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Respiratory Fungal Infections in Cystic Fibrosis: Diagnostic and Therapeutic Challenges

C. Schwarz[1,2] · J. G. Mainz[3,4] · E. Romanowska[1,2] · S. Rosseau[1,5,6] · H. Schuette[1,5] · L. Martin-Souto[7] · A. Ramirez-Garcia[7] · P. Eschenhagen[1,2]

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Abstract

Purpose of Review In cystic fibrosis (CF), the main focus in bronchopulmonary infections is on bacterial pathogens, as they significantly influence lung function and the exacerbation rate. In the last decade, fungal respiratory diseases have been increasingly investigated for their impact on the clinical course of people with CF. This review aims to highlight recent findings in diagnostics and therapeutic approaches in terms of fungal infections in CF.

Recent Findings We reviewed over 100 publications on fungal species in CF. Studies showed that *Aspergillus* spp. negatively impact lung function in patients with CF. A summary of these investigations showed that fungal diseases in patients with CF present as colonization, sensitization, bronchitis, pneumonia, allergic bronchopulmonary aspergillosis, and aspergilloma. Two significant fungal infections, pneumonia and bronchitis, are now well-defined, and several studies have established treatment options. The following pathogens are considered the primary cause of bronchitis, i.e., *Aspergillus* spp. and *Exophiala* spp., and of pneumonia, i.e., *Scdedosporium* spp., *Apergillus* spp., *Trichosporon* spp., and *Candida* spp. The main therapeutic innovations highlighted were real-time PCR techniques, DNA chips, and antigen-reactive T cell enrichment assay (ARTE).

Summary Respiratory fungal infections in CF are a complex task in terms of definition and therapy.

Keywords Respiratory fungal infections · Fungal bronchitis · Fungal pneumonia · Combination therapy · Susceptibility testing

Introduction

A new era for people with cystic fibrosis (pwCF) has started with the approval and use of highly effective CFTR modulator therapy. In clinically stabalized patients with increasing pulmonary function and an significantly reduced exacerbation rate, the role of bronchopulmonary exacerbations or infections needs to be discussed in a new setting, in particular with regard to fungal infections. In this context, the current challenges in diagnosis and therapy are to be adressed [1–3]. In fact, diagnosis of invasive fungal infections (IFI) is often delayed, resulting in inadequate treatment. Fortunately, several studies have been conducted regarding fungal colonization and infection of the airways in pwCF to bring light into a complex topic.

It is known that *Aspergillus fumigatus* (*Af*) for filamentous fungi and *Candida albicans* for yeasts remain by far the most common fungal species in pwCF, but the pattern of fungal species associated with CF has considerably diversified

✉ C. Schwarz
pddrschwarz@gmail.com

1 HMU-Health and Medical University, Potsdam, Germany

2 Klinikum Westbrandenburg, Division Cystic Fibrosis, CF Center Westbrandenburg, Potsdam, Germany

3 Universitätsklinikum Brandenburg, Brandenburg a. d, Havel, Germany

4 Klinikum Westbrandenburg, CF Center Westbrandenburg, Brandenburg a. d, Havel, Germany

5 Klinikum Ernst Von Bergmann, Potsdam, Germany

6 Klinikum Ernst Von Bergmann, Bad Belzig, Germany

7 Department of Immunology, Microbiology and Parasitology, University of the Basque Country (UPV/EHU), Leioa, Spain

recently [4–13]. Thus, *besides Af*, some *Scedosporium* species (*Scedosporium boydii, Scedosporium apiospermum, Scedosporium aurantiacum*, *Scedosporium minutisporium*) and the closely related species *Lomentospora prolificans* have worldwide been recognized as significant fungal pathogens in CF, potentially causing severe fungal infetions that might be in many cases difficult to treat [11, 14–16]. In addition, very rare fungal species such as *Exophiala dermatitidis*, *Arxula adeninivorans*, and *Trichosporon mycotoxinivorans* have also been described causing severe fungal pneumonia in pwCF [17–22].

In addition to fungal infections, pwCF commonly experience allergic bronchopulmonary aspergillosis (ABPA) and bronchitis [23–27]. The diagnosis and treatment of *Af*-related conditions remains a challenge in CF due to overlapping features with other disease and absence of clinical guidelines for these conditions outside of ABPA. The impact of *Aspergillus* spp. is of high importance and was evidenced in a study from Germany where presentation with a significantly lower forced expiratory volume in 1 s (FEV_1) in pwCF was associated with detection of *Aspergillus* spp. in respiratory samples. In particular, patients without chronic *Pseudomonas aeruginosa* (*Pa*) infection had a significantly lower FEV_1 in association with one or at least two positive *Aspergillus* spp. Cultures per year ($P < 0.0001$). Pulmonary exacerbations requiring antibiotic treatment were experienced by significantly more pwCF with at least two positive *Af* cultures (53.9% vs. 39.5% with no *Aspergillus* spp. diagnosis in 2016 and 73.9% vs. 59.2% in 2017; $p < 0.0001$, respectively) [5]. In addition, a current study from the USA has shown that in people with CF, TH2 inflammation based on serum absolute eosinophil counts and IgE correlated with pulmonary exacerbations requiring hospitalizations and/or intravenous antibiotics, independent of bacterial airway colonization. Furthermore, lung function decline correlated with increased IgE and serum absolute eosinophil counts [28].

Although many patients receive CFTR modulator therapy, the European Cystic Fibrosis Society (ECFS) registry data from 2018, 2019, and 2020 show no recent significant differences in proportion of pwCF with ABPA diagnosis, with 4.75% reported in 2018, 5.12% in 2019, and 4.55% in 2020, respectively [29]. Despite the increasing number of studies adressing fungal pulmonary diseases in CF, there is still a great heterogeneity in the availability of diagnostic tools and diagnostic and therapeutic guidelines [30]. In this review, the definition of fungal infection will be described as well as the diagnostic and therapeutic approaches and challenges.

In this review, both the definition of fungal infections and the diagnostic and therapeutic approaches and challenges will be described.

In general in CF, different entities or categories of fungal pulmonary diseases have been discovered [15, 17, 24, 26, 27, 31–35]. In addition to fungal airway colonization, host responses can be divided into two different groups: the allergic and the infectious groups. The allergic group comprises with sensitization, ABPA, or allergic bronchopulmonary mycosis (ABPM). The infectious group includes fungal pulmonary infection, fungal bronchitis, and aspergilloma [7, 8, 15, 26, 27, 31, 36–39] (Fig. 1).

The Challenge to Detect Fungal Species in Cystic Fibrosis

Culture-Based Methods

Until now, the most common and widely available detection method for fungal species in pwCF is the use of culture-based techniques [30]. In the past, it has already been pointed out that the comparison of data from one study to another might be hampered by the lack of standardization of mycological examination of respiratory secretions [40, 41]. For instance, some laboratories might inoculate the respiratory samples on agar slants which offer a more limited surface for fungal growth compared to agar plates. Some slow-growing or low-prevalent fungal species may therefore be missed in case of mixed fungal populations, which are common in the CF airways. Likewise, great variations may be seen from one laboratory to another in the volume and processing of the sample being analyzed, as well as in the number of culture media inoculated and in incubation time and temperature [40]. Moreover, the microbiological follow-up of CF patients is usually limited to an exhaustive bacteriological analysis of respiratory secretions, and for mycological analysis, samples are inoculated only on Sabouraud dextrose agar supplemented with antibiotics (SDA) [30, 42, 43]. The frequency of indications for excluding or diagnosing fungal species varies between countries [30]. As these differences exist, it is impossible to establish consensus definitions of chronic colonization of the airways with fungal species. Only if respiratory samples are analyzed in a regular and standardized manner to detect bacteria and fungi at the same time can chronic colonizations could be defined. In addition to the lack of standarization, differences in the population studied, in environmental exposure and in lifestyle of the patients, possibly account for part of the differences that can be seen in the prevalence of filamentous fungi from one study to another [44–46].

Nevertheless, limitations in the biological diagnosis need to be considered as attested by the recent publication from Reece et al. [47] or the comparison of current practices in clinical laboratories in the UK [48]. This was perfectly illustrated by Hong et al. [43] in Baltimore (MA, USA). During 1 year, consecutive sputa ($n = 487$) collected from outpatients were inoculated in parallel on bacteriological

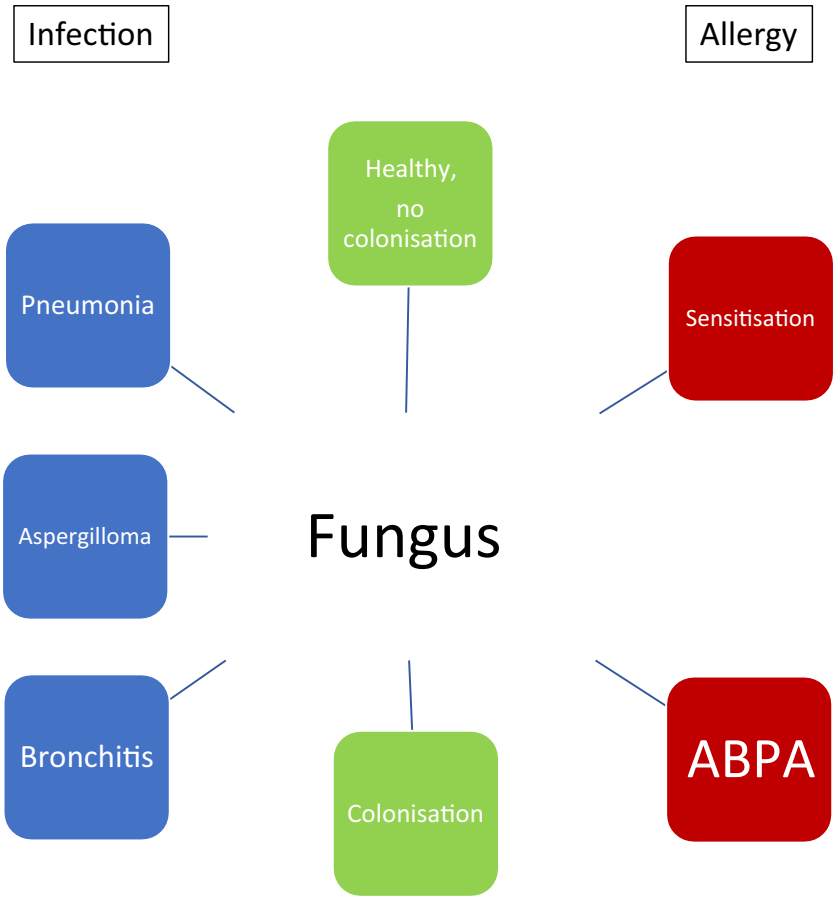

Fig. 1 Fungi can cause different disease entities in people with CF. One direction are allergic entities with fungus sensitization and allergic bronchopulmonary aslergillosis (or allergic bronchopulmonary mycosis caused by fungi other than *Aspergillus*). The second direction of fungal entity is infection with fungal bronchitis, aspergilloma, and fungal pneumonia. Between these two directions are people with CF who are not colonized with fungi and others who are only colonized with fungi

agar plates, which were incubated for 3 days at 37 °C, or on SDA, brain–heart infusion (BHI) agar plates supplemented with antibiotics and inhibitory mold agar (IMA) plates which were incubated for 2 weeks at 30 °C. Only 48 of the samples (9.8%) revealed to be positive for fungi using bacteriological media, whereas they were about 120 (from 23.8 to 24.8%) on each of the three mycological media incubated at 30 °C. Interestingly, 32% of the samples revealed the presence of fungi when combining the results obtained on SDA, BHI agar, and IMA and the prevalences of *Aspergillus* and *Scedosporium/Lomentospora* species were found to be twofold to threefold higher than their reported prevalences in the USA (40.8% vs. 20.4% and 5.2% vs. 1.9%, respectively). Likewise, only 3 out of the 20 *Scedosporium/Lomentospora* and 1 out of the 17 *Trichosporon* positive samples were detected using bacteriological media, and none of the *Exophiala* was a positive sample. Of note, samples were not digested with a mucolyticum nor sonicated or diluted before inoculation of the plates and no *Scedosporium*-selective culture media were used [43]. Similar results have been reported previously in Germany [49, 50].

Regarding the detection procedures, many countries currently have their own recommendations on detection of fungal species in respiratory samples in pwCF, which vary greatly between them [6]. Nevertheless, this needs to be updated to allow a correct overview of the fungal biota colonizing the CF lungs and airways. In addition, consensus is needed for the frequencies of testing as, for example, fungal surveillance is not routinely performed in most clinical centers in the USA [30]. Therefore, to address these challenges in detecting fungi, improved and consented guidelines for mycological examination of sputum samples from CF patients are urgently needed [51]. With this aim, two multicenter studies were conducted recently within the Fri-CF (fungal respiratory infections in cystic fibrosis) working group launched by both the European Confederation of Medical Mycology (ECMM) and the International Society for Human and Animal Mycology (ISHAM). The studies encompassed 7 CF-care centers from France for the Muco-Fong project [52] and 19 laboratories (9 from France and 4 from Italy, as well as laboratories from Spain, the UK, Belgium, Austria, Greece and Australia, one each) for the MFIP project [53]. In this study, the same procedure was used for the mycological examination of samples, including inoculation on a wide range of culture media and incubation of agar plates at two different temperatures. From the analysis of the obtained data, a combination of three to four culture media was proposed [52, 53]. In a Dutch study,

the inclusion of prior digestion of the sample with a mucolyticum, increased inoculum size, additional culture media (SDA, B + medium, Sce-Sel + and dichloran-glycerol agar), and longer incubation time (3 weeks) was tested [54], noting a marked increase in both the frequency and the diversity of molds. Nevertheless, some semi-selective culture media were not evaluated in these studies, such as dichloranrose bengal-chloramphenicol agar, the aforementioned inhibitory mold agar, or the Scedo-Select III and SceSel + culture [4, 43, 49, 55–57]. To enhance the recovery of *Exophiala dermatitidis* from sputum samples, *Burkholderia cepacia*-selective agar can also be used [58].

In fact, all growing fungi should be identified, which is not the case presently, for example, because of a lack of consideration of some fungi as potential pathogens, misidentifications using conventional methods, or limits of commercially available databases for MALDI-TOF mass spectrometry. For example, in a questionnaire survey of current practices in UK CF centers, only 7 out of the 11 respondents performed species identification for *Exophiala* species and only 2 for non-*Candida* yeasts [59]. Likewise, some molds colonizing the CF airways may be misidentified on the basis of the sole macroscopic and microscopic morphology or when using only commercially available databases for MALDI-TOF mass spectrometry. This is particularly true for *Rasamsonia* species which are frequently misidentified as *Penicillium* or *Paecilomyces* species [60–62]. Further multicenter studies comparing these innovative and new culture media with those previously selected [52, 53] and using gold-standard procedures for species identification should be conducted in order to provide evidence-based guidelines in CF.

Culture-Independent Methods

In addition to culture-based methods, molecular approaches are currently being developed based on real-time PCR techniques, or on DNA chips. One limitation of these possible new approaches will be the differenciation between infection, colonization, and transient carriage of recently inhaled fungal spores [12, 51, 63, 64]. If necessary, next-generation sequencing technique for secondary identification of the isolated fungi may be used in reference laboratories [65]. Genotyping of fungal isolates can be applied to describe transmission patterns between patients or to identify an environmental source of contamination at home of the patients, as well as to differenciate between a transient carriage of always distinct genotypes unable to establish in the respiratory tract and a true chronic colonization with the same genotype recovered by culture from successive samples.

In addition, serological tests (for detection of specific IgG antibodies) may be useful, for culture-positive patients, to differenciate an airway colonization from a respiratory infection [24, 26, 66–68]. Unfortunately, serological methods for CF-related fungi apart from *Aspergillus* and *Candida* are not commercially available.

The Growing Problem of Resistance to Antifungals

Once fungal species are detected, in vitro antifungal susceptibility testing of mold isolates is mandatory because of primary resistance of some fungal species and increasing occurrence of acquired resistance in other species. However, it is not known wether the results can be transferred from in vitro to in vivo, and there might be discrepancies, in particular in triazoles.

Analysis of transcriptomic changes induced in *S. apiospermum* in response to the particular physicochemical environment encountered by the fungus in the CF airways revealed reprogramming of genes involved in the synthesis of some important cell wall or membrane components, including genes encoding not only the glycosylphosphatidylinositol anchor and sphingolipids, but also ergosterol, with the downregulation of five of the genes involved in ergosterol biosynthesis pathway [69]. These metabolic changes could explain the discrepancies aforementioned regarding triazole drugs, since environmental conditions in CF lungs may lead to a drastic reduction in the ergosterol content of the plasma membrane, maintaining fungal growth despite the inhibition of the azole target, the 14 alpha-demethylase encoded by the gene Cyp51A. In agreement with these data, Mello et al. [70, 71] reported that cultivation of some *Scedosporium* species under 5% carbon dioxide in a medium mimicking the biochemical composition and viscosity of the CF bronchial mucus resulted in increased resistance to triazole drugs compared to reference growth conditions. In addition, this reseach group could show the influence of biofilm on resistance in *Scedosporium* species [71].

As azoles are used intensively to treat invasive and chronic aspergillosis, the likelihood for selection of resistant isolates is high. Analysis of isolates collected from respiratory samples of CF patients revealed resistance rates in cyp51A ranging from 5.3 to 13.2%. For example, a resistance rate of up to 8.2% was found for *Af* isolates collected from pwCF in Italy [72]. In the same line, in Germany, 2888 *Af* isolates from 961 CF patients were screened prospectively and 101 isolates from 51 of these patients (5.3% of the patients) were found to be azole resistant [73]. Likewise, in the UK, 167 *Af* isolates collected from 135 pwCF were investigated; resistance to at least one azole drug was confirmed in 22 out of these isolates (13.2%) [74]. Finally, during a 1-year period, similar results were reported in France and in the Netherlands. All *Af* isolates collected from pwCF in a French reference CF center were investigated prospectively; 23 out of the 355 isolates studied (6.5%) were

found to be resistant to at least one triazole drug, leading to a prevalence of 6.8% (6/88 patients) [75]. In the Netherlands, azole-resistant *Af* isolates were cultured from 7.3% (10/137) of the CF patients [76]. In patients with CF, azole-resistant *A. fumigatus* strains may be selected during the course of azole therapy, but they also from azolenaïve pwCF, in relation with the extensive use of triazole fungicides in agriculture. The acquired resistance to azole drugs in the course of azole therapy is usually due to point mutations in the cyp51A gene sequence, but analysis of resistant isolates from environmental origin might reveal other mutations such as TR46/Y121F/T289A [73, 75, 77].

The Challenge to Define and Treat Fungal Pneumonia

As bronchopulmonary infections or exacerbations in CF are usually caused by bacteria, no commonly agreed upon definition for pulmonary infections existed in the past. However, in recent years, a definition has been developed and established for fungal pulmonary infection and published by international experts in the field [11, 37, 78, 79]. This definition is as follows:

1. Increased sputum production
2. Multiple isolation of the same fungal species from sputum or bronchoalveolar lavage (≥ twice over a 6-month period)
3. Pulmonary infiltrate(s) on chest computertomography- or magnet resonace imaging or chest X-ray
4. Treatment failure by antibiotic therapy (≥ 2 × antibiotic treatment, duration ≥ 2 weeks)
5. Unclear cause for lung function decline (exclusion of newly diagnosed CF-related diseases, e.g., CF-related diabetes mellitus)
6. Exclusion of new/other bacteria (e.g., non-tuberculous mycobacteria or *Pa*)
7. Exclusion of ABPA/ABPM

Diagnosis of Fungal Pneumonia

Although this definition probably helps to identify patients with fungal pneumonia, it can still be challenging to confirm the diagnosis. CT or MRI imaging might be a very helpful tool in diagnosing fungal pneumonia. Typical findings for this specific diagnosis — as shown and described in two previous publications [15, 17] — may be ground glass density surrounding a nodule, also known as "halo sign," or consolidations with internal ground glass density. But other findings like semi-solid and ground glass nodules and, in particular, peripheral well-circumscribed nodules might also be specific hints for severe non-specific pulmonary infections in CF [80, 81]. The definitive diagnosis of IFI is by microbiology or histopathology. However, as many CF patients receive highly effective CFTR modulator therapy, less sputum is produced which makes appropriate collection of sputum samples for analysis difficult, while bronchoalveolar lavage or induced sputum is not feasible in a routine setting. For this reason, new methods to detect microbiological colonization or infections with bacteria or fungi are needed.

A novel diagnostic tool has been proposed in recent years relying on the measurement of fungus-specific host response. This antigen-reactive T cell enrichment assay (ARTE) might be useful to measure fungus-specific T cell responses during the onset of fungal infections [38, 39, 82, 83]. This method reflects the direct host–pathogen reaction, therefore identifying the relevant pathogen in usually co-colonized pwCF could be possible despite negative culture-based detection methods or PCR-based methods from low quality samples. In addition, an easier and less time-consuming diagnostic tool is the above mentioned fungus-specific IgG. In a recent publication, it could be shown that *Af*-specific IgG might be helpful in the diagnosis and treatment follow-up of *Af* entities as *Af-specific* IgG decreases after therapy [24].

Serological techniques such as galactomannan in blood bronchoalveolar lavage or other *Aspergillus* antigens measured by lateral flow devices have also helped to achieve aspergillosis diagnosis within hours with a specificity and sensitivity of more than 80%, in particular in severe, life-threatening cases [84–86]. Although not commercially available, some serological methods have recently also been prposed for *Scedosporium/Lomentospora*. Two of them based on ELISA system using a total extract or recombinant proteins as antigen [67, 87]. An additional, newly developed assay based on a rapid dot immobinding assay (DIA), which allows the detection of serum IgG against a total extract of these fungi in less than 15 min with a sensitivity and specifity of 90.48% and 79.30%, respectively [123].

Af or non-*Aspergillus* species pneumonia is a new disease entity in pwCF which is not easy to diagnose as bacteria are the main reason for infections with similar symptoms [88, 89], and it therefore needs the attention of radiologists and clinicians. Usually, the gold standard would be transbronchial biopsy of the region with infiltrate to verfy fungal growth via direct detection [90]. In CF, this intervention is usally too dangerous as pneumothorax might cause severe clinical detoriation [91].

Tratment of Fungal Pneumonia

The treatment of these infections might cause some challenges in this patient population. Infections with *Af* is usually treated with monotherapy using voriconazole, posaconazole, isavuconazole, caspofungin, micafungin, or lipidic amphotericin B [15, 92]. Susceptibility testing is

considered important if clinical response is lacking, particularly as azole resistance has recently been reported [72, 76]. However, treatment of fungal pulmonary infections in CF remains challenging when they are caused by multi-resistant fungi such as *Scedosporium* species and *L. prolificans* or the emerging *Rasamsonia* species. These fungi exhibit a primary resistance or low susceptibility to most available antifungal drugs [9, 60, 93]. In recent years, a lot of efforts have been made performing studies to address this issue. First of all, for all antifungal treatments, susceptibility testing is mandatory as resistance can occur [9, 11, 94]. Single studies on *Scedosporium/Lomentospora* infections recommend the option of combined anti-fungal therapy. A combination of two or even three different antifungals might be necessary and is therefore recommended for the initial treatment phase [11, 15, 16, 79, 95]. Ideally, it should combine an oral azole (voriconazole, posaconazole, isavuconazole), an intravenous echinocandin (e.g. caspofungin or micafungin), and inhaled lipidic amphotericin B. In *Rasamsonia* infections, micafungin would be preferred in combination, and in infections with *L prolificans,* terbinafine might be susceptible as well as miltefosine, in combination with an echinocandin and azole [11, 15, 78, 96]. In the case of *E. dermatitidis,* antifungal therapy recommendations are mainly focused on therapy with amphotericin B, flucytosine, or azoles [19, 20, 37], but susceptibility testing should also be performed initially as different resistance might occur [18, 19, 97–100]. In the rare cases caused by *T. mycotoxinivorans*, recommended treatment regimes are a combination of amphotericin B (intravenous or inhaled) and an azole as with monotherapy; however, breakthrough infections might occur [21, 101–104]. As recommended for other fungi, it is also crucial to performe susceptibility testing as resistance might occur and cause treatment failure [21, 22, 101, 102, 105].

Candida spp. are the most common yeast isolated in respiratory samples in pwCF, being isolated in a study period of 6 years in microbiology institutes from 9 CF centers in Europe with a range (in mean) of 33.8% up to 77.9% [6]. However, infections caused by this fungus are only rarely described [6, 15]. In fact, the pathogenicity of *Candida* spp. in CF and their influence on disease progression is less clearly understood than with filamentous fungi and continues to be debated. In the late 1990s, registry data from 7010 pwCF showed the association of *Candida* spp. and lower FEV_1(91), although it is unknown whether his was due to a direct effect of *Candida* spp.; an observation for its predilection for damaged pulmonary parenchyma or a finding associated with antibiotic treatment used more often in those with greater disease severity is unknown. More recently, the potential of *Candida* spp. to cause lung function decline, implicating a significant impact in CF, was also demonstrated as well as that the yeast can cause both localized and systemic infections and induce oral and genital thrush, vascular access device-related infections, and post-transplantation complications (108; 109). However, whether treatment against *Candida* spp. influences the course of disease or the drop in lung function remains unknown and needs further investigation. In the very rare cases of highly probable pulmonary infection due to *Candida* spp., the accurate identification of the infecting *Candida* species is crucial in determining which antifungal agent to use, because of the occurrence of fluconazole-resistant *Candida* species [92]. In *C. albicans* infections, it is recommended to start with an azole, preferably fluconazole, and to modify treatment if needed according to susceptibility tests. Echinocandins (e.g., caspofungin, anidulafungin, micafungin) are effective drugs for *Candida glabrata* and *Candida tropicalis* infections. Amphotericin B is also useful for *Candida* spp. infections but has the disadvantage of nephrotoxicity, hypokalemia and acute infusion-related side effects [13]. However, *Candida* spp. are still rarely identified as causing acute pulmonary infection in CF requiring treatment, and further research is needed to determine their true position as pathogenic organisms in CF.

Clinicians have to be aware that all detected fungal species in respiratory samples of pwCF should be considered as potentially pathogenic. The first in-human detection of *A. adeninivorans* in a pwCF causing a life-threatening invasive pulmonary infection underlines this hypothesis [17].

An additional important question for clinicians is whether colonization without clinical implications should be treated to prevent clinical deterioration and chronic lung damage, in particular if multi-resistant fungi such as *L. prolificans* are detected, which could be a potential contraindication for lung transplantation. IFI pose a serious threat in CF. Nevertheless, it has been shown that it is complicated to diagnose them and that early initiation of appropriate antifungal therapy is vital for a good outcome [16, 37, 48, 108–112].

The Challenge to Diagnose and Treat *Af* Bronchitis

Besides ABPA, other diseases, such as aspergilloma, invasive pulmonary aspergillosis, hypersensitivity diseases, and *Af* bronchitis, may occur [25, 26, 31, 113, 114]. *Af* bronchitis was first described in 2006 [31]. The main problem in a clinical setting is to distinguish between harmless colonization and clinically relevant bronchitis.

Aspergillus spp. or non-*Aspergillus* spp. bronchitis is defined as by *Aspergillus*-positive sputum cultures, respiratory exacerbations unresponsive to antibiotic treatment and successful antifungal treatment, as well as exclusion of ABPA [31]. Further specifications and a modified definition of *Aspergillus* bronchitis were published in 2013

already by Baxter et al. [26] including a cutoff index of > 0.5 for sputum galactomannan, positive *Af*-specific PCR, and elevated levels for specific serum IgG antibodies (75 mg/l), in combination with not elevated total serum IgE (whereas a total serum IgE level > 500 IU/ml indicates ABPA) [115] and specific serum IgE [26]. Thus, repeatedly detected *Aspergillus* spp. in sputum samples without hypersensitivity to *Aspergillus* spp. and with persistent respiratory symptoms are features of *Aspergillus* bronchitis in patients with CF [113].

In a recent study, *Aspergillus*-specific IgGm3 was evaluated in pwCF with ABPA, bronchitis and pneumonia. The results showed that *Aspergillus*-specific IgGm3 is a good individual biomarker to follow up the patient during and after treatment in order to access treatment response [24]. Once the diagnosis is confirmed, treatment should be started with an azole but susceptibility testing is always recommended [72, 76, 116, 117]. As there are no prospective studies on *Aspergillus* spp. bronchitis, the duration of treatment is unclear. In a German single center study, the duration of antifungal treatment was 4 ± 1.6 weeks with a range between 2 and 6 weeks. But the treatment in this small study significantly reduced cough ($P = 0.0067$), sputum production ($P < 0.0001$) and lung function measures ($P = 0.0358$) but did not increase physical capacity ($P = 0.0794$) [27]. In some cases, it might be indicated to continue the treatment longer, and it is not yet known which biomarker might be the most suitable to decide the duration of antifungal treatment, but as mentioned above, the Af-IgG would be a fast and low-cost marker [24].

General Challenges in the Antifungal Treatment of pwCF

CF presents some aspects different from other diseases that make it essential to adjust doses of azoles and to monitor levels in those drugs with a low volume of distribution. These following factors result in a need for potentially higher dosing and highlight the importance of therapeutic drug monitoring delayed absorption of oral antifungal drugs due to pancreatic insufficiency and fat malabsorption, increased total body volume leading to higher volume distribution with low drug concentrations, and enhanced hepatic and renal drug clearance due to hypermetabolic state [118, 119]. In addition, pwCF have usually many co-medications, particularly drugs with severe interactions [120]. Drug–drug interactions with CFTR modulators are typical, and in patients with elaxaftor, tezacaftor, and ivacaftor, the modulator therapy needs to be adjusted to a lower dosage [1, 121, 122].

Mycobiome

To understand more about fungal species in the airways, analysis of the mycobiome becomes important. With new methods such as qPCR and rRNA analyses, it is now feasible to gain data in terms of the total fungal load in particular of the lower airways.

Two metrics, α-diversity and β-diversity, are used to simplify the high-dimension data associated with microbiome analysis into a single metric. Alpha diversity describes the ecological diversity within a certain niche using the Shannon diversity index. This index includes many parameters such as the number of taxa (richness) and the distribution (evenness and dominance) of each taxon. Beta diversity is a measure of the degree of difference in membership or structure between two or more microbiota with many equations and indices existing to measure β-diversity [123, 124]. In a current study from the USA, in the bronchoalveolar lavage of people with CF, differences were observed in the abundance of *Aspergillus* and *Candida* in people with CF [125]. More studies are needed to understand the mycobiome in people with CF, in particular in terms of treatment with highly effective modulator therapy. Those studies usually focus only on bacteria such as *Pseudomonas aeruginosa* or *Staphylococcus aureus*.

Conclusion

In summary, fungal pulmonary infections are a complex task in terms of definition and therapy. But the efforts of world-wide collaborations (e.g., ECFS Fungal Pathogens Working Group and ISHAM/ECCMID Fungal Respiratory Infections in CF Group) in the last years have led to (i) new diagnostic approaches for detecting the causative fungal pathogen, (ii) new definitions of fungal pneumonia, (iii) definition for fungal bronchitis, and (iv) new recommendations for antifungal treatments. Using these established definitions for different categories of fungal diseases is now strongly recommended and should be taken into account if patients are deterioriating without responding to antibiotic treatment. Furthermore, there is a need for better data to determine predictive risk factors, causality, and biomarkers that enable monitoring and response to treatment. In addition, there is a need for more prospective and interventional studies in this area in CF.

Finally, clinicians should appreciate the findings of fungal species and should keep in mind that these fungi might cause a significant infection in pwCF that needs to be treated in an appropriate way. In response to rising concerns about antifungal restistances, very potent drugs with

fosmanogepix, ibrexafungerp, olorofim, opelconazole, and rezafungin [126] are in the pipeline. A promising drug in CF seems to be olorofim, an orotamide, which in the future will play a central role in the treatment of multi-resistant mold infections, including azole-resistant aspergillosis, *L. prolificans*, and endemic mycoses.

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