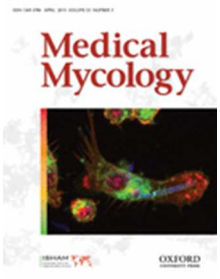

Aspergillus colonisation and antifungal immunity in cystic fibrosis patients.

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Abstract:	Cystic fibrosis (CF), caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, is the most common inherited life-limiting disease in North European people affecting 90,000 people worldwide. Progressive lung damage caused by recurrent infection and chronic airway inflammation is the major determinant of survival with a median age at death of 29 years. Approximately 60% of CF patients are infected with Aspergillus fumigatus, a ubiquitous environmental fungus, and its presence has been associated with accelerated lung function decline. Half of the patients infected with Aspergillus are <18 years of age. Yet, time of acquisition of this fungus and determinants of CF-related Aspergillus disease severity and progression are not known. CFTR expression has been demonstrated in cells of the innate and adaptive immune system and has shown to be critical for normal function. Research delineating the role of CFTR-deficient phagocytes in Aspergillus persistence and infection in the CF lung, has only recently received attention. In this concise review we aim to present the current understanding with respect to when people with CF acquire infection with A. fumigatus and antifungal immune responses by CF immune cells.

Aspergillus colonisation and antifungal immunity in cystic fibrosis patients.

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Abstract

Cystic fibrosis (CF), caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, is the most common inherited life-limiting disease in North European people affecting 90,000 people worldwide. Progressive lung damage caused by recurrent infection and chronic airway inflammation is the major determinant of survival with a median age at death of 29 years. Approximately 60% of CF patients are infected with *Aspergillus fumigatus*, a ubiquitous environmental fungus, and its presence has been associated with accelerated lung function decline. Half of the patients infected with *Aspergillus* are <18 years of age. Yet, time of acquisition of this fungus and determinants of CF-related *Aspergillus* disease severity and progression are not known.

CFTR expression has been demonstrated in cells of the innate and adaptive immune system and has shown to be critical for normal function. Research delineating the role of CFTR-deficient phagocytes in *Aspergillus* persistence and infection in the CF lung, has only recently received attention. In this concise review we aim to present the current understanding with respect to when people with CF acquire infection with *A. fumigatus* and antifungal immune responses by CF immune cells.

Introduction

In cystic fibrosis (CF), 90% of morbidity and mortality is a consequence of chronic suppurative lung disease.[1] Aggressive targeted and chronic treatment of airway infection is the mainstay of clinical management, reducing lung function decline and improving survival.[2] The introduction of CFTR modulators into clinical practice reduces (but does not abolish) pulmonary exacerbations and rate of lung function decline, and will not reverse existing lung damage (bronchiectasis). Even with new treatment modalities targeting CFTR dysfunction, pulmonary infections will continue to remain the major prognostic problem in CF. Research and treatment has conventionally focussed on the role of bacterial pathogens with little attention being paid to the role of fungal species.

Aspergillus fumigatus is a ubiquitous fungus: inhalation of *Aspergillus* spores and their airway deposition is fact of everyday life. Susceptibility to *Aspergillus*-related lung disease in [3]CF is reflected by clinical phenotypes ranging from persistent *Aspergillus* infection and bronchitis to allergic and airway invasive aspergillosis.[4] About 60% of CF patients are infected with *A. fumigatus* and this has been associated with accelerated lung function decline.[5-8] Little attention has been paid to the role of *A. fumigatus* in the pathogenesis of non-ABPA (allergic bronchopulmonary aspergillosis) *Aspergillus* lung disease in CF, despite its' frequent recovery in respiratory samples.

In vitro studies have demonstrated that CF phagocytes display reduced killing activity against typical CF bacterial pathogens such as *Pseudomonas aeruginosa* and *Burkholderia cepacia*.[9-11] Others have reported defects in a number of host immune mechanisms including the release of antimicrobial peptides, intracellular alkalization, diminished production of hypochlorous acid, and increased release of pro-inflammatory cytokines.[12-14] Only a few studies can be found in the literature addressing innate antifungal immune

responses by CFTR-defective phagocytes and epithelial cells against *A. fumigatus*.[15-17] In addition, 2 studies have addressed the persistent inflammation evoked by *A. fumigatus* (non-allergic) infection in an experimental CF murine model. [18,19]

This concise review summarizes our current understanding addressing the highly relevant questions of when people with CF acquire infection with *A. fumigatus* and why the absence of a functional CFTR protein in immune cells renders them susceptible to develop *Aspergillus* airway disease.

Aspergillus colonisation

The isolation of *Aspergillus* species, in particular *A. fumigatus*, in respiratory secretions of patients with CF is a common occurrence.[20,21] *Aspergillus fumigatus* is a ubiquitous fungus and its conidia are dispersed in the air we breathe. In patients with CF, the impaired mucociliary clearance and airways being filled with thick mucus, the inhaled *Aspergillus* conidia are easily trapped. Numerous studies have reported a high variety in prevalence rates (ranging from 3.2% to 56.7%) and this most likely reflects the differences in culture techniques, the frequency of sampling, the interpretation of the culture results and if fungal cultures are performed as routine practice in clinical management. Authors have aimed to differentiate between transient colonisation (≤ 1/yr) and persistent or chronic (≥2/0.5-1yr) colonisation, although no consensus exists for those definitions, and some studies have used even stricter definitions. Table 1 provides an overview of studies in which either results of fungal cultures were reported as a secondary outcome or in which fungal colonisation was the primary focus of the study.[6,22-57] Colonisation with *Aspergillus* species may be an apparently isolated finding, but may also be seen in the context of fungal sensitization,

ABPA or *Aspergillus* bronchitis. The majority of the studies summarized in table 1 lack information on this important aspect, and make it difficult to assess *Aspergillus* colonisation on its own as being harmful in terms of CF lung disease progression. To address this issue, a novel diagnostic classification system of *Aspergillus* infection and disease has been proposed and was consequently used to provide estimates on the prevalence of the different phenotypes of aspergillosis in CF using national and international CF registry data.[7,58] As this novel classification system was tested in a cohort of adult patients, it remains to be seen if these frequencies can be used on the total CF population as almost 50% of CF patients are ≤ 18 yrs of age. The estimated prevalences reported did not include patients colonized with *Aspergillus* in the absence of fungal sensitisation, ABPA or *Aspergillus* bronchitis.

Galactomannan and *Aspergillus* PCR on sputum samples have been proposed to improve the detection of *A. fumigatus* and *Aspergillus* disease in CF.[58] Unfortunately, no definition was proposed how those 2 markers should be included to define persistent colonisation.

Most colonisation studies have focussed on *A. fumigatus* [23-25,27,28,33-36,38,39,42,43,45-49,51-55,57], being the most prevalent *Aspergillus* species encounters in human colonisation and disease, while others have reported *Aspergillus* colonisation without defining the actual species[6,22,26,29,30,32,37,40,41,50,56,59]. Studies reporting on the relative number of *A. fumigatus* versus non-*fumigatus Aspergillus* species have shown that between 36% and 58% of *Aspergillus* colonisation in patients with CF is caused by *A. fumigatus*.[38,40-42,46] Jubin et al showed that mixed *Aspergillus* species colonisation may occur in up to 33% of the patients.[41]

Although *Aspergillus* infection and disease in CF is considered to become a problem during adolescence and adulthood, epidemiological studies about when patients with CF acquire *Aspergillus* colonisation are scarce. Epidemiological studies conducted in France, Australia

and in Greece, reported a mean age of the patients at date of first isolation of *A. fumigatus* to be 12.9 yrs, 9 yrs and 13 yrs, respectively.[33,36,49] Two more recent studies, reported an age of acquisition being 16.4 yrs (median, range 12.2-22.0 yrs) in the US[56], 13.5 yrs (mean, SD +/- 4yrs) in France[57], and 9.0 yrs (median, range 3.1-16.2 yrs) in the UK[51]. Two other studies have broken down the prevalence of *A. fumigatus* colonisation into different age categories showing colonisation rates of 16.4% up to 28% in children < 12 yrs of age.[39,43] By differentiating transient (≤ 1/yr) from persistent (≥2/yr) colonisation, Saunders et al showed that children < 12 yrs of age were only transiently colonized in a minority of the cases (21%), with the majority of children ≥ 12 yrs (59%) being persistently colonised with *Aspergillus*.[51] Cultures from BAL-fluid showed a significantly higher yield of *A. fumigatus* compared to sputum samples taken in the same period.[51] Valuable data were obtained from an Australian study in which infants, diagnosed with CF after newborn screening, underwent bronchoalveolar lavage (BAL) at the age of 3 mo, 1 yr and 2 yrs.[6] *Aspergillus* was cultured from BAL-fluid of 7 out of 56 infants (12.5%).[6] The difficulties in obtaining sputum samples from infants and young children (< 8 yrs) most likely leads to an underestimation of the prevalence of *Aspergillus* colonisation and infection in this age group, and precludes any conclusion about when patients with CF acquire *Aspergillus* infection.

Aspergillus colonisation and lung disease

Several studies have investigated the role of *Aspergillus* colonisation on lung function in CF with conflicting results. Several studies have reported either more pulmonary exacerbations requiring hospitalizations and/or significant lower lung function parameters and/or a steeper lung function decline in *Aspergillus* colonized patients.[5,42,45,55] However, other

studies do not support an independent effect of *Aspergillus* colonisation on lung function.[29,32,37,41,43,54,56]

McMahon showed that *Aspergillus* colonisation, defined as at least two sputum cultures positive for *Aspergillus* spp. at least four weeks apart in the year prior to study inclusion, resulted in more severe and significant bronchiectasis scored on HRCT-chest.[60]

Noni et al showed that paediatric patients (n=121, mean age 14 yrs) with chronic *Aspergillus* colonisation (≥2 positive sputum cultures/yr) had a lower FEV1 at baseline and a faster deterioration of their lung function in the 7 yrs after baseline.[49] In a large retrospective study using registry data from over 16,000 CF patients, lower FEV1 percent predicted was not a predictor of development of persistent *Aspergillus* colonisation.[56]

In a systematic review by Harun et al [61], which included studies describing lung function progression over age in CF patients, only 2 out of 39 publications (between 1990-2015) recognized *Aspergillus* colonisation or disease as a possible risk factor influencing lung function decline.[5,62] This reflects that *Aspergillus* infection and disease has not been recognized as playing a major role in the lung function decline in patients with CF, resulting in the absence of systematic approaches to monitor *Aspergillus* infections and disease longitudinally in CF patients from an early age onwards. A recent survey among paediatric and adult CF consultants in the UK showed that one third of the respondents considered *Aspergillus* colonisation to be potentially harmful and would therefore treat this condition.[63]

Innate fungal immunity

CFTR-defective epithelial cells

Direct experimental evidence of the interaction between CF epithelial cells and *Aspergillus* conidia is limited. However, there is evidence for the contribution of healthy epithelial cells to the innate immune response to *A. fumigatus* and for impairments in epithelial cell function in CF, from which insights can be gained. CFTR protein is highly expressed in lung epithelial cells and its loss leads to reduced airway surface hydration and impaired mucociliary transport.[64] This is significant as *Aspergillus* conidia are regularly inhaled into the airways and the mucociliary escalator is a key mechanism for preventing colonisation. Pentraxin 3 is a soluble pattern recognition receptor that is secreted b epithelial cells in response to *A. fumigatus* [65] and is important for *Aspergillus* clearance.[66] Pentraxin 3 levels have been found to be reduced in sputum from CF patients.[67]

Although not the primary phagocytes of the airway, epithelial cells have been shown in-vitro to phagocytose conidia.[68] Adhesion and internalisation of conidia has been shown to be impaired in CF bronchial epithelial cells.[15] In the same paper authors also demonstrated increased baseline and *Aspergillus*-induced apoptosis, reduced chemokine secretion and impaired killing by CF epithelial cells in-vitro. In an in-vivo murine model, they found impaired clearance of *Aspergillus* conidia and evidence of epithelial necrosis and fibrin deposition in CFTR-deficient but not wild-type mouse airways. Ceramide has been shown to accumulate in CF bronchial and alveolar epithelial cells in mice and humans.[69] In *A. fumigatus* infection, ceramide mediates inflammation and interferes with epithelial killing of the fungus. Inhibition of de-novo ceramide synthesis reduced inflammatory cytokine release, granulocyte infiltration and fungal colonisation in a murine model of *A. fumigatus* infection.[16]

CFTR-defective neutrophils

Human neutrophils express the CFTR protein in phagosomes and in the membranes of secretory vesicles and has shown to play a critical role in regulating antimicrobial neutrophil activities.[70-72] Upregulating of Toll-like receptor (TLR) 5 on human CF airway neutrophils have been described and its signalling results in excessive cytokine production after stimulation with *P. aeruginosa*.[73,74] A small number of functional studies implicate that the absence of a functional CFTR-protein in phagocytes leads to inadequate intraphagosomal chloride transport and less production of hypochlorous acid (HOCl) resulting in a diminished killing of *P. aeruginosa*.[9] A study by Pohl et al. showed that impaired CFTR function in neutrophils leads to decreased release of secondary and tertiary granules due to altered ion homeostasis that is corrected by CFTR potentiator therapy.[75]

We have recently shown that although human CF neutrophils are capable of efficiently phagocytose and kill *A. fumigatus*, this is at a cost of excessive reactive oxygen species (ROS).[17] Previous studies investigating neutrophil ROS production in vitro have shown contrasting results, with ROS production being either increased, decreased or normal in CF neutrophils.[76-78] A possible reason for this inconsistency is that this response is stimulus specific as a range of microbial and non-microbial stimuli were used in these studies. The excessive amount of ROS induced by *A. fumigatus* in CF neutrophils is significantly correlated to disease severity in terms of clinical exacerbations and lung function [Brunel 2018].[17] Our data suggest that the hyper inflammatory response by CF neutrophils upon exposure to *A. fumigatus* is likely to contribute to progressive lung disease.[17] Increased NLRP3 expression in murine cftr-/- neutrophils was demonstrated, while pNLRC4 expression was inherently lower.[19] These combined defects were shown to result in increased neutrophil recruitment and IL-1β release during *Aspergillus* infection in CF mice.[19]

CFTR-defective macrophages

Alveolar macrophages play a key role in controlling pulmonary fungal infections both through direct killing of phagocytosed pathogens and by regulating the inflammatory response generated by inhaled fungal pathogens.[79] There is evidence from work with bacteria that macrophage antimicrobial functions are impaired in CF but there is little experimental data looking at macrophage interactions with fungi in CF.

CFTR expression has been demonstrated in human monocyte-derived macrophages [11] and murine and human alveolar macrophages[80,81]. These authors have reported impaired macrophage killing of *P. aeruginosa* and have proposed impaired phagosomal acidification as the mechanism underlying this defect. However, others have challenged this hypothesis.[82] Impaired autophagy has also been reported in CF macrophages and may contribute to the killing defect towards *B. cenocepacia* as observed in one study.[10] A similar autophagy defect has been reported in lung macrophages isolated from *A. fumigatus* infected CFTR-deficient mice.[19] Recently, correction of an anti-*Pseudomonal* killing defect by treatment of CF macrophages with the CFTR corrector Lumacaftor (VX-809) was reported.[83]

Alveolar macrophages are the primary phagocytes of the airway. Failure to kill phagocytosed conidia will result in germination and fungal escape out of the cell. The hyphal form of *Aspergillus* releases proteases and gliotoxins, causing further damage to the host airway.

As well as failing to kill pathogens effectively, CF macrophages have also been observed to generate hyper-inflammatory responses to infectious stimuli. *B. cenocepacia* induced greater inflammatory cytokine release and inflammatory cell death in monocyte-derived macrophages from CF patients versus healthy controls.[10,84] Inflammatory cytokine release by CF murine and human macrophages stimulated with LPS was increased versus wild type

and healthy controls.[13] In a murine CF model of *A. fumigatus* infection, Ianitti et al observed increased activation of the NLRP3 inflammasome in CF lung macrophages versus wild type controls.[19]

CFTR-defective T-cells

The role of T cells in immunity to pulmonary aspergillosis is well recognised with Th1, Th2, Th17, Th9 and cytotoxic T cells all playing a role.[85-87] Th1 CD4+ T cells enable inflammation and fungal clearance, whilst Th17 cells are important for neutrophil recruitment, Th2 cells for allergic inflammation and Th9 responses play a role in fibrosis. Furthermore, a role for T regulatory (Treg) cells has been described in regulation of innate and adaptive responses.[88] CF lymphocyte unresponsiveness to bacterial pathogens has been long established [89], with a tendency for a pro-allergic Th2 response and increased tendency to develop allergic *Aspergillus* lung disease in patients with CF.[23,90] This is consistent with CF murine models where *A. fumigatus* challenge leads to a shift to IgE, IL-4 and IL-13 production.[91] Furthermore, T helper cells from CF patients have lower levels of interferon-ɣ production and increased IL-10 production.[92] It has been hypothesized that this is a consequence of increased calcium flux across the T cell membrane.[93] Calcium flux has been shown to be regulated by CFTR, and is crucial for T cell activation. Murine studies have specifically demonstrated that T cell receptor stimulation led to increased calcium entry in CFTR-deficient T cells, and increased translocation of the calcineurin-dependent transcription factor NFAT.[94] Thus, excessive calcium flux as a direct consequence of CFTR dysfunction in T cells may be a major contributor to dysregulated immune responses in CF.

Recent studies showed that $CFTR^{-/-}$ mice have increased innate lymphoid cell IL-9 production in response to *A. fumigatus* challenge, which is linked to expansion of Th9 T cells.[87] IL-9

neutralisation led to an improvement in lung immunopathology and fibrosis. Further studies in the murine model of pulmonary aspergillosis showed that CFTR dysfunction led to defective indoleamine 2,3-dioxygenase activity, leading to alterations in tryptophan metabolism with a subsequent imbalance of the Th17/Treg axis and excessive lung inflammation.[18] Therapeutic modulation of this pathway to enhance indoleamine 2,3-dioxygenase enabled resolution of excessive inflammation.[18] Clinical studies have also shown that the co-stimulatory molecule, OX40 ligand, is critical for driving CD4+ Th2 responses in CF patients with ABPA.[95] This correlated with low vitamin D levels in serum. Notably ex vivo therapy with vitamin D led to reduced OX40 ligand expression and reduced Th2 responses.[95] These studies indicate that a better understanding of the hyper-inflammatory T cell responses in CF may open up new immunotherapeutic avenues for the treatment of fungal-driven lung disease.

Summary and Future Directions

Aspergillus colonisation is commonly observed in patients with CF, with *A. fumigatus* most frequently encountered, and can manifest in clinically recognized CF-related *Aspergillus* diseases as ABPA and fungal sensitization. Persistent *Aspergillus* colonisation or *Aspergillus* bronchitis are not well defined and should receive more attention. Only incomplete data exists with respect to *A. fumigatus* colonisation dynamics and time of acquisition. In addition, the impact of persistent *Aspergillus* colonisation (infection) on lung function decline in CF has not been studied sufficiently. A systematic approach in which standardized fungal diagnostic measurements are applied in a longitudinal way to a large cohort of patients with CF, is urgently needed to assess the impact of persistent *Aspergillus*

colonisation. The findings of such investigations will inform clinical decision making with respect to the need of therapeutic interventions.

Treatment of persistent *Aspergillus* colonisation in the absence or presence of clinical symptoms of pulmonary exacerbation and/or a decline in lung function with antifungals has not been properly assessed. The only study which aimed to target persistent *Aspergillus* infection in patients with CF was flawed by largely underdetectable serum itraconazole concentrations.[96] Well-known adverse effects of long term azole antifungal therapies, including the rapid emerging triazole antifungal resistance, toxicity and interaction with CFTR modulators, will challenge the use of azole antifungals for persistent *Aspergillus* colonisation and infection.

Enhanced mechanistic insights in the impairments in innate antifungal immune mechanisms is warranted to identify new targets for non-allergic *Aspergillus* infections in CF patients. Currently, the observed *Aspergillus*-induced inflammatory responses by innate immune cells in both in vitro, ex vivo and experimental models of infection, seem to be a potential target to limit the pathological consequences of CF-related *Aspergillus* infection. A range of anti-inflammatories are being developed for the general treatment of CF-related airway disease.[97] It will be crucial to understand the impact both on reducing hyper-inflammatory responses as well as whether there is an effect on direct antifungal killing mechanisms. The CFTR modulators are the first causative treatment options for CF patients and have achieved significant improvement in lung function and quality of life.[98] One study showed a clear reduction in fungal colonisation in the CF lung - an effect that may be associated with the mitigation of the impaired innate immune mechanisms observed in CF immune cells.[50] In vitro work, which showed that a defective CFTR is associated with impaired degranulation of

antimicrobial proteins in neutrophils, has demonstrated that ivacaftor is able to correct degranulation and to increase bacterial killing by activation of Rab27a.[75] Another example of a small molecule based therapy targeting both the primary defect in CF as well as the aberrant inflammation and immune response, is thymosin α1. Thymosin α1 is a natural occurring polypeptide, used as an immunomodulator in viral infections, malignancies and immunodeficiencies, and has recently been shown to increase CFTR maturation and to reduce inflammation in preclinical models of disease.[99] Its mode of action seems to be the induction of IDO1 which in turns induces autophagy and favourable influencing the balance of protein folding versus degradation of the CFTR.[99]

There is considerable interest in immunotherapeutic approaches to the treatment of fungal disease.[100] Recombinant cytokines have been used extensively in transplant-related fungal disease[100], and recently successful use of interferon-gamma in CF-related fungal disease was reported.[101] Targeting the increased inflammasome activation and IL-1β release with the IL-1-receptor antagonist Anakinra, has shown promising results in pre-clinical models of disease.[19] Improved understanding of *Aspergillus* disease pathogenesis in CF patients will lead to new therapeutic targets and should eventually result in new management options.

References

1. Elborn JS. Cystic fibrosis. *Lancet*. 2016;388(10059):2519-2531.

2. Elborn JS, Bell SC, Madge SL, et al. Report of the european respiratory society/european cystic fibrosis society task force on the care of adults with cystic fibrosis. *Eur Respir J*. 2016;47(2):420-428.

3. Skov M, Koch C, Reimert CM, Poulsen LK. Diagnosis of allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis. *Allergy*. 2000;55(1):50-58.

4. King J, Brunel SF, Warris A. Aspergillus infections in cystic fibrosis. *J Infect*. 2016;72 Suppl:S50-5.

5. 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(1):171-176.

6. Ramsey KA, Ranganathan S, Park J, et al. Early respiratory infection is associated with reduced spirometry in children with cystic fibrosis. *Am J Respir Crit Care Med*. 2014;190(10):1111-1116.

7. Armstead J, Morris J, Denning DW. Multi-country estimate of different manifestations of aspergillosis in cystic fibrosis. *PLoS One*. 2014;9(6):e98502.

8. Luong ML, Chaparro C, Stephenson A, et al. Pretransplant aspergillus colonization of cystic fibrosis patients and the incidence of post-lung transplant invasive aspergillosis. *Transplantation*. 2014;97(3):351-357.

9. Painter RG, Bonvillain RW, Valentine VG, et al. The role of chloride anion and CFTR in killing of pseudomonas aeruginosa by normal and CF neutrophils. *J Leukoc Biol*. 2008;83(6):1345-1353.

10. Assani K, Tazi MF, Amer AO, Kopp BT. IFN-gamma stimulates autophagy-mediated clearance of burkholderia cenocepacia in human cystic fibrosis macrophages. *PLoS One*. 2014;9(5):e96681.

11. Del Porto P, Cifani N, Guarnieri S, et al. Dysfunctional CFTR alters the bactericidal activity of human macrophages against pseudomonas aeruginosa. *PLoS One*. 2011;6(5):e19970.

12. Painter RG, Marrero L, Lombard GA, Valentine VG, Nauseef WM, Wang G. CFTR-mediated halide transport in phagosomes of human neutrophils. *J Leukoc Biol*. 2010;87(5):933-942.

13. Bruscia EM, Zhang PX, Satoh A, et al. Abnormal trafficking and degradation of TLR4 underlie the elevated inflammatory response in cystic fibrosis. *J Immunol*. 2011;186(12):6990-6998.

14. Mueller C, Braag SA, Keeler A, Hodges C, Drumm M, Flotte TR. Lack of cystic fibrosis transmembrane conductance regulator in CD3+ lymphocytes leads to aberrant cytokine secretion and hyperinflammatory adaptive immune responses. *Am J Respir Cell Mol Biol*. 2011;44(6):922-929.

15. Chaudhary N, Datta K, Askin FB, Staab JF, Marr KA. Cystic fibrosis transmembrane conductance regulator regulates epithelial cell response to aspergillus and resultant pulmonary inflammation. *Am J Respir Crit Care Med*. 2012;185(3):301-310.

16. Caretti A, Torelli R, Perdoni F, et al. Inhibition of ceramide de novo synthesis by myriocin produces the double effect of reducing pathological inflammation and exerting antifungal activity against A. fumigatus airways infection. *Biochim Biophys Acta*. 2016;1860(6):1089-1097.

17. Brunel SF, Willment JA, Brown GD, Devereux G, Warris A. Aspergillus-induced superoxide production by cystic fibrosis phagocytes is associated with disease severity. *ERJ Open Res*. 2018;4(2):10.1183/23120541.00068-2017. eCollection 2018 Apr.

18. Iannitti RG, Carvalho A, Cunha C, et al. Th17/treg imbalance in murine cystic fibrosis is linked to indoleamine 2,3-dioxygenase deficiency but corrected by kynurenines. *Am J Respir Crit Care Med*. 2013;187(6):609-620.

19. Iannitti RG, Napolioni V, Oikonomou V, et al. IL-1 receptor antagonist ameliorates inflammasome-dependent inflammation in murine and human cystic fibrosis. *Nat Commun*. 2016;7:10791.

20. Lipuma JJ. The changing microbial epidemiology in cystic fibrosis. *Clin Microbiol Rev*. 2010;23(2):299-323.

21. Liu JC, Modha DE, Gaillard EA. What is the clinical significance of filamentous fungi positive sputum cultures in patients with cystic fibrosis? *J Cyst Fibros*. 2013;12(3):187-193.

22. Nelson LA, Callerame ML, Schwartz RH. Aspergillosis and atopy in cystic fibrosis. *Am Rev Respir Dis*. 1979;120(4):863-873.

23. Laufer P, Fink JN, Bruns WT, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis. *J Allergy Clin Immunol*. 1984;73(1 Pt 1):44-48.

24. Bauernfeind A, Bertele RM, Harms K, et al. Qualitative and quantitative microbiological analysis of sputa of 102 patients with cystic fibrosis. *Infection*. 1987;15(4):270-277.

25. Mroueh S, Spock A. Allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *Chest*. 1994;105(1):32-36.

26. Flume PA, Egan TM, Paradowski LJ, Detterbeck FC, Thompson JT, Yankaskas JR. Infectious complications of lung transplantation. impact of cystic fibrosis. *Am J Respir Crit Care Med*. 1994;149(6):1601-1607.

27. 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(4):387-397.

28. Becker JW, Burke W, McDonald G, Greenberger PA, Henderson WR, Aitken ML. Prevalence of allergic bronchopulmonary aspergillosis and atopy in adult patients with cystic fibrosis. *Chest*. 1996;109(6):1536-1540.

29. Milla CE, Wielinski CL, Regelmann WE. Clinical significance of the recovery of aspergillus species from the respiratory secretions of cystic fibrosis patients. *Pediatr Pulmonol*. 1996;21(1):6-10.

30. Burns JL, Emerson J, Stapp JR, et al. Microbiology of sputum from patients at cystic fibrosis centers in the united states. *Clin Infect Dis*. 1998;27(1):158-163.

31. Burns JL, Van Dalfsen JM, Shawar RM, et al. Effect of chronic intermittent administration of inhaled tobramycin on respiratory microbial flora in patients with cystic fibrosis. *J Infect Dis*. 1999;179(5):1190-1196.

32. Bargon J, Dauletbaev N, Kohler B, Wolf M, Posselt HG, Wagner TO. Prophylactic antibiotic therapy is associated with an increased prevalence of aspergillus colonization in adult cystic fibrosis patients. *Respir Med*. 1999;93(11):835-838.

33. Cimon B, Carrere J, Vinatier JF, Chazalette JP, Chabasse D, Bouchara JP. Clinical significance of scedosporium apiospermum in patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis*. 2000;19(1):53-56.

34. Hodson ME, Gallagher CG, Govan JR. A randomised clinical trial of nebulised tobramycin or colistin in cystic fibrosis. *Eur Respir J*. 2002;20(3):658-664.

35. 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(1-2):19-23.

36. Skov M, McKay K, Koch C, Cooper PJ. Prevalence of allergic bronchopulmonary aspergillosis in cystic fibrosis in an area with a high frequency of atopy. *Respir Med*. 2005;99(7):887-893.

37. Chotirmall SH, Branagan P, Gunaratnam C, McElvaney NG. Aspergillus/allergic bronchopulmonary aspergillosis in an irish cystic fibrosis population: A diagnostically challenging entity. *Respir Care*. 2008;53(8):1035-1041.

38. 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(2):123-127.

39. Paugam A, Baixench MT, Demazes-Dufeu N, 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:S32-6.

40. 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(1):166-76.e1.

41. Jubin V, Ranque S, Stremler Le Bel N, Sarles J, Dubus JC. Risk factors for aspergillus colonization and allergic bronchopulmonary aspergillosis in children with cystic fibrosis. *Pediatr Pulmonol*. 2010;45(8):764-771.

42. Sudfeld CR, Dasenbrook EC, Merz WG, Carroll KC, Boyle MP. Prevalence and risk factors for recovery of filamentous fungi in individuals with cystic fibrosis. *J Cyst Fibros*. 2010;9(2):110-116.

43. 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(9):1381-1386.

44. Wainwright CE, Vidmar S, Armstrong DS, et al. Effect of bronchoalveolar lavage-directed therapy on pseudomonas aeruginosa infection and structural lung injury in children with cystic fibrosis: A randomized trial. *JAMA*. 2011;306(2):163-171.

45. Fillaux J, Bremont F, Murris M, et al. Assessment of aspergillus sensitization or persistent carriage as a factor in lung function impairment in cystic fibrosis patients. *Scand J Infect Dis*. 2012;44(11):842-847.

46. Gungor O, Tamay Z, Guler N, Erturan Z. Frequency of fungi in respiratory samples from turkish cystic fibrosis patients. *Mycoses*. 2013;56(2):123-129.

47. Fillaux J, Bremont F, Murris M, et al. Aspergillus sensitization or carriage in cystic fibrosis patients. *Pediatr Infect Dis J*. 2014;33(7):680-686.

48. Masoud-Landgraf L, Badura A, Eber E, Feierl G, Marth E, Buzina W. Modified culture method detects a high diversity of fungal species in cystic fibrosis patients. *Med Mycol*. 2014;52(2):179-186.

49. Noni M, Katelari A, Dimopoulos G, Doudounakis SE, Tzoumaka-Bakoula C, Spoulou V. Aspergillus fumigatus chronic colonization and lung function decline in cystic fibrosis may have a two-way relationship. *Eur J Clin Microbiol Infect Dis*. 2015;34(11):2235-2241.

50. Heltshe SL, Mayer-Hamblett N, Burns JL, et al. Pseudomonas aeruginosa in cystic fibrosis patients with G551D-CFTR treated with ivacaftor. *Clin Infect Dis*. 2015;60(5):703-712.

51. Saunders RV, Modha DE, Claydon A, Gaillard EA. Chronic aspergillus fumigatus colonization of the pediatric cystic fibrosis airway is common and may be associated with a more rapid decline in lung function. *Med Mycol*. 2016;54(5):537-543.

52. Mirkovic B, Lavelle GM, Azim AA, et al. The basophil surface marker CD203c identifies aspergillus species sensitization in patients with cystic fibrosis. *J Allergy Clin Immunol*. 2016;137(2):436-443.e9.

53. Gernez Y, Waters J, Mirkovic B, et al. Blood basophil activation is a reliable biomarker of allergic bronchopulmonary aspergillosis in cystic fibrosis. *Eur Respir J*. 2016;47(1):177-185.

54. Reece E, Segurado R, Jackson A, McClean S, Renwick J, Greally P. Co-colonisation with aspergillus fumigatus and pseudomonas aeruginosa is associated with poorer health in cystic fibrosis patients: An irish registry analysis. *BMC Pulm Med*. 2017;17(1):70-017-0416-4.

55. Brandt C, Roehmel J, Rickerts V, Melichar V, Niemann N, Schwarz C. Aspergillus bronchitis in patients with cystic fibrosis. *Mycopathologia*. 2018;183(1):61-69.

56. Hong G, Psoter KJ, Jennings MT, et al. Risk factors for persistent aspergillus respiratory isolation in cystic fibrosis. *J Cyst Fibros*. 2018.

57. Coron N, Pihet M, Frealle E, et al. Toward the standardization of mycological examination of sputum samples in cystic fibrosis: Results from a french multicenter prospective study. *Mycopathologia*. 2018;183(1):101-117.

58. Baxter CG, Dunn G, Jones AM, et al. Novel immunologic classification of aspergillosis in adult cystic fibrosis. *J Allergy Clin Immunol*. 2013;132(3):560-566.e10.

59. Wainwright CE, Quittner AL, Geller DE, et al. Aztreonam for inhalation solution (AZLI) in patients with cystic fibrosis, mild lung impairment, and P. aeruginosa. *J Cyst Fibros*. 2011;10(4):234-242.

60. McMahon MA, Chotirmall SH, McCullagh B, Branagan P, McElvaney NG, Logan PM. Radiological abnormalities associated with aspergillus colonization in a cystic fibrosis population. *Eur J Radiol*. 2012;81(3):e197-202.

61. Harun SN, Wainwright C, Klein K, Hennig S. A systematic review of studies examining the rate of lung function decline in patients with cystic fibrosis. *Paediatr Respir Rev*. 2016;20:55-66.

62. Mastella G, Rainisio M, Harms HK, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis. A european epidemiological study. epidemiologic registry of cystic fibrosis. *Eur Respir J*. 2000;16(3):464-471.

63. Boyle M, Moore JE, Whitehouse JL, Bilton D, Downey DG. The diagnosis and management of respiratory tract fungal infection in cystic fibrosis: A UK survey of current practice. *Med Mycol*. 2018.

64. Boucher RC. Airway surface dehydration in cystic fibrosis: Pathogenesis and therapy. *Annu Rev Med*. 2007;58:157-170.

65. Chen F, Zhang C, Jia X, et al. Transcriptome profiles of human lung epithelial cells A549 interacting with aspergillus fumigatus by RNA-seq. *PLoS One*. 2015;10(8):e0135720.

66. Garlanda C, Hirsch E, Bozza S, et al. Non-redundant role of the long pentraxin PTX3 in anti-fungal innate immune response. *Nature*. 2002;420(6912):182-186.

67. Hamon Y, Jaillon S, Person C, et al. Proteolytic cleavage of the long pentraxin PTX3 in the airways of cystic fibrosis patients. *Innate Immun*. 2013;19(6):611-622.

68. Gomez P, Hackett TL, Moore MM, Knight DA, Tebbutt SJ. Functional genomics of human bronchial epithelial cells directly interacting with conidia of aspergillus fumigatus. *BMC Genomics*. 2010;11:358-2164-11-358.

69. Teichgraber V, Ulrich M, Endlich N, et al. Ceramide accumulation mediates inflammation, cell death and infection susceptibility in cystic fibrosis. *Nat Med*. 2008;14(4):382-391.

70. Painter RG, Valentine VG, Lanson NA,Jr, et al. CFTR expression in human neutrophils and the phagolysosomal chlorination defect in cystic fibrosis. *Biochemistry*. 2006;45(34):10260-10269.

71. Bonvillain RW, Painter RG, Adams DE, Viswanathan A, Lanson NA,Jr, Wang G. RNA interference against CFTR affects HL60-derived neutrophil microbicidal function. *Free Radic Biol Med*. 2010;49(12):1872-1880.

72. Zhou Y, Song K, Painter RG, et al. Cystic fibrosis transmembrane conductance regulator recruitment to phagosomes in neutrophils. *J Innate Immun*. 2013;5(3):219-230.

73. Koller B, Kappler M, Latzin P, et al. TLR expression on neutrophils at the pulmonary site of infection: TLR1/TLR2-mediated up-regulation of TLR5 expression in cystic fibrosis lung disease. *J Immunol*. 2008;181(4):2753-2763.

74. Blohmke CJ, Victor RE, Hirschfeld AF, et al. Innate immunity mediated by TLR5 as a novel antiinflammatory target for cystic fibrosis lung disease. *J Immunol*. 2008;180(11):7764-7773.

75. Pohl K, Hayes E, Keenan J, et al. A neutrophil intrinsic impairment affecting Rab27a and degranulation in cystic fibrosis is corrected by CFTR potentiator therapy. *Blood*. 2014;124(7):999-1009.

76. Vaisman N, Kerasin E, Hahn T, Trifon S, Voet H, Tabachnik E. Increased neutrophil chemiluminescence production in patients with cystic fibrosis. *Metabolism*. 1994;43(6):719-722.

77. Houston N, Stewart N, Smith DS, Bell SC, Champion AC, Reid DW. Sputum neutrophils in cystic fibrosis patients display a reduced respiratory burst. *J Cyst Fibros*. 2013;12(4):352-362.

78. McKeon DJ, Cadwallader KA, Idris S, et al. Cystic fibrosis neutrophils have normal intrinsic reactive oxygen species generation. *Eur Respir J*. 2010;35(6):1264-1272.

79. Ibrahim-Granet O, Philippe B, Boleti H, et al. Phagocytosis and intracellular fate of aspergillus fumigatus conidia in alveolar macrophages. *Infect Immun*. 2003;71(2):891-903.

80. Di A, Brown ME, Deriy LV, et al. CFTR regulates phagosome acidification in macrophages and alters bactericidal activity. *Nat Cell Biol*. 2006;8(9):933-944.

81. Deriy LV, Gomez EA, Zhang G, et al. Disease-causing mutations in the cystic fibrosis transmembrane conductance regulator determine the functional responses of alveolar macrophages. *J Biol Chem*. 2009;284(51):35926-35938.

82. Haggie PM, Verkman AS. Defective organellar acidification as a cause of cystic fibrosis lung disease: Reexamination of a recurring hypothesis. *Am J Physiol Lung Cell Mol Physiol*. 2009;296(6):L859-67.

83. Barnaby R, Koeppen K, Nymon A, et al. Lumacaftor (VX-809) restores the ability of CF macrophages to phagocytose and kill pseudomonas aeruginosa. *Am J Physiol Lung Cell Mol Physiol*. 2018;314(3):L432-L438.

84. Kopp BT, Abdulrahman BA, Khweek AA, et al. Exaggerated inflammatory responses mediated by burkholderia cenocepacia in human macrophages derived from cystic fibrosis patients. *Biochem Biophys Res Commun*. 2012;424(2):221-227.

85. Romani L. Immunity to fungal infections. *Nat Rev Immunol*. 2011;11(4):275-288.

86. Carvalho A, De Luca A, Bozza S, et al. TLR3 essentially promotes protective class I-restricted memory CD8(+) T-cell responses to aspergillus fumigatus in hematopoietic transplanted patients. *Blood*. 2012;119(4):967-977.

87. Moretti S, Renga G, Oikonomou V, et al. A mast cell-ILC2-Th9 pathway promotes lung inflammation in cystic fibrosis. *Nat Commun*. 2017;8:14017.

88. Bedke T, Iannitti RG, De Luca A, et al. Distinct and complementary roles for aspergillus fumigatus-specific Tr1 and Foxp3+ regulatory T cells in humans and mice. *Immunol Cell Biol*. 2014;92(8):659-670.

89. Sorensen RU, Stern RC, Polmar SH. Cellular immunity to bacteria: Impairment of in vitro lymphocyte responses to pseudomonas aeruginosa in cystic fibrosis patients. *Infect Immun*. 1977;18(3):735-740.

90. Hartl D, Griese M, Kappler M, et al. Pulmonary T(H)2 response in pseudomonas aeruginosa-infected patients with cystic fibrosis. *J Allergy Clin Immunol*. 2006;117(1):204-211.

91. Muller C, Braag SA, Herlihy JD, et al. Enhanced IgE allergic response to aspergillus fumigatus in CFTR-/- mice. *Lab Invest*. 2006;86(2):130-140.

92. Moss RB, Hsu YP, Olds L. Cytokine dysregulation in activated cystic fibrosis (CF) peripheral lymphocytes. *Clin Exp Immunol*. 2000;120(3):518-525.

93. Balghi H, Robert R, Rappaz B, et al. Enhanced Ca2+ entry due to Orai1 plasma membrane insertion increases IL-8 secretion by cystic fibrosis airways. *FASEB J*. 2011;25(12):4274-4291.

94. Mueller C, Braag SA, Keeler A, Hodges C, Drumm M, Flotte TR. Lack of cystic fibrosis transmembrane conductance regulator in CD3+ lymphocytes leads to aberrant cytokine secretion and hyperinflammatory adaptive immune responses. *Am J Respir Cell Mol Biol*. 2011;44(6):922-929.

95. Kreindler JL, Steele C, Nguyen N, et al. Vitamin D3 attenuates Th2 responses to aspergillus fumigatus mounted by CD4+ T cells from cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *J Clin Invest*. 2010;120(9):3242-3254.

96. 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(4):e36077.

97. Cantin AM, Hartl D, Konstan MW, Chmiel JF. Inflammation in cystic fibrosis lung disease: Pathogenesis and therapy. *J Cyst Fibros*. 2015;14(4):419-430.

98. Wainwright CE, Elborn JS, Ramsey BW, et al. Lumacaftor-ivacaftor in patients with cystic fibrosis homozygous for Phe508del CFTR. *N Engl J Med*. 2015;373(3):220-231.

99. Romani L, Oikonomou V, Moretti S, et al. Thymosin alpha1 represents a potential potent single-molecule-based therapy for cystic fibrosis. *Nat Med*. 2017;23(5):590-600.

100. Armstrong-James D, Brown GD, Netea MG, et al. Immunotherapeutic approaches to treatment of fungal diseases. *Lancet Infect Dis*. 2017;17(12):e393-e402.

101. Eades CP, Armstrong-James DPH, Periselneris J, et al. Improvement in exophiala dermatitidis airway persistence and respiratory decline in response to interferon-gamma therapy in a patient with cystic fibrosis. *J Cyst Fibros*. 2018.

Table 1. Overview of studies reporting *Aspergillus* colonisation rates in patients with cystic fibrosis.

References	Focus	Country	Patient population	Duration and frequency of sampling*	Colonisation rates
Nelson, 1979 [22]	primary	USA	46 pts	once	56.7% Asp colonisation (no info if ABPA or sens)
Laufer, 1984 [23]	primary	USA	100 pts aged 2 – 34 yrs	once	9% Af colonisation overall 10% in those with ABPA
Bauernfeind, 1987 [24]	secondary	Germany	102 pts	22 mo period, multiple samples	5.9% Af colonisation (no info if ABPA or sens)
Mroueh, 1994 [25]	primary	USA	236 pts aged 1 – 41 yrs (mean age 14.5 yrs)	retrospective	25.4% overall Af colonisation 19% isolated Af colonisation 6.5% Af colonisation + ABPA based on single cultures
Flume, 1994 [26]	primary	USA	27 pts prior to lung transplantation	once	63% Asp colonisation (no info if ABPA or sens)
Cimon, J Med Mycol 1995 [in 27]	primary	France	210 pts	once	21.4% Af pos culture (only 1% ABPA in the study population)
Becker, 1996 [28]	primary	USA	49 adult pts	1 yr cross-sectional	16% Af colonisation (no data if ABPA or sens) based on single cultures
Milla, 1996 [29]	primary	USA	212 paediatric pts > 5 yrs mean age 17.2 +/- 9.2 yrs	multiple 1 yr study period	21.2% at least one Asp pos culture
Burns, 1998 [30]	secondary	USA	465 pts > 6 yrs	once	23.2% Asp colonisation (ABPA or sens not exclusion criteria for

					study)
Burns, 1999 [31]	secondary	USA	520 pts ≥ 6 yrs of age, FEV1 25%-75% predicted	twice (baseline and end of study)	25% Asp colonisation baseline 16.2% wk 20 (end of study) (18% vs 8%, tobra vs placebo)
Bargon, 1999 [32]	primary	Germany	104 adult pts	repeated cultures	41.3% Asp colonisation (no info if ABPA or sens)
Cimon, 2000 [33]	secondary	France	128 pts (98 adults and 30 paediatric pts)	longitudinal, 5 yrs, minimum 4 samples/pt	46.1% at least one Af pos culture (only 4% ABPA in the population studied)
Hodson, 2002 [34]	secondary	UK/Ireland	42 (tobramycin) + 37 (colistin) pts ≥ 6 yrs	once at end of study	5.7% Af pos in tobramycin group 3.2% Af pos in colistin group
Bakare, 2003 [35]	primary	Germany	94 pts, median age 28 yrs	multiple, 6 mo study period	45.7% Af colonisation 24.5% had ≥ 2 Af positive cultures (no info if ABPA or sens)
Skov, 2005 [36]	primary	Australia	270 paediatric and adult pts	2 samples/yr 4 yr study period	Increase from 7.4% to 18.8% (based on single Af pos cultures) ABPA 0.3 to 4%
Chotirmall, 2008 [37]	primary	Irish	50 pts	during exacerbations 5 yrs study period	30% at least one Asp positive culture 20% > 1 Asp pos culture 4% ≥ 10 Asp pos culture 12% ABPA
Valenza, 2008 [38]	primary	Germany	60 adult and paediatric pts [median 18 yrs, 6 -41 yrs]	multiple samples 1 yr study period	58.3% *A. fumigatus* pos 10% non-*fumigatus Aspergillus* pos (based on single positive cultures)
Paugam, 2010 [39]	primary	France	201 adult pts	multiple samples 2 yrs study period	56.7% in total study population (based on one Af pos culture) 28% age group 6-10 yrs

					59% age group 11-15 yrs 75% age group 16-20 yrs 61% age group 21-41 yrs
Nagano, 2010 [40]	secondary	Ireland	77 adult pts, median 28.5 yrs [18-59 yrs]	once	9.1% all Asp species 5.2% *A. fumigatus* (no info ABPA or sens)
Jubin, 2010 [41]	primary	France	85 paediatric pts mean age 8.5 +/- 9 yrs	multiple at least once/yr 6 yrs study period	21% Asp colonisation 14% isolated persistent Asp colonisation 4% Asp colonisation + ABPA
Sudfeld, 2010 [42]	primary	US	614 pts	10 yr study period, cohort	36.3% *A. fumigatus* (single pos culture) 26.1% non-*fumigatus Aspergillus*
De Vrankrijker, 2011 [43]	primary	Netherlands	259 pts non-ABPA 106 pts 0-12 yrs 99 pts 13-24 yrs 54 pts ≥25 yrs	multiple in one yr	23.6% (>50% of cultures Af pos) 16.4% age group 0-12 yrs 54.1% age group 13-24 yrs 29.5% age group ≥ 25 yrs
Wainwright, 2011 [44]	secondary	Australia	153 pts, BAL fluid from children < 5 yrs	during pulmonary exacerbations	9% Asp colonisation/infection
Fillaux, 2012 [45]	primary	France	206 pts median age 16.3 yrs [range 9.8-23.6]	routine samples, median follow-up 3.6 yrs [range 2.1–8.7]	18% persistent carriage = 3 Af pos cultures in 6 mo (non-ABPA or sens) 62.1% at least one Af pos culture 27.1% persistent carriage period at least once
Güngör, 2013 [46]	primary	Turkey	48 pts, mean age 11.6 yrs [range 2-38 yrs]	at least 3 sputum/deep throat swab samples	10.4% one *A. fumigatus* pos culture 8.3% non-*fumigatus Aspergillus* (no info ABPA or sens)

Fillaux 2014 [47]	primary	France	117 paediatric pts median age 9.9 yrs [IQR 6.0-13.2] at end of study	routine 8 yrs period	38.5% (= 3 Af pos cultures in 6 mo) without sensitization
Masoud-Landgraf, 2014 [48]	secondary	Austria	113 pts, median 20.3 yrs [range 2-57]	multiple samples 1 yr study period	78.8% Af pos cultures (no info ABPA or sens)
Noni, 2015 [49]	primary	Greece	121 paediatric pts	routine	32.2% one Af pos culture 11.6% chronic colonisation
Ramsey, 2014 [6]	primary	Australia	56 infants ≤ 2yrs of age	3 BAL samples 2 yrs study period	12.5% Asp colonisation
Heltshe, 2015 [50]	secondary	USA	151 pts: 38 pts 6-11 yrs 32 pts 12-17 yrs 81 pts ≥18 yrs	3 cultures as per study protocol	~15% and ~7.5% Asp pos cultures before and after ivacaftor (no info ABPA or sens)
Saunders, 2016 [51]	primary	UK	45 children < 18 yrs of age	multiple	29% Af pos BAL culture 14% Af pos sputum 42% one Af pos culture 22% persistent colonisation
Mirkovic, 2016 [52]	primary	Ireland	48 pts	8 sputum samples 2 year period	60.9% in Af sens pts 24% in non-sens pts (at least one pos)
Gernez, 2016 [53]	primary	US & Ireland	48 and 26 adult pts	At least 2 pos sputum cultures within previous 2 yrs	US 27.1% Af pos Ireland 30.8% Af pos
Reece, 2017 [54]	primary	Ireland	CF registry 749 pts median 18.1 yrs [range 4-	retrospective, 1 yr study period	11% overall Af colonisation (5% persistent and 6% intermittent) ABPA 5.9%[#]

			69]		≤ 10 yrs of age: 0 – 10% 11-20 yrs of age: 10-30% 21-30 yrs: 7-18% ≥31 yrs: 0-18%
Brandt, 2018 [55]	primary	Germany	CF Registry 2599 pts (mean age 21 yrs +/- 12yrs)	1 yr study period	32.5% at least one Af pos culture/yr (no info if ABPA or sens)
Hong, 2018 [56]	primary	US	CF Foundation registry 16,095 pts (6 -45 yrs)	Retrospect 6 yrs study period at least 2 cultures/yr, Asp prevalent cases excluded	27.9% Asp pos overall 9.6% persistent Asp pos (≥2 pos cultures during 12 mo; of which 5.4% ABPA) 18.4% transient of which 3.4% ABPA
Coron, 2018 [57]	primary	France	243 pts > 6 yrs (mean age 21.2 +/- 8.7 yrs)	prospective, samples annually or during exacerbation 3 yr study period	35.4% one Af pos culture adults 35.6% children 34.8%

*based on sputum samples unless indicated otherwise; #patients intermittently or persistently colonised with *A. fumigatus* showed no increased prevalence of ABPA; Af, Aspergillus fumigatus; Asp, Aspergillus species; ABPA, allergic bronchopulmonary aspergillosis; sens, fungal sensitisation; pos, positive;