Aspergillus and cystic fibrosis: old disease – new classifications

Imogen C. Felton[a,b] *and Nicholas J. Simmonds*[a,b]

Purpose of review
Aspergillus pulmonary infection has traditionally been recognized as a clinical spectrum of increasing pathogenicity, encompassing saprophytic airways colonization historically regarded of doubtful clinical significance, to allergic bronchopulmonary aspergillosis, chronic cavitatory and life-threatening invasive disease in the immunocompromised host. Whilst the latter two categories are rarely encountered in cystic fibrosis (CF), there is recognition of an extending spectrum of disease yet to be reflected in consensus management guidelines. The purpose of this review is to provide an up-to-date overview of this extending spectrum, with a focus on disease categories and their clinical significance.

Recent findings
Conflicting evidence regarding the clinical significance of *Aspergillus* colonization and sensitization in CF, alongside the emergence of a novel disease category 'Aspergillus bronchitis', has led to proposals for the reclassification of *Aspergillus* disease. In addition, lack of standardization and poor sensitivity of culture-dependent mycology techniques renders clinical and epidemiological interpretation of these isolates challenging.

Summary
The role of *Aspergillus* in the absence of established CF-allergic bronchopulmonary aspergillosis remains unclear. The following review discusses new approaches proposed to categorise the extended spectrum of CF *Aspergillus* disease, highlighting the need for enhanced microbiological investigation and serological monitoring of patients in light of evidence which differentiates colonization from categories of greater pathogenic potential.

Keywords
Aspergillus – spectrum, bronchitis, colonization, fungal biomarkers, sensitization

INTRODUCTION

The autosomal recessive condition, cystic fibrosis (CF), is the most common hereditary disease in white populations [1]. More than 1900 mutations have been identified in the gene which encodes the cystic fibrosis transmembrane conductance regulator (CFTR) protein [1,2], a chloride channel on the apical surface of many epithelial cells [3]. CFTR mutations lead to variable injury of organs in which this gene is expressed [4].

Within the lungs, abnormal chloride ion transport causes dehydration of the airway surface liquid layer [5], resulting in viscous sputum which facilitates the entrapment of airborne bacteria and fungal spores [2,6]. A rich environment for the growth of these microorganisms is created and favours repeated lower respiratory tract infection [7]. Although CF is a multisystem disorder, it is the pulmonary manifestations of this disease which cause over 90% of morbidity and mortality [1]. Whilst the acquisition of bacterial pathogens, *Pseudomonas aeruginosa* and *Burkholderia cenocepacia*, has an established negative influence on the prognosis of these patients [8,9], far less is known about the impact of chronic infection with *Aspergillus* species.

[a]Adult Cystic Fibrosis Centre, Royal Brompton and Harefield NHS Foundation Trust, Royal Brompton Hospital and [b]National Heart and Lung Institute, Imperial College London, London, UK

Correspondence to Nicholas J. Simmonds, MD (Res), MRCP, MBBS, BMed Sci, Consultant Respiratory Physician and Honorary Senior Lecturer, Adult Cystic Fibrosis Centre, Royal Brompton and Harefield NHS Foundation Trust, Royal Brompton Hospital, Sydney Street, London, SW3 6NP, UK. Tel: +44 207 351 8997; e-mail: n.simmonds@imperial.ac.uk

Curr Opin Pulm Med 2014, 20:632–638
DOI:10.1097/MCP.0000000000000106

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

KEY POINTS

- There is an extending spectrum of clinically significant CF *Aspergillus* disease in addition to ABPA.
- Dynamic shifts occur in patients between nondisease and disease-associated categories.
- The use of enhanced mycological techniques and regular serological surveillance will aid accurate clinical phenotyping according to new classification models.

ASPERGILLUS AND CYSTIC FIBROSIS

Fungi are frequently cultured from the respiratory tract of CF patients [10], and have been associated with risk factors such as increasing age [11,12], prolonged and prophylactic antibiotic exposure [12,13], and corticosteroid treatment [10,14–17]. The most frequently isolated filamentous fungal species are *Aspergillus fumigatus* and *Scedosporium apiospermum* and the major yeast, *Candida albicans* [10].

The ubiquitous, airborne conidia of *A. fumigatus* have a diameter small enough (2 to 3 μm) to reach the alveoli of the lung [18], the majority of which are cleared by the ciliary action of the airway epithelium and phagocytosed by cells of the innate immune system [19]. These host-defence mechanisms are significantly compromised in CF, thereby enabling the persistence of anergic spores to germinate by producing septate, acutely-branching, filamentous hyphae which are capable of immune activation and tissue invasion [20,21] (Fig. 1). The release of toxins and virulence factors, such as gliotoxin, proteases and melanin, has been shown to inhibit ciliary activity, damage epithelial tissue and aid biofilm formation [19,22,23].

A range of interactions occur within CF which are clinically categorized as follows:

(1) colonization,
(2) serological sensitization,
(3) allergic bronchopulmonary aspergillosis (ABPA), which is recognized as the most common *Aspergillus*-associated disorder in CF patients [4],
(4) Aspergillus bronchitis, the most recent category added to this spectrum,
(5) Aspergilloma.

The clinical significance and precise mechanisms which influence an individual's predisposition towards, and/or dynamic shifts between the range of *Aspergillus* complications are not fully understood, but interplay between prevailing 'host–pathogen' conditions is proposed to play a part.

This review article provides an erudite overview of the clinical significance of *Aspergillus* disease categories in CF, with a focus on novel microbiological detection techniques and clinical classification.

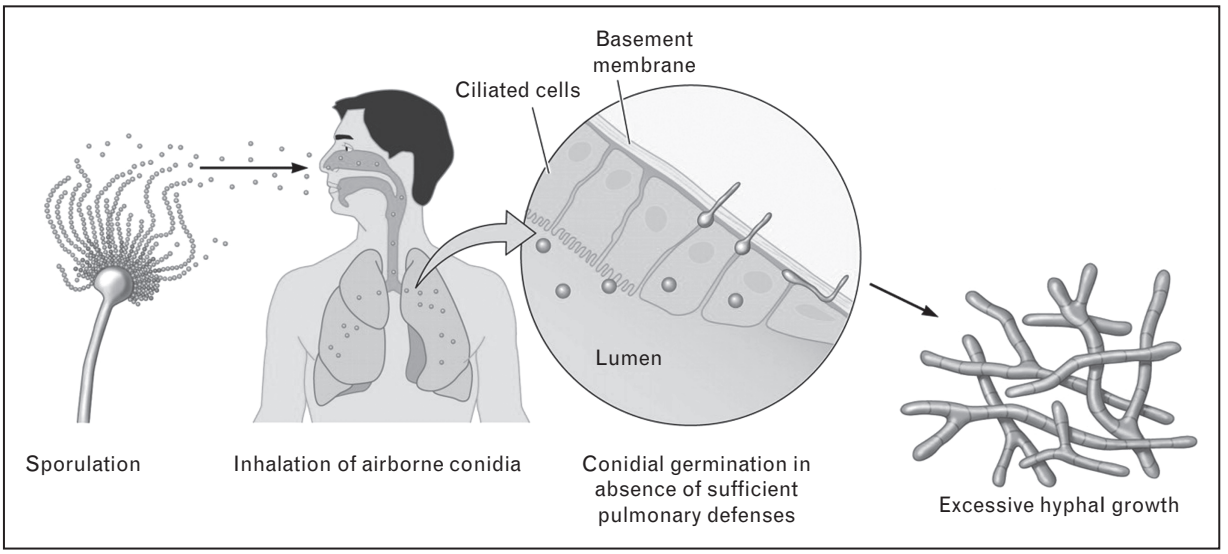

FIGURE 1. Infectious life cycle of *A. fumigatus*. Airborne *Aspergillus* conidia are ubiquitous in the environment. Inhalation may result in conidial establishment in the airway and subsequent germination. Either successful suppression by competent innate immunity pathways and sufficient airway clearance ensues with resultant significant inflammation, or uncontrolled hyphal growth with persistent immune activation and/or tissue invasion [21]. Adapted with permission from [21].

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

ASPERGILLUS DETECTION

To date, medical mycology research in CF has predominantly focused on the species *A. fumigatus* which has a prevalence of 6–60% reported in the different populations studied [10,24–32,33▪].

Conventional techniques

Recent culture-based studies have reported that both the prevalence and diversity of fungi are increasing in CF airways [10,15,17]. It is clear, however, that prevalence rates vary widely between studies. In addition to patient-associated factors and geographic variance, this has been attributed to a lack of standardized mycological methods [15,24,34]. The aim of the largest CF mycology study to date (https://www.ecfs.eu/special-projects/mucofong-international-project), incorporating 21 CF centres across Europe, has been to evaluate and confirm the detection sensitivity of different fungal culture methods, assessing selective media, extended duration and different incubation temperatures [25,35,36]. Previous studies have shown that prolonged incubation increases sample prevalence of *Aspergillus* spp. by 17% [37]. Results from this recent international study are eagerly awaited, and will inform the collation of an optimal, consensus approach for CF specimen mycology.

Molecular detection

In order to establish whether *Aspergillus* spp. have a pathogenic role in lung function decline, detection methods must first be accurate and reliable. Highly sensitive, molecular microbiology approaches such as *Aspergillus*-specific PCR assays have been developed which have highlighted and confirmed the comparative poor sensitivity of conventional culture-dependent methods [35,38,39].

The development of other molecular identification strategies has made steady progress in research laboratories over recent years. Sequencing of the internal transcribed spacer (ITS) regions, located between the 18S and 28S rRNA gene subunits of the fungal ribosomal RNA operon, has emerged as the gold standard for identification of a broad range of fungal species, enabling a non-species-biased, comprehensive illustration of the fungal microbiota present [40]. Primers designed to align in the adjacent, highly conserved small (18S), intercalary 5.8S and large subunit (28S) rRNA genes allow amplification of the entire ITS region or its component ITS1 or ITS2 regions which possess enough sequence variation to distinguish between species or species groups [41,42].

Fungal biomarkers

Highly sensitive PCR assays may well detect *Aspergillus* DNA, but this test's clinical utility may be confounded as DNA from both actively growing hyphae and inactive spores will be detected. This highlights the need for multimodal investigations of disease activity in order to draw meaningful clinical conclusions regarding the detection of *Aspergillus* spp. and the contribution towards disease progression.

As spores, the conidial forms of *A. fumigatus* are anergic to the immune system due to the presence of surface hydrophobins [43]. Upon germination however, the morphology becomes hyphal and the pathogenicity of this species is altered. Furthermore, germination is associated with the production of allergens and virulence factors such as melanin and the ability to form hydrophobic, antifungal biofilms [19,44,45]. It is the germinating conidia and hyphae, as opposed to quiescent spores, which are detected by pattern-recognition receptors on epithelial cells subsequently responsible for the production of proinflammatory cytokines and chemokines such as interleukin-6, tumor necrosis factor-alpha, and interleukin-8 [46,47]. At a mechanistic level, *A. fumigatus* toxins including proteases and the fungal secondary metabolite gliotoxin have been shown to induce airway inflammation and tissue damage *in vitro* [48] inhibiting ciliary activity [22,23].

The presence of filamentation may be a key factor in enabling continued interaction between the host and *Aspergillus* species. Galactomannan, a component in the cell wall of *Aspergillus* spp., is released during hyphal growth and not by dormant spores. In-vitro models of the lung show that galactomannan levels correspond to hyphal invasion across endothelium into alveoli [49]. With reference to the proposed new classification of *Aspergillus* disease in CF by Baxter *et al.* [50▪▪], the use of a commercially available galactomannan antigen assay in sputum differentiated patients with simple colonization and sensitization from those with a more infective spectrum of disease (discussed in more detail later in this review). However, further validation is required of this assay in CF sputum as false-positive results have been reported with bronchoalveolar lavage and serum from patients receiving intravenous beta-lactam antibiotics and the potential confounding effect of concurrent nebulized and/or oral antibiotics must be evaluated [51]. Additionally, this antigen is not specific for *Aspergillus* species, with cross-reactivity in other fungi reported [52].

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Nonetheless, these studies suggest a role for interactions exerted at different stages of fungal germination in the pathobiology of CF *Aspergillus* disease, as adherent inactive spores may play no role in disease [53]. This correlation has diagnostic potential and casts a renewed light upon more traditional pathology laboratory-staining techniques such as Grocott's methenamine silver cytology stain which after preparation with potassium hydroxide will render fungal cell walls dark brown through the Argentaffin reaction, defining fungal morphology. Similarly Calcofluor White, a fluorescence stain which highlights chitin in fungal cell walls, delineates spores from septate branching hyphae directly in sputum and may also be used in tandem alongside highly sensitive PCR-based assays as an important corollary in the clinical interpretation of *Aspergillus* species [54]. Histopathologic examination of samples may, therefore, play an important role in defining the diagnostic significance of positive culture isolates or results from PCR testing.

CLINICAL SPECTRUM OF ASPERGILLUS DISEASE IN CYSTIC FIBROSIS

The genus *Aspergillus* alone comprises almost 200 species, several of which have been associated with human mycoses. Invasive aspergillosis and chronic necrotizing pneumonia occur in the context of immunosuppression and other chronic pulmonary conditions, such as chronic obstructive pulmonary disease, but are thought to be rare in CF and so will not be addressed in this review [55].

Colonization and sensitization

The term 'colonization' implies the presence of a microorganism which provokes neither symptoms nor immunologic response; nonetheless, colonization may be viewed as a reservoir for potential outgrowth and infection.

In a CF registry study from the United States, the recovery rate of *A. fumigatus* from sputum more than doubled from 1995 to 2005 [15]. More recently, a Canadian CF registry review comparing adult and paediatric patients revealed that the overall prevalence of *Aspergillus* spp. had increased from 8% in 2001 to 18% in 2009 [56].

Sensitization in this context refers to elevated IgE-mediated responses to *Aspergillus* in the absence of the full cohort of ABPA diagnostic criteria. There are usually no discrete clinical symptoms associated with colonization or *Aspergillus* sensitization alone. In the context of CF patients who chronically experience a heavy burden of respiratory symptomatology, the clinical challenge is to decipher at which point these isolates may contribute to a progressive pathogenic burden. Recent studies have focused on defining the clinical significance of these 'innocent saprophytic bystanders', culminating in conflicting reports thus far regarding the contribution towards airway inflammation and lung function decline.

A retrospective study of Canadian paediatric patients in 2010 first provided evidence that *A. fumigatus* airways colonization may act as an independent risk factor for hospital admissions and reduced respiratory function [30]. However, this was confounded by the patients with a higher frequency of pulmonary exacerbations as they would have received more antibiotics and consequently an increased risk of fungal colonization. Accordingly, adjustment for these variables weakened the epidemiological association [30].

Furthermore, a recent prospective study of 55 adult patients in the United Kingdom provided evidence which did not support the link between *Aspergillus* colonization alone and progressive pulmonary disease. However, the association of decreased lung function parameters and *A. fumigatus* sensitization was observed [33▪,50▪▪]. Interestingly, this group found no association between *A. fumigatus* colonization and the development of serological sensitization.

Clinical interpretation of *Aspergillus* colonization and markers of serological sensitization amidst these contradictory reports remains challenging; one such difficulty is the varied definitions of clinical significance applied to colonization and serological sensitization, with variable *Aspergillus*-specific-IgE and IgG cut-off levels having been proposed [50▪▪,57,58].

At present, the optimal management of *Aspergillus* colonization is unknown as there is limited trial evidence available. A recent placebo-controlled trial of antifungal treatment reported a nonsignificant trend towards poorer lung function in the patient group receiving itraconazole compared with placebo. However, this study was hampered by failure to achieve therapeutic itraconazole levels in 43% of its patients and limited by a small study size ($n = 35$) [56].

Allergic bronchopulmonary aspergillosis

Amongst the range of mycoses associated with *Aspergillus* species, 6–25% of patients with CF develop ABPA [4] for whom there is an established association with lung function decline and unstable disease [4,59].

ABPA is caused by the exaggerated hypersensitivity reaction to potent antigens produced by a

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

variety of fungi, most frequently *A. fumigatus* in individuals with preexisting lung disease such as asthma and CF. Antigen presenting cells and T-lymphocytes of the adaptive immune system are stimulated. A Th-2 CD4+ mediated local inflammatory response results in the production of *Aspergillus*-specific IgE and IgG, causing type I and type III hypersensitivity reactions, respectively. If untreated, the persistent inflammation results in progressive bronchiectasis and fibrosis [60,61].

Because of the unreliability of distinguishing new radiological features alone on a background of established structural lung disease in CF, the characteristic immunological response in CF-ABPA is the focus of diagnostic criteria proposed by the Cystic Fibrosis Foundation Consensus Conference [62] (Table 1).

Interestingly, whilst *Aspergillus*-airway prevalence rates are reportedly increasing in CF patients, rates of ABPA remain comparatively stable. The factors leading to ABPA are not fully understood, but respiratory tract isolation of *A. fumigatus* is not considered a major criterion for diagnosis [62]. Nonetheless, detection of *Aspergillus* species may raise suspicion of other clinical syndromes within the spectrum and guide further investigations which would aid more sensitive clinical stratification and patient phenotyping.

Aspergillus bronchitis

A case series by Shoseyov *et al.* [63] in 2006 first described the syndrome 'Aspergillus bronchitis' in a cohort of six CF patients whose sputum repeatedly cultured *A. fumigatus*, without strong serological evidence of ABPA in whom progressive clinical deterioration was abated by antifungal therapy. The clinical syndrome may be characterized by marked small-airways inflammation and precipitous lung function decline in addition to the expectoration of dark, viscous sputum. More recently, this new class of *Aspergillus* disease has been defined by Chrdle *et al.* [64] as follows: first, symptomatic chronic lower airway disease (symptoms of 'chronic bronchitis'), second, detection of *Aspergillus* spp. in sputum or BAL by culture or real-time validated PCR, and third, detection of *Aspergillus*-specific IgG antibodies. The introduction of this novel category has enabled the clinical distinction between asymptomatic *Aspergillus* colonization, IgE-mediated *Aspergillus* sensitization and ABPA, facilitating a rationale for antifungal treatment of those affected.

More recently, longitudinal work by Baxter *et al.* has made a significant contribution to the understanding of this condition and proposed additional biomarkers for a new classification of the CF *Aspergillus* disease continuum (Table 2), as well as providing evidence for the dynamic shifts exhibited by individuals between disease classes over time [50▪▪]. Using the proposed new classification criteria, this group was able to identify Aspergillus bronchitis in 30% ($n = 39$) of their adult CF cohort; however, a relatively low cut-off level of significance was applied to *Aspergillus*-specific-IgG measurement (75 mg/l) which requires validation in future prospective studies [50▪▪].

In their 2-year prospective study of CF patients not receiving antifungal triazole therapy, significant lung function decline was observed in patients grouped into classes 2, 3 and 4 when compared with class 1 which was independent of baseline patient characteristics. Subsequently, they have proposed a division of pathogenicity in this spectrum with the 'nondiseased' class 1 characterized by a lack of immunologic response, negative sputum galactomannan, with or without *Aspergillus* in sputum and a 'disease-associated' *Aspergillosis*-spectrum encompassing ABPA, sensitization and Aspergillus bronchitis.

The group's use of sputum galactomannan highlights the potential utility of sputum biomarkers for

Table 1. Cystic fibrosis foundation consensus conference: ABPA in CF diagnostic criteria

	Classic Case	Minimal Criteria
1	Acute or subacute clinical deterioration and decline in pulmonary function not attributable to another cause	Acute or subacute clinical deterioration and decline in pulmonary function not attributable to another cause
2	Elevated serum IgE concentrations >1000 IU/ml	Elevated serum IgE concentrations >500 IU/ml
3	Immediate skin prick test reactivity to *Aspergillus* antigen or presence of specific IgE antibodies to *A. fumigatus*	Immediate skin prick test reactivity to *Aspergillus* antigen or presence of specific IgE antibodies to *A. fumigatus*
4	Precipitating antibodies to *A. fumigatus* or elevated serum IgG to *A. fumigatus*	And ONE of the following: a) Precipitating antibodies to *A. fumigatus* or elevated serum IgG to *A. fumigatus*, OR b) New or recent pulmonary infiltrates
5	New or recent pulmonary infiltrates	

Reproduced with permission from [62].

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Table 2. Modified table illustrating *Aspergillus* immunologic and sputum biomarker value ranges, from proposed classification criteria for spectrum of CF *Aspergillus* disease, Baxter *et al.* [50▪▪]

	Class 1 Nondisease colonization	Class 2 Serologic ABPA	Class 3 Aspergillus sensitization	Class 4 Aspergillus bronchitis
Aspergillus-IgG	Normal range	Elevated	Normal range	Elevated
Aspergillus-IgE	Normal range	Elevated	Elevated	Normal range
Total IgE	Normal range	Elevated	Elevated	Normal range
Aspergillus PCR	+/−	Positive	+/−	Positive
Sputum galactomannan	Negative	Positive	Negative	Positive

ABPA, allergic bronchopulmonary aspergillosis.
Reproduced with permission from [50▪▪].

Aspergillus-disease activity in addition to culture-independent molecular detection techniques.

Aspergilloma

Parenchymal cavities within non-CF lung disease, commonly seen as a sequelae of prior tuberculosis infection, provide sites for the aggregation of consolidated *Aspergillus* hyphae and subsequent formation of Aspergilloma. Despite their comparative infrequent occurrence in CF [55], clinical significance is manifest as episodic and sometimes large volume haemoptysis, as a consequence of hyphal angio-invasion into bronchial arteries. Predominantly detected in the upper zones on chest radiography, they may be identified by the 'air-crescent sign' on computed tomography [65]. Bronchial artery embolization and in rare situations surgical resection remain the mainstay of therapeutic and preventive management for these cases.

CONCLUSION

Although evidence supports the association of reduced lung function with *Aspergillus* sensitization in CF, this has not been definitively established for colonization [28,30,66]. Currently, antifungal treatment in this context is of unproven benefit [56]. *In-vitro* evidence of excessive virulence associated with *Aspergillus* hyphal morphology after germination serves to highlight the importance of determining the most effective monitoring and treatment strategies to reduce clinical decline in this patient population.

The proposal of defining 'nondisease' and three distinct 'disease' categories within the extended CF-*Aspergillus* spectrum serves as a timely and informative update to previously published guidelines on CF-ABPA over a decade ago [62]. Future prospective studies will validate new classification criteria and further the development of diagnostic testing with the use of highly sensitive detection assays alongside immunologic and sputum biomarkers, enabling the clinician to accurately classify these patients, thus ultimately guiding the need for treatment.

Acknowledgements

The authors would like to acknowledge the academic support of Professor Diana Bilton, Dr Michael Loebinger, Professor Miriam Moffatt and Professor William Cookson.

Conflicts of interest

The authors declare that there are no conflicts of interest relating to this article.

REFERENCES AND RECOMMENDED READING

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

1. Dodge JA, Lewis PA, Stanton M, Wilsher J. Cystic fibrosis mortality and survival in the UK: 1947–2003. Eur Respir J 2007; 29:522–526.
2. Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. N Engl J Med 2005; 352:1992–2001.
3. Boucher RC. An overview of the pathogenesis of cystic fibrosis lung disease. Adv Drug Deliv Rev 2002; 54:1359–1371.
4. Accurso FJ. Update in cystic fibrosis 2006. Am J Respir Crit Care Med 2007; 175:754–757.
5. Matsui H, Grubb BR, Tarran R, *et al.* Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. Cell 1998; 95:1005–1015.
6. Ratjen F, Doring G. Cystic fibrosis. Lancet 2003; 361:681–689.
7. O'Sullivan BP, Freedman SD. Cystic fibrosis. Lancet 2009; 373:1891–1904.
8. Jones AM, Dodd ME, Govan JR, *et al. Burkholderia cenocepacia* and *Burkholderia multivorans*: influence on survival in cystic fibrosis. Thorax 2004; 59:948–951.
9. Klepac-Ceraj V, Lemon KP, Martin TR, *et al.* Relationship between cystic fibrosis respiratory tract bacterial communities and age, genotype, antibiotics and *Pseudomonas aeruginosa*. Environ Microbiol 2010; 12: 1293–1303.
10. Pihet M, Carrere J, Cimon B, *et al.* Occurrence and relevance of filamentous fungi in respiratory secretions of patients with cystic fibrosis: a review. Med Mycol 2009; 47:387–397.
11. Saunders RMD, Claydon A, Gaillard E. Chronic A. fumigatus colonisation of the cystic fibrosis airway is common and may be associated with a more rapid decline in lung function. J Cyst Fibros 2011; 10 (Suppl 1):S37.
12. de Vrankrijker AM, van der Ent CK, van Berkhout FT, *et al. Aspergillus fumigatus* colonization in cystic fibrosis: implications for lung function? Clin Microbiol Infect 2011; 17:1381–1386.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

13. Cheer SM, Waugh J, Noble S. Inhaled tobramycin (TOBI): a review of its use in the management of *Pseudomonas aeruginosa* infections in patients with cystic fibrosis. Drugs 2003; 63:2501–2520.
14. Vandeputte P, Ferrari S, Coste AT. Antifungal resistance and new strategies to control fungal infections. Int J Microbiol 2012; 2012:713687.
15. Nagano Y, Millar BC, Johnson E, *et al.* Fungal infections in patients with cystic fibrosis. Rev Med Microbiol 2007; 18:11–16. .
16. Bargon J, Dauletbaev N, Kohler B, *et al.* Prophylactic antibiotic therapy is associated with an increased prevalence of *Aspergillus* colonization in adult cystic fibrosis patients. Respir Med 1999; 93:835–838.
17. Sibley CD, Parkins MD, Rabin HR, *et al.* A polymicrobial perspective of pulmonary infections exposes an enigmatic pathogen in cystic fibrosis patients. Proc Natl Acad Sci U S A 2008; 105:15070–15075.
18. Askew DS. *Aspergillus fumigatus*: virulence genes in a street-smart mold. Curr Opin Microbiol 2008; 11:331–337.
19. Latge J. The pathobiology of *Aspergillus fumigatus*. Trends Microbiol 2001; 9:382–389.
20. Aimanianda V, Bayry J, Bozza S, *et al.* Surface hydrophobin prevents immune recognition of airborne fungal spores. Nature 2009; 460:1117–1121.
21. Dagenais TR, Keller NP. Pathogenesis of *Aspergillus fumigatus* in invasive aspergillosis. Clin Microbiol Rev 2009; 22:447–465.
22. Amitani R, Taylor G, Elezis EN, *et al.* Purification and characterization of factors produced by *Aspergillus fumigatus* which affect human ciliated respiratory epithelium. Infect Immun 1995; 63:3266–3271.
23. Tomee JF, Wierenga AT, Hiemstra PS, Kauffman HK. Proteases from *Aspergillus fumigatus* induce release of proinflammatory cytokines and cell detachment in airway epithelial cell lines. J Infect Dis 1997; 176:300–303.
24. Masoud-Landgraf L, Badura A, Eber E, *et al.* Modified culture method detects a high diversity of fungal species in cystic fibrosis patients. Med Mycol 2014; 52:179–186.
25. Bakare N, Rickerts V, Bargon J, Just-Nubling G. Prevalence of *Aspergillus fumigatus* and other fungal species in the sputum of adult patients with cystic fibrosis. Mycoses 2003; 46:19–23.
26. Lipuma JJ. The changing microbial epidemiology in cystic fibrosis. Clin Microbiol Rev 2010; 23:299–323.
27. Nagano Y, Millar BC, Goldsmith CE, *et al.* Emergence of *Scedosporium apiospermum* in patients with cystic fibrosis. Arch Dis Child 2007; 92:607.
28. Milla CE, Wielinski CL, Regelmann WE. Clinical significance of the recovery of *Aspergillus* spp. species from the respiratory secretions of cystic fibrosis patients. Pediatr Pulmonol 1996; 21:6–10.
29. Valenza G, Tappe D, Turnwald D, *et al.* Prevalence and antimicrobial susceptibility of microorganisms isolated from sputa of patients with cystic fibrosis. J Cyst Fibros 2008; 7:123–127.
30. Amin R, Dupuis A, Aaron SD, Ratjen F. The effect of chronic infection with *Aspergillus fumigatus* on lung function and hospitalization in patients with cystic fibrosis. Chest 2010; 137:171–176.
31. Jubin V, Ranque S, Stremler Le Bel N, *et al.* Risk factors for *Aspergillus* colonization and allergic bronchopulmonary aspergillosis in children with cystic fibrosis. Pediatr Pulmonol 2010; 45:764–771.
32. Paugam ABM, Demazes-Dufeu N, Burgel PR, *et al.* Characteristics and consequences of airway colonization by filamentous fungi in 201 adult patients with cystic fibrosis in France. Med Mycol 2010; 48 (Suppl 1): S32–S36.
33. ▪ Baxter CG, Moore CB, Jones AM, *et al.* IgE-mediated immune responses and airway detection of *Aspergillus* and *Candida* in adult cystic fibrosis. Chest 2013; 143:1351–1357.

This article highlighted that *Aspergillus* sensitization and not colonization was associated with increased rates of pulmonary exacerbations and greater lung function decline in contrast to earlier studies.

34. Borman AM, Palmer MD, Delhaes L, *et al.* Lack of standardization in the procedures for mycological examination of sputum samples from CF patients: a possible cause for variations in the prevalence of filamentous fungi. Med Mycol 2010; 48 (Suppl 1):S88–S97.
35. Baxter CG, Jones AM, Webb K, Denning DW. Homogenisation of cystic fibrosis sputum by sonication: an essential step for *Aspergillus* PCR. J Microbiol Methods 2011; 85:75–81.
36. Nagano Y, Millar BC, Goldsmith CE, *et al.* Development of selective media for the isolation of yeasts and filamentous fungi from the sputum of adult patients with cystic fibrosis (CF). J Cyst Fibros 2008; 7:566–572.
37. Mortensen KL, Johansen HK, Fuursted K, *et al.* A prospective survey of *Aspergillus* spp. in respiratory tract samples: prevalence, clinical impact and antifungal susceptibility. Eur J Clin Microbiol Infect Dis 2011; 30:1355–1363.
38. Nagano Y, Elborn JS, Millar BC, *et al.* Comparison of techniques to examine the diversity of fungi in adult patients with cystic fibrosis. Med Mycol 2010; 48:166.e1–176.e1.
39. Nagano Y, Elborn JS, Millar BC, *et al.* Development of a novel PCR assay for the identification of the black yeast, *Exophiala (Wangiella) dermatitidis* from adult patients with cystic fibrosis (CF). J Cyst Fibros 2008; 7:576–580.
40. Schoch CL, Seifert KA, Huhndorf S, *et al.* Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proc Natl Acad Sci U S A 2012; 109:6241–6246.
41. Balajee SA, Sigler L, Brandt ME. DNA and the classical way: identification of medically important molds in the 21st century. Med Mycol 2007; 45:475–490.
42. Henry T, Iwen PC, Hinrichs SH. Identification of *Aspergillus* species using internal transcribed spacer regions 1 and 2. J Clin Microbiol 2000; 38:1510–1515.
43. Aimanianda V, Bayry J, Bozza S, *et al.* Surface hydrophobin prevents immune recognition of airborne fungal spores. Nature 2009; 460:1117–1121.
44. Fedorova ND, Khaldi N, Joardar VS, *et al.* Genomic islands in the pathogenic filamentous fungus *Aspergillus fumigatus*. PLoS Genet 2008; 4:e1000046.
45. Mowat E, Rajendran R, Williams C, *et al. Pseudomonas aeruginosa* and their small diffusible extracellular molecules inhibit *Aspergillus fumigatus* biofilm formation. FEMS Microbiol Lett 2010; 313:96–102.
46. Balloy V, Sallenave JM, Wu Y, *et al.* Aspergillus fumigatus-induced interleukin-8 synthesis by respiratory epithelial cells is controlled by the phosphatidylinositol 3-kinase, p38 MAPK, and ERK1/2 pathways and not by the Toll-like receptor-MyD88 pathway. J Biol Chem 2008; 283:30513–30521.
47. Bellanger AP, Millon L, Khoufache K, *et al. Aspergillus fumigatus* germ tube growth and not conidia ingestion induces expression of inflammatory mediator genes in the human lung epithelial cell line A549. J Med Microbiol 2009; 58 (Pt 2):174–179.
48. Coughlan CA, Chotirmall SH, Renwick J, *et al.* The effect of *Aspergillus fumigatus* infection on vitamin D receptor expression in cystic fibrosis. Am J Respir Crit Care Med 2012; 186:999–1007.
49. Hope WW, Kruhlak MJ, Lyman CA, *et al.* Pathogenesis of *Aspergillus fumigatus* and the kinetics of galactomannan in an in vitro model of early invasive pulmonary aspergillosis: implications for antifungal therapy. J Infect Dis 2007; 195:455–466.
50. ▪▪ Baxter CG, Dunn G, Jones AM, *et al.* Novel immunologic classification of aspergillosis in adult cystic fibrosis. J Allergy Clin Immunol 2013; 132:560–6e10.

This important prospective study utilized sputum *Aspergillus*-specific PCR testing and measurements of sputum galactomannan for the first time, in addition to immunologic markers of *Aspergillus* sensitization and infection to create a novel classification system of the extended *Aspergillus* disease spectrum in CF.

51. Boonsarngsuk V, Niyompattama A, Teosirimongkol C, Sriwanichrak K. False-positive serum and bronchoalveolar lavage *Aspergillus* galactomannan assays caused by different antibiotics. Scand J Infect Dis 2010; 42:461–468.
52. Tortorano AM, Esposto MC, Prigitano A, *et al.* Cross-reactivity of *Fusarium* spp. in the *Aspergillus* galactomannan enzyme-linked immunosorbent assay. J Clin Microbiol 2012; 50:1051–1053.
53. Chaudhary N, Datta K, Askin FB, *et al.* Cystic fibrosis transmembrane conductance regulator regulates epithelial cell response to *Aspergillus* and resultant pulmonary inflammation. Am J Respir Crit Care Med 2012; 185: 301–310.
54. Felton IC, Benson S, Alshafi K, *et al.* Evaluation of fungal diagnsotic stains and prolonged sputum culture in cystic fibrosis fungal disease. J Cyst Fibros 2014; 13 (Suppl 2):S73.
55. Chotirmall SH, Al-Alawi M, Mirkovic B, *et al. Aspergillus*-associated airway disease, inflammation, and the innate immune response. Biomed Res Int 2013; 2013:723129.
56. Aaron SD, Vandemheen KL, Freitag A, *et al.* Treatment of *Aspergillus fumigatus* in patients with cystic fibrosis: a randomized, placebo-controlled pilot study. PLoS One 2012; 7:e36077.
57. Kanthan SK, Bush A, Kemp M, Buchdahl R. Factors affecting impact of *Aspergillus fumigatus* sensitization in cystic fibrosis. Pediatr Pulmonol 2007; 42:785–793.
58. O'Driscoll BR, Powell G, Chew F, *et al.* Comparison of skin prick tests with specific serum immunoglobulin E in the diagnosis of fungal sensitization in patients with severe asthma. Clin Exp Allergy 2009; 39:1677–1683.
59. de Almeida MB, Bussamra MH, Rodrigues JC. Allergic bronchopulmonary aspergillosis in paediatric cystic fibrosis patients. Paediatr Respir Rev 2006; 7:67–72.
60. Soubani AO, Chandrasekar PH. The clinical spectrum of pulmonary aspergillosis. Chest 2002; 121:1988–1999.
61. Thia LP, Balfour Lynn IM. Diagnosing allergic bronchopulmonary aspergillosis in children with cystic fibrosis. Paediatr Respir Rev 2009; 10:37–42.
62. Stevens DA, Moss RB, Kurup VP, *et al.* Allergic bronchopulmonary aspergillosis in cystic fibrosis: state of the art: Cystic Fibrosis Foundation Consensus Conference. Clin Infect Dis 2003; 37 (Suppl 3):S225–S264.
63. Shoseyov D, Brownlee KG, Conway SP, Kerem E. Aspergillus bronchitis in cystic fibrosis. Chest 2006; 130:222–226.
64. Chrdle A, Mustakim S, Bright-Thomas RJ, *et al.* Aspergillus bronchitis without significant immunocompromise. Ann N Y Acad Sci 2012; 1272:73–85.
65. Abramson S. The air crescent sign. Radiology 2001; 218:230–232.
66. Maiz L, Cuevas M, Quirce S, *et al.* Serologic IgE immune responses against *Aspergillus fumigatus* and *Candida albicans* in patients with cystic fibrosis. Chest 2002; 121:782–788.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.