Prevalence and Factors Associated with Isolation of *Aspergillus* and *Candida* from Sputum in Patients with Non-Cystic Fibrosis Bronchiectasis

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Key Words

Aspergillus prevalence · *Candida albicans* prevalence · Fungal isolation · Fungal persistence · Non-cystic fibrosis bronchiectasis · Fungal colonization

Abstract

Background: Information on the role of fungi in non-cystic fibrosis (CF) bronchiectasis is lacking. **Objectives:** Our aim was to determine the prevalence of and factors associated with the isolation and persistence of fungi from sputum in these patients. **Methods:** We performed a multicenter observational study comprising adult patients with non-CF bronchiectasis. Persistence of *Aspergillus* spp. and *Candida albicans* was defined as the presence of ≥ 2 positive sputum cultures taken at least 6 months apart within a period of 5 years. **Results:** A total of 252 patients (62.7% women with a mean $\pm$ SD age of 55.3 ± 16.7 years) were included in the study. All patients had at least 1 sputum sample cultured for fungi, with a mean $\pm$ SD of 7 ± 6 cultures per patient. Eighteen (8.7%) and 71 (34.5%) patients had persistent positive cultures for *Aspergillus* spp. and *C. albicans*, respectively. Patients with persistence of *Aspergillus* spp. and *C. albicans* were older and had more daily purulent sputum. In addition, patients with persistent *C. albicans* had worse postbroncho-

dilator forced expiratory volume in the first second (FEV₁), more frequent cystic bronchiectasis, and more hospital-treated exacerbations. They were also more frequently treated with long-term antibiotics. Multivariate analysis showed that daily purulent sputum (OR = 3.75, $p = 0.045$) and long-term antibiotics (OR = 2.37, $p = 0.005$) were independently associated with persistence of *Aspergillus* spp. and *C. albicans*, respectively. **Conclusions:** Isolation and persistence of *Aspergillus* spp. and *C. albicans* are frequent in patients with non-CF bronchiectasis. Daily purulent sputum and chronic antibiotic treatment were associated with persistence of *Aspergillus* spp. and *C. albicans*, respectively.

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Introduction

Non-cystic fibrosis (CF) bronchiectasis is a chronic airway disease characterized by progressive dilation and destruction of the bronchial tree. Associated dysfunctional mucociliary clearance leads to persistent bacterial infection, chronic inflammation of the bronchial tree, and progressive tissue destruction [1].

In patients with non-CF bronchiectasis, the airway is usually colonized by bacterial pathogens and other mi-

croorganisms, such as yeasts and filamentous fungi. In healthy individuals, fungal spores are quickly cleared by the mucociliary escalator and phagocytosed by cells of the innate immune system. However, in patients with chronic lung conditions such as bronchiectasis, impaired mucociliary clearance and mucus plugging can lead to the persistence of spores in the respiratory tract [2, 3].

The prevalence of colonization of the airways by *Aspergillus* spp. and *Candida albicans* in patients with CF is high [4–6] and can vary according to geography, local surveillance methods for endobronchial fungal infections, and the definition of fungal colonization, infection, and persistence [6, 7]. Although risk factors for persistence of these fungi in the airways of patients with CF have been investigated [4, 8], they have yet to be clarified. In the case of non-CF bronchiectasis, published data on the prevalence and fungus-associated factors are lacking. Therefore, the aim of this study was to determine the prevalence and factors associated with the isolation and persistence of fungi from sputum in patients with non-CF bronchiectasis.

Materials and Methods

Study Population

The study population comprised 296 consecutive patients, all of whom were aged ≥ 18 years and had been diagnosed with non-CF bronchiectasis of widely varying causes, with radiological extension and clinical and functional impairment. Patients were evaluated over a 5-year period between December 31, 2002, and December 31, 2010. The exclusion criteria were CF and not having at least 1 sputum sample cultured for fungi during a clinically stable phase within a period of 5 years after the diagnosis.

According to the recommendations of the Spanish Society of Pulmonology and Thoracic Surgery, the causes ruled out in idiopathic bronchiectasis were as follows: immune deficiencies with evidence of defective antibody production, gastroesophageal reflux disease, allergic bronchopulmonary aspergillosis, mycobacterial infection, CF, primary ciliary dyskinesia, and $\alpha 1$ -antitrypsin deficiency [9]. Patients with allergic bronchopulmonary aspergillosis ($n = 4$) [10] were excluded because they comprise a subgroup that differs from patients with other causes of non-CF bronchiectasis in terms of the prevalence and pathogenic significance of *Aspergillus* colonization. CF was ruled out by 2 negative sweat test results in patients with bronchiectasis of unknown cause or with a clinical presentation compatible with CF. The study was approved by the Ethics and Research Committee of each center (registration number of the coordinating center: 0088-89-2011).

Study Design

We performed an observational study of historical cohorts from 4 Spanish centers that were homogeneous in terms of the participating specialists and the type of outpatient clinic.

Patients were selected for 2 separate analyses. For the primary analysis, we compared patients who had at least 1 filamentous fungus or yeast in sputum over the course of the study with those who had not. For the secondary analysis, we compared patients with persistent *Aspergillus* spp. and *C. albicans* and patients without persistent *Aspergillus* spp. and *C. albicans*.

Data Collection

Data were collected from all patients during a 4-week clinically stable phase and included the following: age, gender, body mass index, etiology, smoking habit (pack-years), dyspnea according to the modified Medical Research Council scale, patient-reported macroscopic appearance of sputum (mucoid, mucopurulent, or purulent), type of bronchiectasis (cystic, noncystic), radiological findings (number of lobes affected by bronchiectasis), presence of respiratory insufficiency (defined as an oxyhemoglobin saturation $< 90\%$ when breathing room air), spirometry [forced vital capacity (FVC) and postbronchodilator forced expiratory volume in the first second (FEV₁), as both absolute values and % predicted], and peripheral levels of C-reactive protein (IU/ml) as a marker of systemic inflammation. We also recorded hospitalizations secondary to severe exacerbation, number of exacerbations, and number of courses of oral corticosteroids administered for exacerbations. A stable clinical condition was defined as the absence of clinical criteria of exacerbation and no requirement for antibiotics or corticosteroids in the preceding 4 weeks [11]. Exacerbation was defined as the acute onset and persistence of changes in sputum characteristics (increased volume, thicker consistency, greater purulence, or hemoptysis) and/or increased breathlessness unrelated to other causes [12].

All variables were obtained a maximum of 6 months after the radiological diagnosis of bronchiectasis, except for hospitalizations, number of exacerbations, and number of courses of oral corticosteroids for exacerbations, which were obtained during the year after the radiological diagnosis.

Long-term treatments (inhaled or oral antibiotics, oral macrolides, and corticosteroids) taken for at least 1 year after the radiological diagnosis of bronchiectasis were recorded.

Diagnosis of Bronchiectasis

Bronchiectasis was diagnosed by a high-resolution computed tomography scan of the chest and interpreted by radiologists experienced in respiratory disorders. Images were obtained in full inspiration (1-mm collimation and 10-mm intervals from the apex to the base of the lungs). The presence of bronchiectasis was based on the criteria published by Naidich et al. [13]. The extent of the bronchiectasis was evaluated according to the number of lobes and segments affected, with the lingula and middle lobe considered as independent lobes.

Microbiology

Only sputum samples with more than 25 polymorphonuclear leukocytes and fewer than 10 squamous cells on Gram stain were considered valid samples and processed for bacterial and fungal culture. For fungal isolation, the samples were plated on Sabouraud chloramphenicol plates and incubated at 30 and 37°C. Cultures were evaluated for growth every 24 h during the first 7 days and weekly till 28 days. If filamentous fungi were detected, colonies were cultured in potato dextrose agar and Czapek agar, and subcultures were incubated at 30, 37, and 42°C in order to identify the species.

Bacterial chronic lung infection was defined as isolation of the same potentially pathogenic microorganism after the diagnosis of bronchiectasis in >50% of respiratory cultures during the 5-year study [14]. Persistent *Aspergillus* spp. and *C. albicans* were defined as the presence of 2 or more positive sputum cultures for *Aspergillus* spp. and *C. albicans*, respectively, taken at least 6 months apart over a period of 5 years.

Statistical Analysis

The data for patients with at least 1 filamentous fungus or yeast isolated in sputum and for patients with persistent *Aspergillus* spp. and *C. albicans* were presented and analyzed separately. The statistical analysis was performed using SPSS, version 15.0 (SPSS, Chicago, Ill., USA). All data were expressed as mean $\pm$ SD or median (IQR) for quantitative variables and as absolute values and percentages for qualitative variables. The normality of the distribution was assessed using the Kolmogorov-Smirnov test. Nonparametric tests were used in the absence of a normal distribution. The t test or Mann-Whitney test was used to compare 2 means, and the χ^2 test or Fisher's exact test were used for qualitative or dichotomous variables. Variables of clinical interest (according to the current literature or researchers' opinion) and those that presented statistically significant differences in the univariate analysis were included as independent variables in 2 logistic regression models based on the backward stepwise technique (Wald test). The dependent variables were isolation of at least 1 filamentous fungus or yeast and persistent *Aspergillus* spp. or *C. albicans*. In the case of elevated collinearity between 2 variables (Spearman or Pearson correlation coefficient >0.6), the variable with greater clinical significance was chosen, based on the judgment of the authors. The odds ratio (OR) and 95% confidence intervals (CI) for the independent variables were also calculated, and p values $\leq$ 0.05 were considered significant.

Results

Study Population

The initial sample comprised 296 patients with non-CF bronchiectasis. Of the 296 patients screened, 252 met the eligibility criteria and were included in the study (fig. 1). The baseline characteristics are shown in tables 1 and 2. The mean $\pm$ SD age was 55.3 $\pm$ 16.7 years (62.7% females). All patients had respiratory secretions cultured for fungi with a mean $\pm$ SD of 7 $\pm$ 6 cultures per patient. No patients received antifungal treatment during the study period. The most frequent etiology of bronchiectasis was postinfectious (36.1%). The etiology was unknown in 35.7% of cases. Cystic bronchiectasis was detected in 57 patients (27.1%).

Prevalence of Fungal Species

Filamentous fungi were identified at least once from respiratory tract secretions in 65 patients (25.8%) and yeasts were identified in 114 patients (45.2%). *Aspergillus*

Fig. 1. Flowchart of the study population. ABPA = Allergic bronchopulmonary aspergillosis.

spp. was identified at least once from respiratory tract secretions in 61 patients (24.2%) and *C. albicans* was identified in 114 patients (45.2%). Nine patients were infected with both. In 26 patients, the isolates of *Aspergillus* spp. were further classified into distinct fungal species. *Aspergillus fumigatus* isolates were identified in 20 patients (7.9%) and *Aspergillus niger* in 6 (2.4%). Other fungal species were recovered from 13 (5.2%) patients and included *Penicillium* (3 patients), *Mucor* (1 patient), *Scedosporium apiospermum* (2 patients), *Fusarium* (2 patients), *Saccharomyces cerevisiae* (1 patient), *Alternaria* (1 patient), and *Rhodotorula* (1 patients). One patient had *S. apiospermum* and *Mucor*, and another had *Penicillium* and *Mucor*. Eight patients had *Aspergillus* spp. and other filamentous fungi. The patient with *S. cerevisiae* also had *C. albicans*. Eighteen patients (8.7%) were considered to have persistent *Aspergillus* spp. and 71 (34.5%) persistent *C. albicans*.

Cohort Characteristics and Risk Factors for Isolation of Fungi

For the primary analysis, we compared the characteristics of patients who had at least 1 filamentous fungus or yeast with those of patients who had not. When comparing individual characteristics in patients with at least 1 filamentous fungal species, significant differences were only found in the macroscopic appearance of sputum (mucopurulent or purulent appearance; $p = 0.01$). Isolation of yeast was associated with older age ($p = 0.019$), female gender ($p = 0.049$), higher dyspnea ($p = 0.025$),

Table 1. Baseline characteristics of the study cohort (n = 252)

Age, years	55.3 ± 16.7
Female gender	158 (62.7)
Body mass index	24.8 ± 4.6
Etiology	
Idiopathic	90 (35.7)
Postinfectious	91 (36.1)
COPD	18 (7.1)
Systemic diseases	27 (10.7)
Ciliary dyskinesia	10 (4)
Immunodeficiency	9 (3.6)
Other	7 (2.8)
Smoking history (pack-years)	
Never smoked	170 (70.4)
Exsmoker	60 (24.4)
Current smoker	13 (5.2)
Dyspnea (mMRC)	
0	83 (34.5)
1	66 (27.5)
2	55 (22.9)
3	27 (11.3)
4	9 (3.8)
Appearance of sputum	
Mucoid	81 (35.8)
Mucopurulent or purulent	145 (64.2)
Cystic bronchiectasis	62 (24.7)
Number of affected lobes	2.6 ± 1.2
Respiratory insufficiency	23 (9.1)
FVC, % predicted	75.7 ± 23.2
Postbronchodilator FEV ₁ , % predicted	68.6 ± 22
CRP, IU/ml	3.5 ± 5.9
Hospitalizations	0.6 ± 1.1
Exacerbations	3 ± 2.1
Courses of oral corticosteroids	0.4 ± 1.2
Long-term antibiotic treatment	83 (32.9)
Long-term corticosteroids	7 (4)
Long-term macrolide treatment	28 (11.1)

Values are expressed as means ± SD or as numbers (%). COPD = Chronic obstructive lung disease; mMRC = modified Medical Research Council; CPR = C-reactive protein.

worse macroscopic appearance of sputum ($p = 0.009$), more cystic bronchiectasis ($p = 0.048$), and worse postbronchodilator FEV₁, % predicted ($p = 0.017$). These patients were also more frequently colonized with *Pseudomonas aeruginosa* ($p = 0.029$) and less frequently colonized with *Haemophilus influenzae* ($p = 0.027$). Furthermore, they took more long-term oral and inhaled antibiotics ($p = 0.009$) and had significantly more hospital-treated exacerbations ($p = 0.041$).

After adjustment for confounders, the variables that significantly increased the odds of yeast isolation were older age, macroscopic appearance of sputum (mucopu-

Table 2. Microbiological characteristics of the study cohort (n = 252)

	n (%)
Chronic bacterial infection	
<i>P. aeruginosa</i>	104 (41.3)
<i>H. influenzae</i>	55 (21.8)
Chronic bacterial infection, other PPMs	22 (8.7)
Isolation of <i>Aspergillus</i> spp.	61 (24.2)
Isolation of <i>C. albicans</i>	114 (45.2)
PPMs = Potentially pathogenic microorganisms.	

Table 3. Multiple logistic regression analysis of risk factors for isolation of yeast^a (n = 114)

	Adjusted OR (95% CI)	p value
Age (years)	1.02 (1.01–1.04)	0.02
Macroscopic appearance of sputum (mucopurulent or purulent)	1.41 (1.1–1.99)	0.049
Postbronchodilator FEV ₁ (% predicted)	0.98 (0.97–0.99)	0.002
Long-term antibiotic treatment	2.04 (1.1–3.8)	0.024

Adjusted ORs are presented for the most significant predictors. Backward stepwise logistic regression was used to obtain the strongest predictors.

^a The variables entered in the fully adjusted model were age, gender, dyspnea, macroscopic appearance of sputum, onset of cystic bronchiectasis, postbronchodilator FEV₁ (% predicted), chronic *P. aeruginosa* infection, chronic *H. influenzae* infection, number of hospitalizations, and long-term antibiotic treatment.

ulent or purulent), worse postbronchodilator FEV₁, and use of chronic antibiotics (table 3).

For the secondary analysis, we compared the characteristics of the patients according to the persistence of *Aspergillus* spp. and *C. albicans* (table 4).

Patients with persistent *Aspergillus* spp. were significantly older (67 vs. 56 years; $p = 0.029$) and produced sputum with a worse macroscopic appearance (83.3% of patients with mucopurulent or purulent sputum vs. 61.8%; $p = 0.002$). No differences were observed with respect to functional impairment, number of pulmonary exacerbations, and long-term antibiotic treatment. Patients with persistent *C. albicans* were also older (62 vs. 54 years; $p = 0.012$) and had more advanced lung disease in terms of lung function [FVC, % predicted, 68.1 vs. 78.8 ($p = 0.003$) and FEV₁, postbronchodilator, % predicted, 58.4 vs. 71.0 ($p = 0.012$)] and more frequent radiological

Table 4. Characteristics of patients with and without persistent *Aspergillus* spp. and *C. albicans* in respiratory secretions

	Persistent <i>Aspergillus</i> spp.			Persistent <i>C. albicans</i>		
	patients without (n = 188)	patients with (n = 18)	p value	patients without (n = 135)	patients with (n = 71)	p value
Age, years	56 (42; 67)	67 (51.3; 73.3)	0.029	54 (40; 68)	62 (50; 70)	0.012
Female gender	121 (62.8)	12 (66.7)	0.743	91 (67.4)	38 (53.5)	0.050
Body mass index	25 (21; 28)	26 (21; 27)	0.947	25 (21; 28)	25 (22; 28)	0.467
Smoking history (pack-years)			0.686			0.842
Never smoked	136 (72.3)	13 (72.2)		96 (71.1)	52 (73.2)	
Exsmoker	41 (21.8)	5 (27.8)		32 (23.7)	15 (21.2)	
Current smoker	11 (5.9)	0		7 (5.2)	4 (5.6)	
Dyspnea (mMRC)			0.179			0.017
0	58 (32.6)	3 (18.8)		48 (36.9)	13 (20.3)	
1	51 (28.7)	5 (31.3)		38 (29.2)	19 (29.7)	
2	45 (25.3)	3 (18.8)		26 (20.1)	21 (32.8)	
3	16 (9)	5 (31.3)		16 (12.3)	7 (10.9)	
4	8 (4.5)	0		2 (1.5)	4 (6.3)	
Appearance of sputum			0.002			0.008
Mucoid	66 (38.2)	3 (16.7)		51 (41.5)	18 (26.5)	
Mucopurulent or purulent	107 (61.8)	15 (83.3)		72 (58.5)	50 (73.5)	
Cystic bronchiectasis	48 (25.5)	4 (22.2)	1.000	29 (21.5)	24 (33.8)	0.049
Number of affected lobes	2 (2; 3)	3 (2; 2.3)	0.286	2 (2; 3)	3 (2; 4)	0.129
Respiratory insufficiency	19 (10.1)	0	0.384	9 (6.7)	9 (12.7)	0.147
FVC, % predicted	74.2±22.6	71.3±21	0.615	78.8±22.8	68.1±19.9	0.003
Postbronchodilator FEV ₁ , % predicted	67.3±22.5	78.2±73	0.285	71.0±21	58.4±22	0.012
CRP	1 (0; 5)	2 (1; 2)	0.555	1 (0; 5)	1 (0; 3)	0.610
Chronic bacterial infection						
<i>P. aeruginosa</i>	83 (44.1)	9 (50)	0.633	52 (38.5)	39 (54.9)	0.024
<i>H. influenzae</i>	43 (22.9)	4 (22.2)	1.000	38 (28.1)	9 (12.7)	0.012
Chronic bacterial infection, other PPMs	15 (11.7)	2 (12.5)	0.870	10 (11.8)	8 (14.5)	0.280
Isolation of <i>Aspergillus</i> spp.	n.a.	n.a.	n.a.	33 (24.4)	23 (32.4)	0.223
Isolation of <i>C. albicans</i>	91 (48.4)	12 (66.7)	0.139	n.a.	n.a.	n.a.
Hospitalizations	0 (0; 1)	1 (0; 1.3)	0.105	0 (0; 1)	0 (0; 1)	0.049
Exacerbations	3 (2; 4)	2.5 (2; 4.5)	0.772	2 (2; 4)	4 (2; 6)	0.051
Courses of oral corticosteroids	0 (0; 0)	0 (0; 4)	0.074	0 (0; 0)	0 (0; 1)	0.001
Long-term antibiotic treatment	68 (36.2)	9 (50)	0.247	41 (30.4)	36 (50.7)	0.004
Long-term corticosteroid treatment	5 (3.8)	2 (20)	0.077	4 (3.9)	3 (7.5)	0.402
Long-term macrolide treatment	24 (12.8)	2 (11.1)	1.000	20 (14.8)	5 (7)	0.120

Values are expressed as medians (IQR), numbers (%), or means ± SD. mMRC = Modified Medical Research Council; CRP = C-reactive protein; PPMs = potentially pathogenic microorganisms; n.a. = not assessed.

evidence of cystic bronchiectasis (33.8 vs. 21.5%; $p = 0.049$). Their sputum had a significantly worse macroscopic appearance (73.5% of patients with mucopurulent or purulent sputum vs. 58.5%; $p = 0.008$), and their dyspnea score was higher ($p = 0.017$). In addition, patients with persistent *C. albicans* had significantly more hospital-treated exacerbations ($p = 0.049$), received more courses of oral corticosteroids for exacerbations ($p = 0.001$), and were prescribed significantly more long-term oral and inhaled antibiotics ($p = 0.004$). Of note, they

were more frequently colonized with *P. aeruginosa* (54.9 vs. 38.5%; $p = 0.024$) and less frequently with *H. influenzae* (12.7 vs. 28.1%; $p = 0.012$). Moreover, patients with persistent *C. albicans* had more exacerbations, and the difference tended toward statistical significance ($p = 0.051$).

Logistic regression was used to determine the most significant predictors of fungal persistence. The significant variables entered into the multiple logistic regression models for *Aspergillus* were age and macroscopic appear-

Table 5. Multiple logistic regression analysis of risk factors for persistence of *Aspergillus* spp. and *C. albicans*

	Adjusted OR (95% CI)	p value
Factors associated with <i>Aspergillus</i> spp. ^a		
Macroscopic appearance of sputum (mucopurulent or purulent)	3.75 (1.03; 13.14)	0.045
Factors associated with <i>C. albicans</i> ^b		
Long-term antibiotic treatment	2.37 (1.30; 4.17)	0.005

Adjusted ORs are presented for the most significant predictors. Backward stepwise logistic regression was used to obtain the strongest predictors.

^a The variables entered in the fully adjusted model were age and macroscopic appearance of sputum.

^b The variables entered in the fully adjusted model were age, dyspnea, macroscopic appearance of sputum, onset of cystic bronchiectasis, postbronchodilator FEV₁ (% predicted), chronic *P. aeruginosa* infection, chronic *H. influenzae* infection, number of hospitalizations, and long-term antibiotic treatment.

ance of sputum; the variables entered for *C. albicans* were age, dyspnea, appearance of sputum, presence of cystic bronchiectasis, FEV₁ (postbronchodilator, % predicted), chronic *P. aeruginosa* infection, chronic *H. influenzae* infection, number of hospitalizations, and chronic antibiotic use. In the final model, after adjustment for confounders, the only factor that significantly increased the odds of persistence of *Aspergillus* was mucopurulent or purulent sputum. In the case of *C. albicans*, the only variable that significantly increased the odds of persistence was long-term antibiotic therapy (table 5).

Discussion

To our knowledge, this is the first study to analyze the prevalence of and factors associated with the isolation and persistence of fungi in a large series of patients with non-CF bronchiectasis. Our data show high rates of fungal isolation and persistence in respiratory secretions, where *Aspergillus* spp. and *C. albicans* were the most commonly isolated microorganisms. According to our results, the presence of mucopurulent or purulent sputum is associated with persistence of *Aspergillus* spp.; long-term antibiotic therapy is associated with persistence of *C. albicans*.

In addition to bacterial colonization, the lower airways of patients with bronchiectasis could be predisposed to

fungal growth owing to impaired mucociliary clearance, mucus plugging, and the ability of fungi to evade or interfere with host defenses [15]. The associated factors and the clinical significance for both filamentous fungi and yeasts in this group of patients remain unclear.

In the present study, prevalence rates recorded for isolation of *Aspergillus* spp. and *C. albicans* were high, in fact, much higher than the prevalence rate for single isolation of this fungus reported by other authors [16–18] and more consistent with the prevalence in patients with chronic obstructive pulmonary disease [19] and CF [2], where the prevalence of *Aspergillus* ranges from 6 to 58% and that of *C. albicans* stands at 50%. Although culture of yeast in sputum is a common practice in people receiving inhaled corticosteroids, the high rate of *C. albicans* isolation in patients with bronchiectasis cannot be explained by use of this medication, since inhaled corticosteroids are not administered routinely in patients with bronchiectasis [9, 20].

We found that purulent sputum was independently associated with the persistence of *Aspergillus*. Although there are no published data on factors associated with *Aspergillus* in patients with non-CF bronchiectasis, Angrill et al. [16] reported an association between chronic expectoration and colonization by potentially pathogenic bacteria in non-CF patients, probably because of the propensity of bacteria and fungi to grow in thick secretions in chronic airway diseases [2, 9]. The clinical factors associated with isolation of *Aspergillus* in patients with CF are open to debate. It has been suggested that increasing age [5, 8, 21, 22], more severe lung disease [8, 21], and azithromycin therapy [23] are risk factors for isolating filamentous fungi in CF. Moreover, chronic antibiotic treatment [8, 22, 24, 25] was also associated with the persistence of *Aspergillus* spp. In addition, culture of *A. fumigatus* from sputum samples is a common feature of chronic obstructive pulmonary disease, but the clinical significance of this finding remains uncertain [19].

Apart from the well-known occurrence in patients with asthma and CF, *Aspergillus* hypersensitivity can also occur in patients with chronic obstructive pulmonary disease [26] and pulmonary tuberculosis-related fibrocavitary disease [27]. The presence of *Aspergillus* hypersensitivity correlates with reduced lung function in patients with asthma and pulmonary tuberculosis-related fibrocavitary disease [27–30], while this correlation is more equivocal in patients with CF bronchiectasis [31, 32].

However, the clinical relevance and the therapeutic implications of *Aspergillus* sensitization in these diseases

require further investigation. Unfortunately, we did not perform allergy testing to investigate the association between sensitization to *A. fumigatus*, *A. fumigatus* culture, and pulmonary function.

Our results revealed a univariate association between respiratory impairment and persistence of *C. albicans*. Furthermore, patients with persistent *C. albicans* in their respiratory secretions had significantly more hospital-treated exacerbations, more frequently received oral corticosteroids for exacerbations, and were prescribed more long-term antibiotic treatment. Although patients with more severe disease are more frequently treated with chronic antibiotics than patients with less severe lung impairment, the results obtained in the multivariate analysis suggest that the association between chronic antibiotic therapy and persistence of *C. albicans* cannot be explained by increased disease severity. In addition, the frequent chronic antibiotic treatment prescribed to our patients could explain the higher ranges of persistence of *C. albicans* we detected compared with other authors [16–18]. Although there are no data on long-term antipseudomonal treatment in these reports, the small number of patients colonized with *Pseudomonas* spp. makes it unlikely that this treatment was administered.

Despite the lack of published data on non-CF bronchiectasis, our results are in keeping with those from previous reports on CF, which suggest that long-term antibiotic therapy and frequent courses of antibiotics can lead to increased isolation of and colonization by *C. albicans* [24, 33]. Moreover, in the univariate analysis, we found that persistence of *C. albicans* was significantly associated with colonization by *P. aeruginosa* but not with colonization by *H. influenzae*. Chronic antibiotic treatment is widely used to treat infection by *P. aeruginosa* but not infection by *H. influenzae*. Antipseudomonal treatment may lead to increases in the sputum density of patients harboring *C. albicans* without eradicating *P. aeruginosa*. The relationship between these organisms has been described elsewhere [34, 35], and in vitro studies showed that *P. aeruginosa* can form dense biofilms on *C. albicans* hyphae and does not bind to or kill *C. albicans* [36].

We found an increased persistence of *C. albicans* – but not *Aspergillus* spp. – in non-CF bronchiectasis patients receiving long-term antibiotic therapy, possibly because sputum sampling can significantly underestimate persistence of *Aspergillus*, as suggested in previous works in CF involving serum immune responses to this fungus [5, 29, 37, 38] or DNA-based techniques [39]. Another potential explanation could be that chronic antibiotics are not really a risk factor for persistence of *Aspergillus*.

Apart from the logical limitations related to the inclusion of a historical series in our analyses, there are other limitations which are worthy of being mentioned. First, it is still unknown whether a positive sputum culture of filamentous fungi or yeasts represents true bronchial colonization. Second, although the optimal definition of fungal infection in the lower airways is unclear, more samples are recommended for defining other chronic lung infections, such as *Pseudomonas*, in CF patients [40]. Furthermore, our study has other methodological limitations: contamination by *C. albicans* was not assessed by physical examination (*Candida* is a commensal in the oral cavity), and patients were not tested for HIV.

In summary, we found a high prevalence of persistence of *Aspergillus* spp. and *C. albicans* in clinically stable patients with non-CF bronchiectasis. We also observed that the presence of daily sputum purulence was associated with persistence of *Aspergillus* and that long-term antibiotic treatment was associated with persistence of *C. albicans*. Our results highlight the need to better understand the relevance of fungi in non-CF bronchiectasis.

Further prospective studies are necessary to evaluate the epidemiologic trends, associated factors, and the clinical implications of fungal persistence in non-CF bronchiectasis. Also, the effect of fungal colonization and sensitization on airway inflammation and lung function in these patients should be explored.

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References

- Cole PJ: Inflammation: a two-edged sword – the model of bronchiectasis. *Eur J Respir Dis Suppl* 1986;147:6–15.
- 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;12:187–193.
- King P, Holdsworth S, Freezer N, et al: Bronchiectasis. *Intern Med J* 2006;36:729–737.
- Chotirmall SH, O'Donoghue E, Bennett K, et al: Sputum *Candida albicans* presages FEV₁ decline and hospital-treated exacerbations in cystic fibrosis. *Chest* 2010;138:1186–1195.
- Máiz L, Cuevas M, Lamas A, et al: *Aspergillus fumigatus* and *Candida albicans* in cystic fibrosis: clinical significance and specific immune response involving serum immunoglobulins G, A, and M (in Spanish). *Arch Bronconeumol* 2008;44:146–151.
- Baxter CG, Rautema R, Jones AM, et al: Intravenous antibiotics reduce the presence of *Aspergillus* in adult cystic fibrosis sputum. *Thorax* 2013;68:652–657.
- Amin R, Dupuis A, Aaron SD, et al: The effect of chronic infection with *Aspergillus fumigatus* on lung function and hospitalization in patients with cystic fibrosis. *Chest* 2010;137:171–176.
- Sudfeld CR, Dasenbrook EC, Merz WG, et al: Prevalence and risk factors for recovery of filamentous fungi in individuals with cystic fibrosis. *J Cyst Fibros* 2010;9:110–116.
- Vendrell M, de Gracia J, Oliveira C, et al: Diagnosis and treatment of bronchiectasis. Spanish Society of Pneumology and Thoracic Surgery (in Spanish). *Arch Bronconeumol* 2008;44:629–640.
- Agarwal R, Chakrabarti A, Shah A, et al: ABPA complicating asthma ISHAM working group: Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. *Clin Exp Allergy* 2013;43:850–873.
- Gaga M, Bentley AM, Humbert M, et al: Increases in CD4⁺ T lymphocytes, macrophages, neutrophils and interleukin 8 positive cells in the airways of patients with bronchiectasis. *Thorax* 1998;53:685–691.
- Angrill J, Agustí C, Torres A: Bronchiectasis. *Curr Opin Infect Dis* 2001;14:193–197.
- Naidich DP, McCauley DI, Khouri NF, et al: Computed tomography of bronchiectasis. *J Comput Assist Tomogr* 1982;6:437–444.
- Lee TW, Brownlee KG, Conway SP, et al: Evaluation of a new definition for chronic *Pseudomonas aeruginosa* infection in cystic fibrosis patients. *J Cyst Fibros* 2003;2:29–34.
- 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.
- Angrill J, Agustí C, de Celis R, et al: Bacterial colonisation in patients with bronchiectasis: microbiological pattern and risk factors. *Thorax* 2002;57:15–19.
- King PT, Holdsworth SR, Freezer NJ, et al: Microbiologic follow-up study in adult bronchiectasis. *Respir Med* 2007;101:1633–1638.
- Nicotra MB, Rivera M, Dale AM, et al: Clinical, pathophysiologic, and microbiologic characterization of bronchiectasis in an aging cohort. *Chest* 1995;108:955–961.
- Bafadhel M, McKenna S, Agbetile J, et al: *Aspergillus fumigatus* during stable state and exacerbations of COPD. *Eur Respir J* 2014;43:64–71.
- Pasteur MC, Bilton D, Hill AT; British Thoracic Society Bronchiectasis non-CF Guideline Group: British Thoracic Society guideline for non-CF bronchiectasis. *Thorax* 2010;65(suppl 1):i1–i58.
- Milla CE, Wielinski CL, Regelman WE: Clinical significance of the recovery of *Aspergillus* species from the respiratory secretions of cystic fibrosis patients. *Pediatr Pulmonol* 1996;21:6–10.
- 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.
- 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.
- 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;179:1190–1196.
- Bargon J, Daulebaev N, Köhler 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.
- Agarwal R, Hazarika B, Gupta D, et al: *Aspergillus* hypersensitivity in patients with chronic obstructive pulmonary disease: COPD as a risk factor for ABPA? *Med Mycol* 2010;48:988–994.
- Dhooria S, Kumar P, Saikia B, et al: Prevalence of *Aspergillus* sensitisation in pulmonary tuberculosis-related fibrocavitary disease. *Int J Tuberc Lung Dis* 2014;18:850–855.
- Agbetile J, Fairs A, Desai D, et al: Isolation of filamentous fungi from sputum in asthma is associated with reduced post-bronchodilator FEV₁. *Clin Exp Allergy* 2012;42:782–791.
- Fairs A, Agbetile J, Hargadon B, et al: IgE sensitization to *Aspergillus fumigatus* is associated with reduced lung function in asthma. *Am J Respir Crit Care Med* 2010;182:1362–1368.
- Agarwal R, Noel V, Aggarwal AN, et al: Clinical significance of *Aspergillus* sensitisation in bronchial asthma. *Mycoses* 2011;54:e531–e538.
- Wojnarowski C, Eichler I, Gartner C, et al: Sensitization to *Aspergillus fumigatus* and lung function in children with cystic fibrosis. *Am J Respir Crit Care Med* 1997;155:1902–1907.
- Máiz 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.
- Blaschke-Hellmessen R, Spitzer H, Paul KD, et al: Mycological surveillance of children with cystic fibrosis (in German). *Mycoses* 1991;34(suppl 1):43–47.
- McAlester G, O'Gara F, Morrissey JP: Signal-mediated interactions between *Pseudomonas aeruginosa* and *Candida*. *J Med Microbiol* 2008;57(Pt 5):563–569.
- Nseir S, Jozefowicz E, Cavestri B, et al: Impact of antifungal treatment on *Candida-Pseudomonas* interaction: a preliminary retrospective case-control study. *Intensive Care Med* 2007;33:137–142.
- Hogan DA, Kolter R: *Pseudomonas-Candida* interactions: an ecological role for virulence factors. *Science* 2002;296:2229–2232.
- el-Dahr JM, Fink R, Selden R, et al: Development of immune responses to *Aspergillus* at an early age in children with cystic fibrosis. *Am J Respir Crit Care Med* 1994;150:1513–1518.
- 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.
- 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;48:166–176.
- Pressler T, Bohmova C, Conway S, et al: Chronic *Pseudomonas aeruginosa* infection definition: EuroCareCF Working Group report. *J Cyst Fibros* 2011;10(suppl 2):S75–S78.