L TR46/Y121F/T289A
Morio et al ⁸⁴	France Single center (Nantes)	50	EUCAST CYP51A sequencing	9/58 isolates in 4/50 (8%) patients	TR34/L98H (n=5) M220T (n=4) G54R
Mortensen et al ⁷⁷	Denmark Single center (Copenhagen)	133	EUCAST (itraconazole screening agar) CYP51A sequencing	6/133 (4.5%) patients	TR34/L98H (n=2) M220I M220K Y431C
Terpstra et al ⁸⁵	The Netherlands Multicenter	653	CYP51A PCR	59/1,072 (5.5%) isolates	TR34/L98H predominantly

Note: ^aFour of eleven patients had isolates with itraconazole MICs of 2 mg/L, indicating intermediate susceptibility to itraconazole.

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; N/A, not available; EUCAST, European Committee on Antimicrobial Susceptibility Testing; PCR, polymerase chain reaction; MICs, minimum inhibitory concentrations.

A. fumigatus isolates. Mortensen et al found that all six patients with azole-resistant *A. fumigatus* isolates had been previously treated with azoles;⁷⁷ the authors reported a trend toward patients with the TR34/L98H phenotype having less azole exposure than the patients with M220 substitutions, suggesting a role for therapeutic azole exposure in the emergence of azole-resistant isolates in these latter patients.⁷⁷ Burgel et al reported that azole-resistant *A. fumigatus* isolates were found in five of 25 (20%) subjects with prolonged exposure to itraconazole within the previous 3 years, whereas a single azole-resistant (TR34/L98H) isolate was found in 84 patients without previous therapeutic azole exposure.¹⁴ Altogether these data strongly suggest that although azole-resistant isolates could be acquired through exposure to environmental strains (eg, TR34/L98H or more recently identified TR46/Y121F/T289A),⁷⁸ prolonged treatment with azoles is associated with in vivo selection of *A. fumigatus* strains exhibiting CYP51A mutations and azole resistance. Although most data on *A. fumigatus* resistance were obtained for itraconazole, an important aspect is the emergence of cross-resistance among *A. fumigatus* isolates. For example, *A. fumigatus* isolates resistant to itraconazole have been reported to be often resistant to posaconazole¹⁴ and/or voriconazole.⁷⁹ Further, a recent study suggests that in vitro exposure of *A. fumigatus* isolates to voriconazole induces resistance to amphotericin B.⁸⁰ The emergence of azole-resistant *A. fumigatus* isolates could become a major concern in patients with CF and severe respiratory disease. Invasive aspergillosis is a frequent complication in CF patients undergoing lung transplantation, and Luong et al have recently shown that the risk of invasive aspergillosis after lung transplantation is increased when the explanted lung shows a positive intraoperative *A. fumigatus* culture.⁸¹ Because azole-resistant *A. fumigatus* isolates are associated with very high mortality (up to 88%) in patients with invasive aspergillosis,⁷⁴ we speculate that the selection of azole-resistant *A. fumigatus* isolates in the pretransplantation period may lead to untreatable invasive aspergillosis in the posttransplantation period.

Conclusion and emerging perspectives

A. fumigatus colonization and/or *A. fumigatus* sensitization with or without significant ABPA is found in a great proportion of CF patients. Although there is no doubt that ABPA is associated with airway damage and lung function decline, the effects of *A. fumigatus* colonization on lung function in the absence of ABPA are less well established. Some retrospective clinical studies found associations of

A. fumigatus colonization with CT scan abnormalities, greater risk of CF exacerbations/hospitalizations, and/or FEV₁ decline, but these findings were not replicated in all studies. The availability of a growing number of oral antifungal triazole drugs together with the results of nonrandomized case series describing some positive effects of azole therapies makes it tempting to treat CF patients with these antifungal drugs. However, the only randomized controlled trial that has used itraconazole in CF patients showed no significant benefit.⁵⁷ At this time, the best criteria for selecting patients who may benefit from azole therapy and the efficacy of this therapeutic approach have yet to be established. A recent study has suggested a novel immunologic classification of *A. fumigatus* in CF patients,¹³ but confirmation of the usefulness of this classification will require further studies. Because triazoles may have significant adverse effects and drug interactions, and because their prolonged use has been associated with the emergence of azole-resistant *A. fumigatus* isolates, it remains unclear whether CF patients benefit or not from azole therapy (Table 3). Progress in the understanding

Table 3 Summary of pros and cons of azole therapy in CF patients

Pros	Cons
ABPA and <i>Aspergillus</i> sensitization are associated with significant decline in lung function in CF patients <i>Aspergillus fumigatus</i> colonization has been associated with lung function decline and increased risk of exacerbations in some (but not all) studies Case reports and cases series have suggested beneficial role of azoles in these patients Azoles are easy to prescribe and to take because they are oral drugs	No randomized controlled trials have evaluated the effects of adding azole to systemic steroids in CF patients with ABPA and/or <i>Aspergillus</i> sensitization The only randomized controlled study evaluating the effects of itraconazole in CF patients with <i>Aspergillus</i> colonization showed no effect on exacerbation rate or FEV ₁
Azoles are usually well tolerated	Therapeutic drug monitoring shows very variable azole concentrations in serum due to poor absorption, drug interactions, and/or poor adherence ⁵⁷ Therapeutic drug monitoring appears mandatory when prescribing azoles Voriconazole exposure is associated with photosensitization and skin cancer Interaction of azoles with inhaled corticosteroids often results in adrenal insufficiency related to increase in serum steroid concentrations
Azoles may have the ability to eradicate <i>A. fumigatus</i> in CF patients	Unproven Prolonged exposure to azoles may results in azole resistance in <i>A. fumigatus</i> isolates

Abbreviations: CF, cystic fibrosis; ABPA, allergic bronchopulmonary aspergillosis; FEV₁, forced expiratory volume in 1 second.

of the role of azoles in CF patients will require carefully designed randomized controlled clinical trials.

Disclosure

The authors report no conflicts of interest in relation to this work.

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