



Short Communication

Chronic *Aspergillus fumigatus* colonization of the pediatric cystic fibrosis airway is common and may be associated with a more rapid decline in lung function

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Received 25 September 2015; Revised 7 December 2015; Accepted 18 December 2015

Abstract

Filamentous fungi are commonly isolated from the respiratory tract of CF patients, but their clinical significance is uncertain and the reported incidence variable. We report on the degree of *Aspergillus fumigatus* airway colonization in a tertiary pediatric CF cohort, evaluate the sensitivity of routine clinical sampling at detecting *A. fumigatus*, and compare lung function of *A. fumigatus*-colonized and non-colonized children.

We carried out an 8-year retrospective cohort analysis using local databases, examining 1024 respiratory microbiological specimens from 45 children. Nineteen (42%) had a positive *A. fumigatus* culture at least once during the 8-year period, with 10 (22%) children persistently colonized. Overall, 29% of 48 bronchoalveolar lavage (BAL) samples tested positive for *A. fumigatus*, compared with 14% of 976 sputum samples. Of 33 children for whom lung function data were available during the study period, seven were classed as having severe lung disease, of whom four (57%) were persistently colonized with *A. fumigatus*.

We conclude that chronic *A. fumigatus* colonization of the CF airway is common, and may be associated with worse lung function. In our practice, BAL appears superior at detecting lower airway *A. fumigatus* compared to sputum samples.

Key words: Children, Filamentous fungi, Sputum, Bronchoalveolar lavage.

Introduction

In cystic fibrosis (CF), acute and chronic respiratory tract infections are the principle cause of progressive decline in res-

piratory function.^{1–3} *Staphylococcus aureus*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa* are the most common pathogens isolated from sputum of CF patients.¹ Another group of organisms frequently isolated from CF

respiratory samples are thermotolerant filamentous fungi^{4,5} such as *Aspergillus fumigatus*, *Scedosporium apiospermum*, and *Aspergillus terreus*.⁴ Most studies have focused on *A. fumigatus* and variable detection rates ranging from 5.9 to 58.3%^{6,7} have been reported, likely the result of differences in the sputum sampling technique and the microbiological processing of samples.^{8,9}

Fungi can cause problems in cystic fibrosis in two ways: (1) a hypersensitivity reaction to *A. fumigatus* including the development of allergic bronchopulmonary aspergillosis (ABPA)^{10,11}; (2) *Aspergillus* bronchitis: infection with *A. fumigatus*, in the absence of ABPA may cause respiratory deteriorations that do not respond to antibiotic treatment, but to antifungal therapy.⁵

Colonization of the airways with *A. fumigatus* has been found to be associated with age in some studies,^{6,12} but colonization may be underestimated in younger children when relying on cough swabs from non-sputum-producing subjects. We undertook an 8-year retrospective cohort analysis of pediatric CF patients to study the rate of *A. fumigatus* airway colonization, determine the sensitivity of different techniques of sputum sampling at detecting *A. fumigatus*, and to compare the lung function of colonized versus non-colonized children.

Materials and methods

Patient population and study design

This is a retrospective cohort study involving 51 children with a confirmed diagnosis of CF (either two positive sweat chloride tests ≥ 60 mmol/L and/or genotype confirmation) treated at our tertiary CF centre between 1 September 2002 to 31 August 2010. Review and anonymised use of patient clinical data were approved by the East Midlands Research Ethics Committee, reference number 12/WM/0285.

We recorded data for every microbiological respiratory sample collected over the study period, including sputum and bronchoalveolar lavage (BAL) samples. National Standards for Microbiology Investigations do not recommend culturing specifically for filamentous fungi from cough swabs. Hence six children were excluded from the study as they only ever had cough swabs taken.

Routine CF care included two-monthly outpatient clinic review where sputum samples were obtained by expectoration; children with either no cough or non-productive cough had a cough swab only sent. Additional samples were collected whenever possible from children who experienced a respiratory exacerbation, and at least twice-weekly samples were sent from children receiving inpatient intravenous antibiotics. Sputum induction was not routinely performed during the study period. BAL was performed in children

where there was unsatisfactory response to two or three courses of appropriate empirical oral antibiotics, and who were unable to expectorate sputum spontaneously.

Microbiological methods

Throughout the entire study period, all respiratory samples from CF patients were processed according to national standard methods for the investigation of BAL, sputum and associated specimens,¹³ which included culture on Sabouraud agar in air at 35–37°C for at least 48 hours for the detection of *Aspergillus*.

Data extraction and definitions

Clinical information was extracted from hospital databases, the medical case notes, and information submitted to the UK CF registry.

Definitions

Persistent *A. fumigatus* colonisation: two or more *A. fumigatus*-positive cultures in any 12-month period.¹⁴ Transient *A. fumigatus* colonization: either one *A. fumigatus*-positive culture during the study period, or *A. fumigatus*-positive cultures separated by at least one year.

Immunological data collected to monitor for ABPA included total immunoglobulin E (IgE), specific IgE to *A. fumigatus*, and IgG precipitins to *A. fumigatus*. A diagnosis of ABPA was considered in patients with a total IgE of > 500 IU/ml and *A. fumigatus*-specific IgE > 0.35 kU/l or IgG precipitins > 90 mg/l¹⁵ in accordance with published minimal diagnostic criteria for diagnosis of ABPA in CF.¹⁶

Statistics

The clinical data are presented as medians and ranges. Comparisons between groups were made using Mann–Whitney U test and Fisher's exact test. A *P*-value of $< .05$ was considered significant.

Results

Data were available and reviewed for 1024 respiratory specimens from 45 children. Specimens comprised 976 sputum samples from 38 patients, and 48 BAL samples from 29 patients. The median patient age for sputum sampling was 11.5 years, compared to a median patient age of 6.6 years for BAL samples. Twenty-two children (49%) had both sputum and BAL samples, 16 (35%) had sputum only, and seven (16%) had BAL sampling only. The demographic data of the study participants are shown in Table 1.

Table 1. Cohort characteristics (n = 45) at the end of the study period (31 August 2010).

	<i>Aspergillus fumigatus</i> never isolated (n = 26)	Transient <i>Aspergillus fumigatus</i> isolated (n = 9)	Persistent <i>Aspergillus fumigatus</i> colonization (n = 10)
Median age (IQR)	7.1 (6.2)	9.5 (6.4)*	16.0 (3.6)*
Female n (%)	15 (57)	4 (44)	6 (60)
Median FEV ₁ %predicted (IQR)	93 (18)	96 (31)	77 (30)
	(n = 15)	(n = 8)	(n = 10)
BMI below 25 th centile n (%)	5 (19)	1 (11)	2 (20)
Pancreatic insufficiency n (%)	22 (85)	9 (100)*	10 (100)*
<i>Pseudomonas</i> isolated n (%)	5 (19)	4 (44)	8 (80)*
<i>S. aureus</i> isolated n (%)	7 (27)	5 (56)	9 (90)*
<i>H. influenzae</i> isolated n (%)	5 (19)	3 (33)	8 (80)*
<i>S. pneumoniae</i> isolated n (%)	1 (4)	2 (22)	5 (50)*
Immunology	n = 24	n = 9	n = 10
Total IgE > 500 IU/ml n (%)	0 (0)	1 (11)	3 (30)*
<i>Aspergillus fumigatus</i> -specific IgE > 0.35 kU/l n (%)	4 (17)	7 (78)*	7 (70)*
IgG precipitins > 90 mg/l n (%)	0 (0)	3 (33)*	5 (50)*

*Statistically significant result, *P*-value <.05, compared to “never isolated” group, using Mann–Whitney *U* test for age and FEV₁ % predicted, and Fisher’s exact test for all other variables.
BMI: body mass index; IQR: interquartile range.

Microbiology

Nineteen children (42%) had a positive *A. fumigatus* culture at least once during the study period, of whom ten (22% of cohort) were “persistently,” and nine (20% of cohort) “transiently” colonized. The median age at first *A. fumigatus*-positive culture was 9.0 years (range 3.1–16.2 years), and it was observed that most of the children over 10 years of age were persistently colonized

with *A. fumigatus* (Figure 1). *A. fumigatus* was the third most commonly isolated organism in our pediatric CF cohort. Other organisms isolated are presented in the online supplement.
A. fumigatus was cultured from a greater proportion of BAL samples (29%) compared with sputum samples (14%). There were eight instances where BAL and sputum were sampled in the same *A. fumigatus*-positive child within two

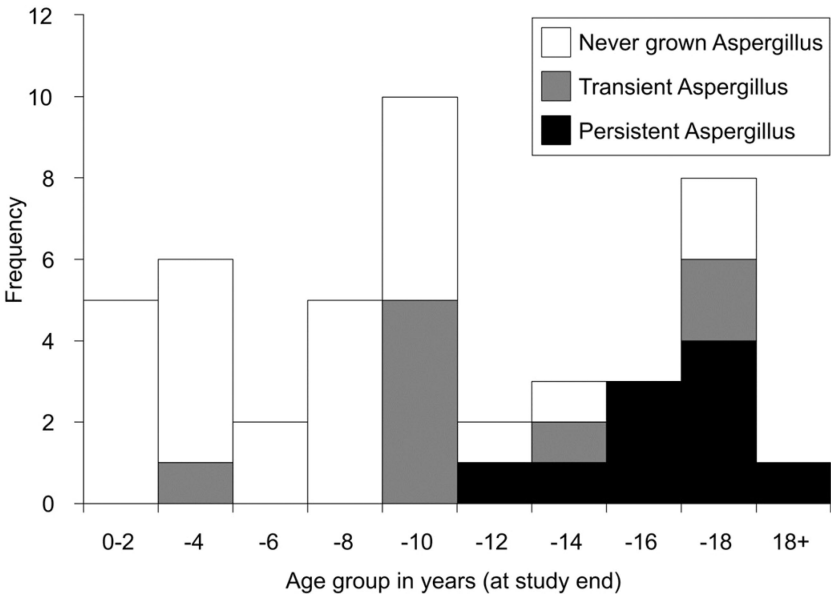


Figure 1. Histogram of ages of the paediatric CF cohort at the close of the study period, demonstrating the relationship between age and *Aspergillus fumigatus* status.

weeks of one another; six of these matched samples were BAL-positive and sputum-negative, one was BAL-negative and sputum-positive, and one was both BAL and sputum-positive.

Immunology

Immunological data were available for 43 patients, 19 of whom were colonized with *A. fumigatus*. During the study period, only four of these 19 patients (9.3%) met the criteria for a laboratory diagnosis of ABPA. Two patients were given a clinical diagnosis of ABPA. One child was treated with oral corticosteroids and intermittent nebulized liposomal amphotericin B. This child still harbored *A. fumigatus* regularly and remained chronically colonised but did not have severe lung disease. The second child was treated with intermittent corticosteroids only and fell into the severe lung disease category. Of the remaining 15 children who were colonized with *A. fumigatus*, 10 were sensitized to *A. fumigatus*. A further four children were sensitized to *A. fumigatus* despite never having *A. fumigatus* isolated during the study period.

Lung function

Lung function data were available for 33 patients; the remaining 12 patients were too young to perform reliable spirometry testing. Patients were classified as having "severe" or "nonsevere" lung disease (Figure 2) accord-

ing to the longitudinal observational data of Schluchter et al.,¹⁷ where "severe" approximates to the lowest quartile of FEV₁ % predicted for age. Seven children from our cohort were classed as having severe lung disease. Of these, five were colonised with *A. fumigatus* (four persistently) of whom four were also sensitized to *A. fumigatus* with one meeting the criteria for a clinical diagnosis of ABPA. Two children with severe lung disease were neither colonized nor sensitized. In 31 children for whom lung function and immunological data were available, we found no significant difference in FEV₁ % predicted between the *Aspergillus*-sensitized (n = 17) and nonsensitized (n = 14) children.

Duration of colonization

Ten children had "persistent" *A. fumigatus* colonization during the study period of whom two were colonised for less than one year. The other eight were colonised over periods ranging from 3.3 to 7.6 years. Duration of *A. fumigatus* colonization was not associated with severity of lung disease, but the number of subjects was too small to perform a meaningful subgroup analysis.

Discussion

A. fumigatus is one of the most common organisms isolated from the sputum of CF patients and colonisation has been associated with worse CF lung disease^{7,18} and more frequent respiratory exacerbations.¹⁴ However, very little

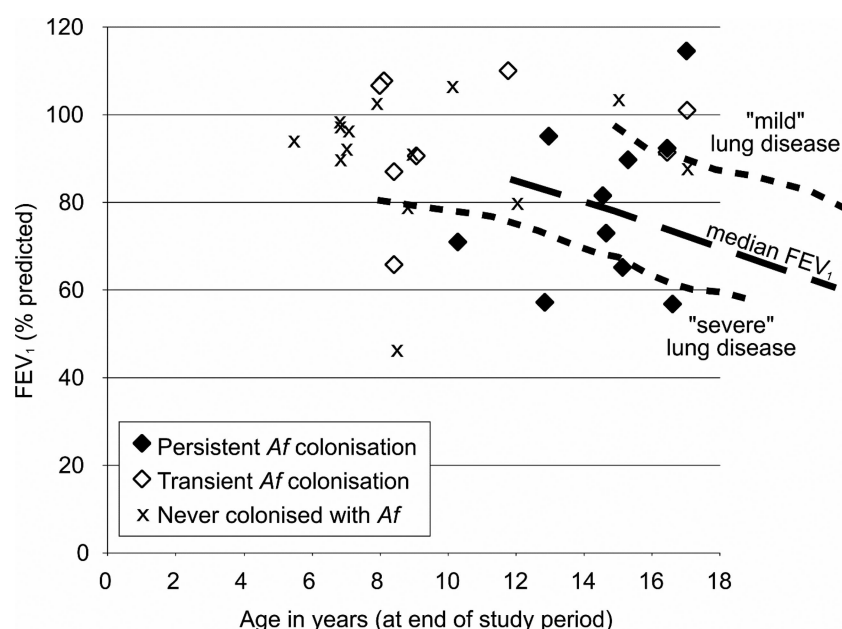


Figure 2. Lung function status of our paediatric CF cohort, plotted according to criteria of Schluchter et al. (2006)¹⁷, grouped into *A. fumigatus*-positive (persistent or transient: see Section 2.3 for definitions) or *A. fumigatus*-negative. Fisher's exact test was used to investigate the association between severe lung disease and persistent *A. fumigatus* colonisation and this was not statistically significant ($p = 0.16$).

data is available in young children with CF and differences in isolation rates between sampling techniques have not been studied. We cultured *A. fumigatus* from the respiratory tract in nearly half of our study population over the 8-year study period with 22% of children fulfilling criteria for persistent *A. fumigatus* colonization.⁷ These findings are similar to recent data reported from France¹⁹ although that study only included children who were able to spontaneously expectorate sputum.

Previous studies reported an association between age and risk of *Aspergillus* sp. Isolation;⁶ however, these studies tended to rely on sputum-producing children and this may underestimate the presence of *A. fumigatus* in younger children. We found that *A. fumigatus* isolation was rare under the age of eight years. This finding is supported by the availability of 19 BAL samples from 15 children under six years, with *A. fumigatus* isolation from only one specimen. The low prevalence of *A. fumigatus* in the airways of young children with CF is consistent with recently published data from annual surveillance BAL sampling in Australian infants, where *Aspergillus* sp. were isolated from seven out of 56 patients.¹⁸ In our children older than 10 years, isolation of *A. fumigatus* was common and the majority of older children eventually became persistently colonized.

BAL may be superior to sputum at identifying *A. fumigatus* colonization. Reviewing the seven sputum cultures taken within two weeks of a BAL containing *A. fumigatus*, we found that the organism was only cultured from one sputum sample. Conversely we only found one negative BAL within two weeks of a positive sputum sample. BAL and sputum samples are processed using equivalent culture methods in our laboratory, and therefore comparisons between BAL and sputum samples are valid and represent genuine differences between the sampling techniques in the detection of *A. fumigatus*.

During initial development of this study, we also reviewed the results of cough swabs ($n = 1679$) for the same cohort, and noted a very low (0.8%) prevalence of *A. fumigatus*. This lower prevalence is based on two factors. First, the historical standard operating procedures for cough swabs did not stipulate routine fungal culture on Sabouraud agar, and this was therefore performed only for some samples, although any observed fungal growth upon nonselective media was reported. Second, there may be a genuine clinical difference between the prevalence of *A. fumigatus* in cough swab specimens compared to sputum and BAL. Clinicians should be aware that both of these factors may contribute to the apparent microbiological absence of *A. fumigatus* in patients who are actually colonized and may wish to check their local microbiological operating procedures.

More than half of the children in our study who were classified as having “severe” lung disease were persistently colonised with *A. fumigatus* and one child had a clinical diagnosis of ABPA. Like others, we found a considerable overlap between *A. fumigatus* isolation and infection and colonization with other important pathogens known to accelerate CF lung disease including *Pseudomonas aeruginosa*,^{20–23} *Staphylococcus aureus*, *Haemophilus influenzae*, or *Streptococcus pneumoniae*. Isolation rates with these organisms are shown in Table 1. These coinfections are likely to be important confounding factors when studying lung function in children with *A. fumigatus* colonization. Nutritional state also needs to be considered in this context. We found no significant relationship between *Pseudomonas aeruginosa* colonization or BMI < 25th centile and “severe” lung disease.

Four children in our study fulfilled the laboratory criteria for ABPA, and two were given a clinical diagnosis and treated accordingly. These figures are in line with previously published data sets.²⁴ In the absence of ABPA, the significance of *A. fumigatus* colonization and the presence of *Aspergillus* bronchitis in CF is controversial.²²

Finally, the clinical significance of *A. fumigatus* sensitization in the absence of other laboratory criteria of ABPA and/or in the absence of *A. fumigatus* isolation is poorly understood. Four of the seven children with severe lung disease were sensitized to *A. fumigatus*. In a cohort of adult CF patients, *A. fumigatus* sensitization (but not microbiological isolation) was found to be associated with a decline in lung function²⁵ as well as increased pulmonary exacerbations.²⁶ The 42% prevalence of *A. fumigatus* sensitization (specific IgE > 0.35 kU/l) in our study is similar to that found in other cohorts, for example 39–46% in mixed pediatric and adult cohorts^{25,27} rising to 66% in an adult-only cohort.²⁸ This is important because the prevalence of raised specific IgE to *A. fumigatus* is much greater than the prevalence of ABPA.^{27,28}

Strengths and limitations

Our findings are limited by the observational and retrospective nature of data collection, and small numbers in the relevant subgroups mean the findings need interpreting with caution. However, the number of children followed was comparable to other published datasets in children and our follow-up study period was especially long. There are few studies on *A. fumigatus* involving young children with CF, and we are not aware of any studies comparing sputum with BAL longitudinally. The small number of patients in the “severe” lung disease category made it difficult to assess the contribution of other confounding factors that may have contributed to poor lung function, such as infection

with other pro-inflammatory pathogens. There were also relatively few examples of paired sputum and BAL samples being taken from the same patient at the same time, resulting in potential sampling bias with paired samples available from sicker patients.

Our study highlights the need to analyze much larger data sets to understand the relationship between *A. fumigatus* sensitization with or without colonization, and severity of CF lung disease. This in turn however requires a harmonized approach to microbiology laboratory standards with respect to *A. fumigatus*, which at present is very variable.⁷ Moreover, data on *A. fumigatus* isolation from sputum is not routinely collected by many national CF databases including the UK CF Trust.

Conclusions

In our practice, BAL appears superior at detecting lower airway *A. fumigatus* compared to sputum samples; therefore, the presence of this organism may be underestimated, particularly in young children. The clinical significance of *A. fumigatus* sensitization with or without airway colonization is poorly understood, but it is possible that current practice may not be providing the optimum treatment for this subgroup of CF patients. Larger prospective observational studies are needed to elucidate the clinical relevance of *A. fumigatus* sensitization and colonization in the absence of ABPA to inform the clinical management of these patients.

Acknowledgements

RVS, DEM, and EAG were involved in study design, data analysis and interpretation. AC provided relevant patient clinical information. RVS wrote the first draft of the manuscript; subsequently all authors contributed to the final submitted version of the manuscript. EAG was responsible for research conduct and co-ordination and is the guarantor for the content of the manuscript. All authors approved submission of the manuscript. This research was funded from a departmental grant from the University of Leicester.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

Supplementary Material

Supplementary material is available at *Medical Mycology* online (<http://www.mmy.oxfordjournals.org/>).

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Supplementary figure. Percentage of paediatric CF cohort from whom organism was isolated from sputum or BAL at any time during study period. *Aspergillus* spp. other than *Aspergillus fumigatus* are listed in “other moulds”. *Pseudomonas* includes both *Pseudomonas aeruginosa* and *Pseudomonas* spp.

