Medical Mycology 2010, **48**(Suppl. 1), S32–S36

informa
healthcare

Original Article

Characteristics and consequences of airway colonization by filamentous fungi in 201 adult patients with cystic fibrosis in France

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A total of 657 sputum samples from 201 cystic fibrosis adult patients were collected during a 24-month period (2005–2006). We retrospectively analyzed the fungal colonization of the respiratory tract of these individuals by linking medical records and microbiological data. Filamentous fungi were isolated from specimens of 65.6% of the patients, with *Aspergillus fumigatus* being the predominant species recovered as it was found in specimens of 56.7% of the patients. We observed no difference for gender, pancreatic status and cirrhosis in patients with or without *A. fumigatus* colonization. We found a higher percentage of recovery of *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and nontuberculous mycobacteria in patients with *A. fumigatus* colonization. During the follow-up period of the study, 8.9% of the patients were diagnosed with allergic bronchopulmonary aspergillosis (ABPA). By a multivariate analysis we demonstrated that *Scedosporium apiospermum* was significantly associated with ABPA (Odds ratio = 13 [2–80]) as opposed to *A. fumigatus* (Odds ratio = 1.58 [0.49–5.05]).

Keywords cystic fibrosis, filamentous fungi, *Aspergillus, Scedosporium*, allergic bronchopulmonary aspergillosis

Introduction

Improvement in the management of patients with cystic fibrosis (CF) has resulted in a marked increase in their life expectancies, with a larger proportion now reaching adulthood. These patients require age-appropriate care. In this context, we have studied the nature and the consequences of fungal respiratory colonization in a cohort of CF adult patients.

Our objective was to investigate, by a retrospective study, the frequency and the characteristics of filamentous fungi isolated from the respiratory samples of patients followed as out-patients, or hospitalized in our adult CF centre during a 2-year period (2005–2006).

Materials and methods

Patients

Data came from patients with CF at our adult CF centre at the Cochin Paris-Descartes University hospital, Paris, France and retrospectively included all patients seen from 1 January 2005–31 December 2006 (2 years). The diagnosis of CF disease was based on clinical features associated with a positive sweat test (chloride > 60 mmol/l), or the presence of two known disease-causing *CFTR* mutations, or abnormal nasal potential difference results. All sputum samples collected from patients during their routine visits to the hospital, as well as during hospitalizations were

Received 19 February 2010; Received in final revised form 2 June 2010; Accepted 21 June 2010

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DOI: 10.3109/13693786.2010.503665

examined for fungi. Patients were excluded from the study if they were unable to produce sputum, or had a history of lung transplantation. We collected 657 sputa from 201 adult patients. The median age of patients in the cohort was 26 years (range 17–65 years).

Design and procedures

We used the CF database of the CF centre to establish the following parameters. Genotype was defined as severe in the case of two *CFTR* severe mutations. Patients were classified as pancreatic insufficient if fecal elastase was <200 μg/g, or fecal fat >6 g/day. Allergic bronchopulmonary aspergillosis (ABPA) status was determined by clinicians [1], based on clinical data and, biologically, on elevated total serum immunoglobulin (IgE) and elevated serum *Aspergillus*-specific IgG and IgE levels.

Culture media and methods

Respiratory samples were cultured on Sabouraud + chloramphenicol + gentamicin agar (bioMérieux, Marne la Coquette, France) incubated for 10 days at 30°C to recover filamentous fungi and were inspected every 1 or 2 days.

Identification

Filamentous fungi were identified on the basis of macroscopic and microscopic morphology.

Statistical analysis

The Fisher test was used for comparison of two variables. Multivariable analysis was also performed to study risk factors for ABPA (gender, cirrhosis, sputum culture positive with *Staphylococcus aureus*, *Stenotrophomonas maltophilia*, *Aspergillus fumigatus* and *Scedosporium apiospermum*).

Results

Clinical and mycological results

A total of 201 adult patients (men 53%) aged 17–65 years (median 26) were included in the study, from whom a total of 657 respiratory samples were collected with a mean of 3.1 cultures per patient (range 1–21). At least one positive culture containing a filamentous fungus was recovered during the 2-year period of the study for 132 patients (65.6%). Thirty seven out of these patients (28%) were colonized by multiple species. Prevalence of the fungal species is summarized in Table 1. *Aspergillus fumigatus* was the most common species found in samples from 56.7% of the patients, followed by *A. flavus* (3.4%), *A. niger* (2.9%), *A. nidulans* (1.9%), *A. versicolor* (0.9%) and *A. terreus* (0.9%). *Scedosporium apiospermum* was recovered from specimens of 3.4% of the patients. In patients with at least one positive culture for one fungal species, the percentage of positive samples indicating colonization by *A. fumigatus* (71%) was significantly higher than those in which non *A. fumigatus Aspergillus* was detected to be colonizing the patients (21%, $P < 10^{-5}$). The proportion of positive samples for *S. apiospermum* was 58%. When comparing the patients with or without positive *A. fumigatus* cultures according to their gender, genotype, pancreatic status, cirrhosis and diabetes (Table 2), we observed a difference only with respect to diabetes, in that a significantly lower proportion of diabetic patient specimens were positive in culture for *A. fumigatus* (19.3% versus 35.6%, $P = 0.007$).

Table 1 Prevalence of filamentous fungal species recovered (2005–2006) and proportion of positive samples according to the species isolated.

Filamentous fungi	Patients ($N = 201$)	Positive samples*
Aspergillus fumigatus	56.7%	71% (322/453)
Aspergillus (non-*fumigatus*)	10.4%	21% (27/125)
Scedosporium apiospermum	3.4%	58% (31/53)

*Ratio between the number of total positive cultures and the total number of cultures in patients who had at least one positive culture.

Bacterial and mycological results

We compared the prevalence of bacteria in the airways of patients with or without culture indication of the presence of *A. fumigatus* (Table 3). In the patients with positive *A. fumigatus* cultures, we observed a higher prevalence of non-tuberculous mycobacteria (13.1% versus 4.6%, $P = 0.03$) and *S. maltophilia* (16.6% versus 8%, $P = 0.05$), as well as a tendency for more frequent isolation of *Pseudomonas aeruginosa* (78.9% versus 68.9%, $P = 0.07$).

ABPA and mycological results

Logistic regression was used to determine the independent effect of gender, cirrhosis, isolation of *S. aureus*, *S. maltophilia*,

Table 2 Presence or absence of *Aspergillus fumigatus* and population characteristics.

	A. fumigatus		
	Presence ($n = 114$)	Absence ($n = 87$)	*P* value
Gender (female)	50.0%	44.8%	NS
Severe genotype	71.9%	72.4%	NS
Pancreatic insufficiency	80.7%	82.7%	NS
Cirrhosis	4.4%	6.9%	NS
Diabetes	19.3%	35.6%	0.007

NS: not significant.

Table 3 Presence or absence of *Aspergillus fumigatus* and isolation of bacteria.

	A. fumigatus		
	Presence ($n = 114$)	Absence ($n = 87$)	*P* value
Pseudomonas aeruginosa	78.9%	68.9%	0.07
Stenotrophomonas maltophilia	16.6%	8%	0.05
Staphyloccocus aureus	68.4%	59.7%	NS
Non-tuberculous mycobacteria	13.1%	4.6%	0.03

NS: not significant.

A. fumigatus and *S. apiospermum* relative to ABPA. Patients with *S. apiospermum* colonization had a significantly elevated risk of ABPA in comparison to patients from whom this fungus was not recovered in culture (RR = 13.04, $P = 0.006$) (Table 4).

Discussion

Airway colonization by filamentous fungi is frequent in CF patients and increases with age. *Aspergillus fumigatus* colonization is uncommon in young children and usually occurs after bacterial infections [2]. In an epidemiological study conducted in France, the mean age at first isolation of *A. fumigatus* was 12.3 years [3]. A recent study found that 28% of the patients aged 6–10 years harboured *A. fumigatus* in comparison to 61% of those aged 21–41 years [4]. Our result of about 58% *A. fumigatus* colonization as indicated by culture is in agreement with those reported during transition from paediatric to adult care [5] and in British adults with CF [6]. In Europe the percentages of CF patients with positive *A. fumigatus* culture from respiratory secretions depends on the country, i.e., 25.4% (61/218) in Denmark [7], 45.7% (43/94) in Germany [8] and 46.1% (59/128) in France [9]. When using a high number of isolates for a single sample and a high-resolution genotyping method (microsatellite-based typing) it was demonstrated that CF patients were colonized with multiple strains and that some genotypes were recurrently isolated and were responsible for prolonged colonization [10].

Table 4 Multivariate analysis: comparison of patients with ABPA ($n = 18$) and without ABPA ($n = 183$).

Risk factor	Odds Ratio	Confidence interval	*P*
Gender (female vs male)	0.33	[0.10–1.07]	NS
Cirrhosis	6.71	[0.87–51.23]	NS
Positive culture :			
Stenotrophomonas maltophilia	1.72	[0.46–6.41]	NS
Aspergillus fumigatus	1.58	[0.49–5.05]	NS
Scedosporium apiospermum	13.04	[2.11–80.42]	0.006

NS: not significant.

Non-*fumigatus Aspergillus* species, such as *A. flavus* and *A. nidulans*, may be isolated transiently from CF respiratory secretions [3], as reflected by the low percentage of positive samples we observed for these species (21%). Therefore, our prevalence of non-*fumigatus Aspergillus* (10.4%) may be underestimated if we consider the results of 25.6% colonization by *A. flavus* and/or *A. terreus* (10 of the 39 patients investigated) observed by Bouchara *et al.* [11] using an oligonucleotide array for direct detection of fungi in sputum samples. However, the high percentage of positive samples of *A. fumigatus* (71%) and *S. apiospermum* (58%) reflected the chronic colonization due to these species.

As already reported in CF [12], we observed that *S. apiospermum* was the second most frequent filamentous fungus isolated from sputa. The incidence of airway colonization by *Scedosporium* spp. varies from 2.3% [13] to 5.4% [14]. In our study *S. apiospermum* was recovered from sputa of 3.4% (7/201) of the patients, but this percentage may be underestimated because Cimon *et al.* [15] have reported positive *Scedosporium* serology in 21.1% (27/128) of their CF patients. In the past decades, *Pseudallescheria/Scedosporium* species have become increasingly recognized as causative agents of significant infections in humans [14] and *S. apiospermum* is now recognized as a complex comprising at least five distinct species [16]. In Australia, it has been observed that a new species called *S. aurantiacum* comprises 45% of the current collection of Australian '*S. apiospermum*' isolates. The epidemiology and clinical relevance of *S. aurantiacum* appear to be similar to that of *S. apiospermum* [17]. However, in our study we did not differentiate species of the *S. apiospermum* complex. *Scedosporium apiospermum sensu lato* is well-known to be responsible for chronic colonization. A genotyping study has demonstrated that colonization was due to a single or a predominant genotype and that the fungus was persistent in the respiratory tract despite antifungal treatments [18]. We must underline that the environmental reservoir of these fungi remains uncertain and the mode of transmission not well defined [19]. Conventional laboratory techniques may fail to detect fungi in patients mainly due to overgrowth by Gram-negative bacteria [20].

We observed no impact of gender or pancreatic insufficiency on *A. fumigatus* colonization, as previously reported by Amin *et al.* [21]. We also did not find an impact of severe genotype or cirrhosis on the colonization by this fungus but we observed less frequent *A. fumigatus* colonization in patients with diabetes. We were quite surprised by this result since diabetes is often considered as a predisposing factor for fungal infections. We have no satisfactory explanation for this observation which needs to be confirmed by a large prospective study.

Regarding the interactions between *A. fumigatus* and bacteria, we observed no difference for *S. aureus* colonization, but a significantly higher percentage of *S. maltophilia* in those instances when *A. fumigatus* was recovered in culture. Occurrence of *A. fumigatus*-positive cultures also tended to be more frequent for those patients in which *P. aeruginosa* was noted in cultures. This finding has already been reported in a case control study of 28 CF patients with *P. aeruginosa* age matched with 28 CF patients without *P. aeruginosa*. Logistic regression analysis showed that the occurrence of *Aspergillus* was a risk factor for developing chronic *P. aeruginosa* infection with an OR of 4.7 (95% CI 1.5–15, $P = 0.008$) [22]. Further, it has been suggested that biofilm formation by *P. aeruginosa* in the respiratory tract may facilitate the colonization by *A. fumigatus*.

Regarding non-tuberculous mycobacteria, there was a trend of higher positive cultures in cases involving the recovery of *A. fumigatus* in cultures (13.1% versus 4.6%, $P = 0.06$). The association between *Aspergillus* and non-tuberculous mycobacteria has been demonstrated in patients with bronchiectasis with an Odds ratio = 5.1 (1.5–17.0) [23]. Non-tuberculous mycobacteria have emerged as 'new' pathogens in CF over the last decade [24], due to improved laboratory techniques for processing CF respiratory specimens [25]. Therefore, the effect of non-tuberculous mycobacteria on the clinical course of CF is poorly understood [26,27] and specific risk factors that contribute to active pulmonary infection have not been identified [27]. In a cohort study there was no statistically significant difference in chest radiographic findings, nor in lung function decline between CF patients colonized or not colonized with non-tuberculous mycobacteria [28].

Logistic regression was used to determine the independent effect of gender, cirrhosis, isolation of *S. aureus*, *S. maltophilia*, *A. fumigatus* and *S. apiospermum* on ABPA. Patients with *S. apiospermum* colonization had a significantly elevated risk of ABPA compared with patients whose specimens did not yield *S. apiospermum* in cultures (RR = 13.04, $P = 0.006$). However, it must be noted that *S. apiospermum* may cause allergic broncho-pulmonary mycoses similar to the well-known ABPA [15]. We observed no association between bronchial colonization with *S. maltophilia* and ABPA, as differs from the results of Ammann *et al*. [29]. A previous study concluded that the frequency of *Aspergillus* isolates did not correlate with the occurrence of ABPA [30] and Milla *et al*. [31] demonstrated that the presence of *Aspergillus* spp. did not independently increase the risk of more severe structural lung damage.

Conclusion

Filamentous fungi are frequently recovered in respiratory cultures from adult patients with CF. *Aspergillus fumigatus* is the major agent colonizing the patients, but apart from ABPA, clinical consequences on the evolution of the disease remain unclear. We observed that colonization with *A. fumigatus* is chronic and more frequently associated with non-tuberculous mycobacteria, *S. maltophilia* and *P. aeruginosa.* These results encourage us to continue our analysis with a prospective study in order to better analyse the sequence of bronchial colonization with bacteria and fungi and to better adapt the antibiotic and antifungal treatments. We observed a significant association between ABPA and the presence of *S. apiospermum* in respiratory secretions and recommend taking into account this emerging species. Considering the small number of *S. apiospermum* isolates in our study, a prospective study will be useful to confirm our results.

Acknowledgements

We thank Thierry Ancelle for his assistance in statistical analysis of our results. We thank Gordon Langsley for editing the English.

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

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