



Review

Taking a look on fungi in cystic fibrosis: More questions than answers

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ABSTRACT

Cystic fibrosis (CF) is one of the most frequent recessive inherited diseases in western countries. Advances in medical care have led to a substantial increase in the life expectancy of CF patients. Survival beyond adolescence has permitted to see fungi not only as late colonizers, but also as potential pathogens responsible of allergic reactions and chronic infections related to lung function deterioration. The role of fungi, nevertheless, has been overlooked until recently. As a result, a number of questions on their epidemiology, clinical significance, or diagnosis, among others, remain unanswered. Besides more in depth studies about the extent of the deleterious effect of fungi on the CF host, new technologies may provide the key to understand its pathogenic role, its interaction with other microbial components of the respiratory microbiota, and should pave the way to define subsets of patients at risk who would benefit from specific therapy. This review is intended to provide a quick overview on what we know about the presence of fungi in the CF airway and its repercussion in the host, and to point out some of the many knowledge gaps needed to understand and advance in the management of fungi in the airway of CF subjects.

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Una mirada a los hongos en la fibrosis quística: más preguntas que respuestas

RESUMEN

La fibrosis quística (FQ) es una de las enfermedades genéticas recesivas más frecuentes en los países occidentales. Los avances médicos han supuesto un notable incremento de la esperanza de vida de estos pacientes. La supervivencia más allá de la adolescencia ha revelado la presencia de los hongos no solo como colonizadores tardíos sino como patógenos potenciales, responsables de reacciones alérgicas e infecciones crónicas relacionadas con el deterioro de la función pulmonar. El papel de los hongos en los pacientes con FQ, no obstante, ha sido pasado por alto hasta fechas recientes. Como consecuencia, quedan por responder numerosas cuestiones sobre su epidemiología, significado clínico o diagnóstico, entre otras. Son precisos estudios en profundidad sobre el verdadero alcance del efecto deletéreo de los hongos en el paciente con FQ. Las nuevas tecnologías pueden resultar claves para entender su papel patogénico, su interacción con otros componentes de la microbiota respiratoria, y para la identificación de los subgrupos de pacientes de más riesgo que se beneficiarían particularmente de las terapias dirigidas. Esta revisión pretende proporcionar una rápida mirada a lo que sabemos actualmente sobre la presencia de los hongos en las vías respiratorias de los pacientes FQ y su repercusión en el huésped, así como señalar las múltiples lagunas de conocimiento que es necesario rellenar para entender y avanzar en el manejo de la enfermedad fúngica respiratoria de los pacientes con FQ.

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Cystic fibrosis (CF) is the most frequent autosomal recessive disease in Caucasian individuals. It ranks among the common-

est "orphan diseases", with an estimated incidence of 10–12.5/100,000 inhabitants in Europe, with a global burden that ranges between 75,000 and 90,000 patients.^{48,56} The physiopathology of this illness (nicely reviewed in Ref. 21) is related to mutations in the gen that encodes the cystic fibrosis transmembrane

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conductance regulator (CFTR), a transmembrane channel present mainly in, but not restricted to, the surface of epithelial cells.¹¹ CFTR alteration has two main consequences: it leads to an increased viscosity of secretions from mucosal surfaces, thus resulting in multi-systemic alterations, and it underlies the altered immune response of these patients. Their effects in the respiratory tract cause 90% of the morbimortality associated to this disease as hyper-viscous secretions, difficult to mobilize, entrap microorganisms that enter the airway. Microorganisms, then, multiply in a pro-inflammatory environment, contributing to potentiate the vicious circle of infection-inflammation that ultimately leads to an irreversible damage of pulmonary structure and function.

Although some ancient references to the “curse of children with salty skin” are thought to refer to cystic fibrosis, it was not until 1938 when the illness was formally described, representing the true starting point for improvement in the knowledge and management of the disease.¹⁰ This has led to a notable increase in the life expectancy of patients, reaching a median of 45 years in 2016, and with approximately 50% of CF patients in industrialized countries being currently older than 18 years.¹⁸ Their increasing lifespan is allowing us to see complex sequences of microbial colonization patterns that evolve throughout decades, and that were previously unnoticed, driving our attention to their long-term potential impact in the lung health. Whereas the role of bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* or *Burkholderia cepacia* complex has been extensively studied and is now well established,³² it remains to be defined the true pathological impact of fungi. There are such extensive gaps in our current knowledge on the subject that this review is just intended to provide a quick overview on what we know about the presence of fungi in the CF airway and its repercussion in the host. Aspects related to microbiological diagnosis of fungal-related pathology in this setting will also be covered, and areas that need to be the subject of more intensive research are summarized at the end of each section.

Fungi in the respiratory tract of CF patients: the new kid on the block?

The convergence of at least three factors may have led to overlook the role of fungi in CF until recently. First, for many years, fungi have been disregarded as contaminants or innocuous colonizers; as a consequence, they were not considered worth to mention in lab reports, making difficult to establish a connection between their presence and the development of pathology, sometimes years after the first isolation. Second, fungal cultures, provided that are ordered by clinicians, have a highly variable and clearly suboptimal yield, making difficult to consider them as a reliable kind of test for use with diagnostic or epidemiological purposes. Third, recognizing a fungal illness in the context of the CF pathological background is extremely difficult due to unavailability of guidelines and lack of robust laboratory tools. Thus, labeling an infectious episode as “fungal-related” is usually made by exclusion of other more common bacterial causes.^{7,65,68,71} In any case, when a fungus is included in lab reports, clinicians tend to feel bewildered, trying to discern to what extent this finding is meaningful for the patient today, or will be relevant in the future, particularly when unfamiliar genera, let's say *Blastobotrys*, *Thichosporon*, *Exophiala* or *Rasamsonia*, are informed. The most immediate consequence of this situation is the poor evidence existing to date on the many epidemiological aspects of fungi in CF that should be at the basis of future research strategies.

How frequent is the presence of fungi in the respiratory tract of CF patients?

Taken together, fungi are as common in the CF airway as some notorious well-known prokaryotes such as *P. aeruginosa* or *S. aureus* but, in contrast to classical bacterial pathogens, fungi are inconsistently reflected in official registries.^{2,18} Despite differences in lab procedures and study designs, positivity rates of cultures of up to 40–85% for yeasts and molds can be found in dedicated papers published from 2010 onwards.^{7,20} In the author's own experience (unpublished data), yeast, as a whole, can be intermittently isolated from respiratory samples in up to 32% of the patients aged 0–4 years. Their isolation rate increases to 53.8% in the 10–14 years age group, and they can be persistently found in at least 10% of patients in the age range of 15–24 years. Results supporting this observation have been recently communicated by Delhaes and co-workers.²⁰ *Candida* is the genus most frequently reported, and *Candida albicans* the commonest species; other ascomycetes and basidiomycetes genera can also be isolated, although much less frequently. Such predominance classically found in mycological cultures has been confirmed by means of more sophisticated molecular methods.⁴² Of interest, isolation of *C. albicans*, but no other yeast species or genera, has been reported to be significantly more frequent in patients above 15.²⁰

Molds are considered “latecomer pathobionts” in CF patients, being isolated for the first time in sputa usually at the mean age of 12.^{58,75} Reports based on culturing LBA samples, nevertheless, point toward an earlier acquisition, which may occur in the very first years of life.⁶⁶ Rates of isolation of filamentous fungi widely oscillate between 0 and 56%; interestingly, this variation depends not only on the culture technique but also on the geographical latitude.^{33,58} The true prevalence and incidence rates, however, are unknown but they are presumed to be high: from studies spanning several years and including multiple serial samples, it can be inferred that the rate of patients positive for selected molds (i.e. *Aspergillus* spp.) ranges between 40 and 50%.⁷⁷ *Aspergillus fumigatus* is almost consistently reported as the most frequent mold species. This observation has been supported by the few ultra-deep sequencing studies published to date.⁸ Other *Aspergillus* species (mainly from the *flavi* and *terrei* sections) and the *Scedosporium apiospermum* species complex usually follow in frequency. Regional differences, however, exist, and geographical and environmental factors have been pointed out as key issues to understand, at least in part, differences in epidemiology.^{20,33,42,51} Besides species commonly reported in cultures, an extensive variety of less common molds can be at least transiently found in the CF airway, as has been unveiled by molecular methods. Their clinical implication has not been defined,^{8,42} nor is known the relationship between degree of colonization and trend to develop fungal-related pathology, the environmental or host-dependent factors that increase the chance of having fungi in the airway, or the dynamics of colonization throughout life.

There remain to be answered questions such as the true prevalence of fungi in the CF airway, the chronology of species, whether the presence of fungi, either intermittent or chronic, is part of the natural microbiological history of the pathologic environment characteristically found in the CF airway, or to what extent it is shaped by the repeated exposure to antibacterials or other agents as suggested.^{43,76} Longitudinal mycobiome studies are expected to provide further insight on this point in the near future.

The CF host-fungi interplay: an indifferent neighborhood or a dangerous liaison?

Provided that the CF host has not undergone a lung transplantation procedure, and do not suffer any other condition that implies deep immunosuppression, presence of fungi in the respiratory tract of CF patients can be associated to asymptomatic colonization or to fungal-related pathology. The last, in turn, can result from a host hyperimmune response (fungal sensitization and allergic bronchopulmonary mycoses), or from a true infection with a variable degree of tissue invasion, as seen in fungal bronchitis and pneumonia. The balance between the fungus and the host response will determine the direction toward the development of any of these three situations.¹³

Colonization has been defined as the presence of a microbe in the host for a variable duration of time.¹² This term tends to be used to indicate a neutral relationship between a microbe and its host, with no specific immune response or, at least, with no deleterious effect on the host or the microbe.^{23,57,74} Nevertheless, it has been extensively used to describe the presence of fungi in the CF airway regardless of its potentially pathogenic role. Despite claimed to be frequent, the true rate of fungal colonization either intermittent or chronic in CF patients is difficult to estimate due to the lack of consistent criteria for defining it across reports, and the variable yield of the non-standardized microbiological techniques. Moreover, as for today, the occurrence of fungi is usually reported in the literature without establishing a connection with a particular clinical outcome and thus, it is commonly interpreted as “colonization”. This represents a major handicap when the long-term impact of fungal presence is to be assessed. Colonization, understood as the non-coincidental presence of a fungus in the CF airway, is related to the ability to form biofilms, and tolerate the human body temperature, but also indicates a better ability of certain fungal species or strains to adapt to the pathological conditions of the CF airway,^{6,35,38,39,41,47,52,63,73} a crucial factor common to other CF pathogens. Colonization may be the first stage of a long-term deleterious effect on the respiratory tract, as fungi may trigger an immunological reaction in a distorted pro-inflammatory background after a no well-defined period of insult, whether intermittent or continuous.⁷⁷ In fact, the presence of *A. fumigatus* in the lower respiratory tract of CF children has been associated to an increased air entrapment and worse lung function in a longitudinal study spanning 8 years.³¹ Thus, the “harmless” status commonly assigned to fungal colonizers probably should be reviewed.

Persistence of strains of some fungi throughout months to years has been proved by means of molecular techniques, opening the door to more extensive studies.^{50,64} Presence for longer than 12 months has been documented mainly for, but not restricted to, yeasts such as *C. albicans* and *Candida dubliniensis* in the very few dedicated reports published to date⁵⁰; unfortunately, no figures on the frequency of occurrence were provided nor was indicated how often patients become recolonized by the same or by other species. In the author's experience, other rare yeast species such as *Trichosporon mycotoxinivorans* and *Blastobotrys adeninivorans* are able to persist for years in the respiratory tract of colonized patients, and it has also been reported for the yeast-like fungus *Exophiala dermatitidis*.³⁰

As opposite to the wide range of transiently found molds, persistent colonization seems to be restricted to a limited number of filamentous species, mainly *A. fumigatus*, *Aspergillus terreus*, *Lomentospora prolificans*, members of the *S. apiospermum* complex and some species of the *Rasamsonia argillacea* complex.⁴⁹ For molds, colonization seems to follow two main patterns: In the first one, patients are not able to clear the fungus, leading to long-term persistence of at least one particular genotype that may be or may be not accompanied by a succession of transient genotypes; the

second pattern corresponds to patients able to clear fungi that get into the airway in succession, so species and genotypes are only sporadically found and, occasionally, are replaced by new ones.^{16,49,64} To date, it is not possible to predict whether a patient is more prone to present any of these patterns, opening a wide field of research on individual susceptibility to fungal colonization.

It is unclear whether the presence of particular bacterial co-pathogens or changes in the bacterial ecosystem driven by antibacterial treatments leads to the creation of niches that can be occupied by fungi, thus favoring colonization.⁴⁴ Moreover, although the presence of molds has been linked to a greater risk of invasive fungal infection after lung transplant, it remains to be ascertain whether, in the long term, persistent colonization of the airway poses the patient at risk of developing fungal-related pathology, or if fungal-related manifestations depend on the genetic background of the host, thus appearing even in cases of sporadic fungal presence.

Fungal sensitization (FS) is defined by the presence of type I skin reaction to fungal allergens, or an elevated serum specific IgE. Among the different types of fungal allergens, proteases are thought to play a preeminent role on the development of this pathology.³ In CF patients, FS is mostly associated to *A. fumigatus*. Although recent evidence points toward the role of other *Aspergillus* species on the etiology of this entity,⁶⁷ it is difficult to define its epidemiology and to ascertain the specific role of other fungi due to the cross reactivity of many fungal allergens,²⁶ and the lack of apparent correlation between colonization and FS.⁵ There are conflicting data on the prevalence of *A. fumigatus* FS in CF patients, although pooled data based on multinational registries and meta-analysis suggest a prevalence of at least 30–39%.^{2,46} Despite figures are affected by the method used to diagnose it, FS seems to be non-significantly more frequent in children than in adults (41.6 vs. 36.1%).⁴⁶ This has led to the suggestion that FS may precede other allergic manifestations such as allergic bronchopulmonary mycoses. Nevertheless, the time sequence has not been proved; moreover, some evidence goes against this hypothesis.⁴ In contrast, there is consensus on the association of *Aspergillus* FS and pulmonary function decline,⁴⁶ so further research on epidemiology, individual susceptibility to fungal allergens, natural history, diagnostic criteria, and management is desirable.

Allergic bronchopulmonary mycosis (ABPM) is a hypersensitivity reaction against fungi. It is commonly linked to *A. fumigatus* but, in rare cases, it can be caused by other yeasts and molds.¹⁵ Impaired clearance of fungal spores, combined with a Th2-mediated hyper-responsiveness to a sustained antigenic fungal insult, is thought to be at the base of its pathophysiology (for a detailed review on the subject, please see Ref. 36). It results in the production of a number of harmful proinflammatory mediators, as well as specific IgE and IgG, which have been claimed to be of diagnostic utility. Persistence of the inflammatory response leads to the development of epithelial damage, bronchiectasis and fibrosis. This condition has been described in a number of chronic pulmonary diseases, but is particularly frequent in CF. HLA-DRB1*1501 and HLA-DRB1*1503 genotypes, the 1082GG genotype of the IL-10 promoter, changes in conformation or affinity of surfactant protein A2, or the allele C for the TLR9 T-1237C polymorphism, among others, have been linked to an increased risk of allergic bronchopulmonary aspergillosis (ABPA), suggesting a genetic basis but, intriguingly, familiar clustering seems to be uncommon²⁹; it is unknown whether these polymorphisms also predispose to allergic pulmonary mycoses caused by non-*A. fumigatus* fungi. Figures on the prevalence of ABPA vary greatly depending on the age of the patients, and the criteria used to define it^{24,25}; up to 50% of published papers in the last 15 years use criteria other than the ones promoted by the Cystic Fibrosis Foundation in 2003, making difficult to draw conclusions about

prevalence.⁴⁶ To add more confusion, albeit current estimates indicate an ABPA prevalence of 10% in CF patients, it has been suggested that, based on serological criteria, it may affect up to 17% of CF individuals.² Frequency seems to be greater in adults than in pediatric patients, although difficulty in diagnosis may cause a delay of up to 10 years in the identification of a case, thus skewing the prevalence toward older patients.^{2,46} Non-*Aspergillus* ABPM in CF seems to be uncommon¹⁵ but it has to be ascertained to what extent the lack of published cases is, in fact, related to its rarity rather than to its difficult diagnosis. Some hallmarks of ABPM are central bronchiectasis, wheezing, new infiltrates on chest imaging, and expectoration of mucous plugs along with marked eosinophilia and elevated total IgE. Of note, differences are found between ABPM of *Aspergillus* and non-*Aspergillus* etiology: as compared to ABPA, non-*Aspergillus* ABPM has been described as having much higher total IgE titers, and a more aggressive presentation, with less than 15% fulfilling the Rosenberg–Patterson criteria, and with less association to bronchial asthma.¹⁵ Ultimately, ABPM seems to lead to a pulmonary tissue deterioration and function decline,⁴⁵ but contradictory findings have come to light recently.³⁷

A modification of current criteria for ABPA diagnosis has been proposed,¹ but it is uncertain whether the new criteria can be adapted to the diagnosis of non-*Aspergillus* ABPM.¹⁹ Probably, further modifications will be needed in the future. In summary, a considerable body of research is needed to identify those patients at higher risk of developing ABPM, even before it manifests, to design appropriate preventive measures addressed to avoid the development of chronic pulmonary damage or, at least, to identify clue points of the pathological process that can be modified via targeted therapy. Beside this, large well-designed studies are needed in order to clarify the role of steroids, immunotherapy, and antifungals in the treatment of these entities, as well as to establish urgently needed management guidelines.^{14,22,27,53,55,59}

Fungal-related bronchitis and fungal pneumonia. True fungal infections are poorly defined in CF. Some loose attempts have been made to define “infection” without making distinction between entities. These definitions usually rely on an increase of signs and symptoms not justified by new bacterial isolates, and/or by lack of response to antibacterial therapy, sometimes mentioning the repeated isolation of a mold.^{44,71} True infections may involve the bronchial wall with or without affecting the underlying lung parenchyma, and tissue invasion by hyphae is at their basis. In a recent paper, Schwarz and coworkers proposed some tentative criteria to guide the diagnosis of fungal pneumonia in this population, including recurrent isolation of the same fungal species, an otherwise unexplained decline of the pulmonary function and increased symptoms, and new infiltrates on chest imaging.⁷⁰ As for today, the most frequent scenario is the diagnosis of a possible/probable fungal infection after exclusion of other potential causes. This carries an evident delay in the establishment of an anti-fungal therapy for which there is no scientific evidence other than isolated reports. Establishing clear definitions, elaborating dedicated guidelines, defining tools for diagnosis, and setting up studies on the most appropriate therapy in each situation are, thus, imperative.

How easy is to make a diagnosis?

In fact, establishing the diagnosis of a fungal-related condition in CF is not easy at all. As is the case of fungal pathologies affecting other patients, the diagnostic armamentarium is poor, and its yield is far from well defined in the specific CF setting. Thus, documenting

a link between pathology and a particular fungus remains an elusive point.

Histological examination is rarely performed despite its potential usefulness. The range of findings that can be observed has been described elsewhere and is out of the scope of this review.¹⁵ However, as taking a biopsy may result in more harm than benefit, it is not currently considered for the diagnosis of pulmonary fungal-related pathology. The mainstay for the detection of fungi in the CF airway is the culture of bronchial secretions, despite its well-known limitations. The performance of cultures is affected by factors as the homogenization technique, volume plated, mycological media chosen, and time and temperature of incubation.⁴⁴ Despite some existing consensus on the necessity of reporting the presence of molds in respiratory samples from CF patients, no uniform opinion exists regarding yeast, with official guidelines omitting recommendations or even advising against reporting them.⁹

Ideally, culture conditions should manage to recover common yeasts that are visible in 24–48 h (i.e. *Candida*, *Trichosporon* or *Blasotobotrys*), as well as quick plate-cover molds such as *Aspergillus*, and relevant slow-growers (*Exophiala*, *Lomentospora*). This may require a combination of selective media and incubation temperatures. Unfortunately, some of the most useful media designed to recover *Exophiala* or *Scedosporium* are not commercially available. Moreover, expanding the amount of mycological media used may increase costs, hampering the general adoption of culture schemes proven to have good performance.¹⁷ Recent studies such as the Mucofong Project or the MFIP, developed under the support of the ECMM-ISHAM Fungal Infections Study Group in Cystic Fibrosis (Fri-CF) have shown the increased yield of mycological cultures performed under well standardized conditions, encouraging the adoption of uniform protocols across centers; although such protocols reach different degrees of complexity, even the most simple and affordable ones result in a clear improvement of results, thus supporting their implementation in Microbiology laboratories dealing with CF samples.^{17,20} In any case, attempts to optimize results through standardized procedures have revealed the need for protocols more elaborated than the ones commonly in use in routine labs, as well as the importance of a staff with a solid mycological background. Improved standardized lab protocols, such as the ones proposed by Coron et al. and Delhaes et al.,^{17,20} should be part of the standard of care in reference centers, although its implementation may take time, and further refinement will probably be required in order to bring them to common practice. Clinical awareness on the appropriateness of routine request for mycological investigation would also be desirable to achieve an appropriate follow-up of the clinical status and the intermittent vs. permanent presence of fungi in respiratory specimens from CF patients.

Little is known on the performance of antigen detection or the added value of biomarker assays in the routine diagnostic workup of these patients. The few studies published to date point toward a poor sensitivity and specificity of galactomannan and/or β -D-glucan detection in serum samples.^{61,72} Nevertheless, their findings are limited by the lack of longitudinal data, and by stratification based on the mere presence/absence of fungi rather than on clinical manifestations. More studies based on clinical stratification of patients are obviously needed to draw solid conclusions.

The diagnostic field is open to the introduction of new techniques. The potential usefulness of fungus-specific lymphocytes analysis by means of the antigen-specific enrichment technique in this setting seems a promising tool of help in cystic fibrosis patients,⁶⁹ although wider prospective studies are necessary to validate this approach. This may also open the door to the use of other immunological-based biomarkers to diagnose fungal related pathology in CF; a deeper knowledge on the immune response to

the many forms of fungal insult seen in these patients has to be reached before arriving to this point.

What do microbiome studies in CF have to tell us about fungi?

Although the word “microbiome” implies a combination of diverse microorganisms (i.e.: bacteria, fungi, virus, etc.) living in a dynamic community and interacting to each other,⁵⁴ our current understanding of what happens inside the respiratory tract of CF patients is partial and has resulted even in contradictory observations.^{28,62} Moreover, with few exceptions^{8,34} most studies have focused on bacteria rather than in the other components assumed to be present. As previously mentioned, reports from a limited number of patients have described the mycobiome of CF patients as dominated by *Candida* species (mainly *C. albicans*, *C. dubliniensis* and *Candida parapsilosis*), followed by the much less represented *Malassezia*⁷⁸; among molds, the dominant presence of *A. fumigatus* seems to be confirmed by molecular methods, although not in all patients.^{8,78} A wide variety of other fungal taxa seems to be transiently present or, even, to represent accidental findings arising from the environment.⁸

Further knowledge needed: the coming future

Studies tend to tackle the mycobiome from the diversity perspective, and some of them try to measure the amount of a specific genus or species present in the sample by using an additional step based on a quantitative PCR, but little is known on the interactions with other fungi or with microorganisms from other kingdoms, the influence of intrinsic or extrinsic factors on their more or less prolonged persistence, their dynamics throughout life and, more importantly, their direct impact on the host respiratory health in the long term.

Can microbiome studies help to solve the unknown on the bacteria-fungi interplay? Is the presence of fungi a long-term consequence of repeated antibiotic treatments? To what extent fungi other than *A. fumigatus* are drivers of function worsening? Which of them have the most deleterious effects, and under what circumstances do so? Probably, ITS-based studies are not appropriate enough to answer these and other questions as they provide limited and qualitative data rather than the quantitative information necessary to understand the balance between components of the microbiota.⁶⁰ It is not improbable that mycobiome studies in this population will shed some light on the need for rapid and reliable identification of specific fungal pathogens associated to deterioration, thus guiding the implementation of specific techniques. However, the information provided by pure descriptions on the mycobiome composition probably will be of limited usefulness on its own. The vast majority of microbiome-based studies just tell us about “who is there” without giving information on “what they are doing there” (transcriptomics), and “why they are doing what they do” (host-to-pathogen and pathogen-to-pathogen interactions). Complementation with information related to microbial expression profiles and the host response across time will be key to our understanding about the role of fungi in the CF airway. Metagenomics and the so-called integrative-omics tools may provide in the near future adequate tools to address these and other questions.⁶⁰ This will also pave the way to the development of diagnostic tools specifically addressed to the early identification of truly harmful fungal agents of components.

Infection is a matter of two. Very little is known on the host response to fungal presence in the airway in CF patients, a population well known to have an abnormal immune system partly related to the CFTR defect present in phagocytic cells among

others.⁴⁰ Recognition of aberrant immune responses will probably be essential to classify patients into high and low-at risk groups to develop fungal-related pathology and to drive selective therapeutic interventions only when needed. The quick expansion of new and more sophisticated technologies combined with powerful computational analysis will be of great help in this setting. Understanding the complexity of microbial communities, including fungi, and their relationship with the host, will probably give the clue on two relevant questions: what to consider deleterious and under what circumstances. Before arriving to this point, standardization of clinical and lab practices through clear and affordable guidelines is urgently needed in order to collect appropriately homogeneous information suitable for rationale analysis across centers. Such analysis is of priority interest as it will provide key information on where to invest research efforts in the most efficacious way. With this aim, the ECMM-ISHAM Fungal Respiratory Infections in Cystic Fibrosis Study Group (Fri-CF; <https://www.isham.org/working-groups/fungal-respiratory-infections-cystic-fibrosis-fri-cf>) was launched in 2006. Their members are based in countries from the five continents, and they actively work together to bring some light to the many unanswered questions on fungal-related pathology in CF, with bi-annual meetings been held, and more than 20 publications on the subject recorded in 2018 as stated in its annual report. The achievements of this free-to-join initiative supports the key idea that, although contributions of individual centers are invaluable, multicentric collaborative approaches involving professionals from diverse areas of expertise will result in greater and quicker advances on the field.

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Conflict of interests

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