

ORIGINAL ARTICLE

Assessment of Aspergillus sensitization or persistent carriage as a factor in lung function impairment in cystic fibrosis patients

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Abstract

Background: Cystic fibrosis (CF) patients presenting with persistent carriage of, or sensitization to, *Aspergillus fumigatus* are often treated with antifungal therapies because the presence of the fungus is commonly thought to impede lung function, even in the absence of allergic bronchopulmonary aspergillosis (ABPA). The aim of this study was to assess *Aspergillus*-related status modulating the forced expiratory volume in 1 s (FEV₁) of CF patients. **Methods:** From 1995 to 2007, 251 patients were evaluated. Demographic data, cystic fibrosis transmembrane conductance regulator gene (CFTR) mutations, body mass index, and FEV₁ were recorded. The presence of *A. fumigatus* and *Pseudomonas aeruginosa* in sputum and the levels of *A. fumigatus* precipitin, total IgE (t-IgE), and specific anti-*A. fumigatus* IgE (Af-IgE) were determined. Patients were divided into 3 groups: (1) ABPA: *A. fumigatus* precipitin ≥ 3 lines, Af-IgE > 0.35 IU/ml, and t-IgE ≥ 500 IU/ml; (2) sensitization: Af-IgE > 0.35 IU/ml but t-IgE < 500 IU/ml; and (3) persistent carriage: Af-IgE ≤ 0.35 IU/ml with either an *A. fumigatus* persistent positive culture or an *A. fumigatus* precipitin ≥ 3 lines, provided this serological finding had been found associated with at least 1 *A. fumigatus*-positive culture. The remaining patients represented the control group. A multivariate analysis was carried out with FEV₁ as the outcome variable. **Results:** ABPA, sensitization, and persistent carriage were significantly associated with a larger decline in FEV₁ compared with the control group, with odds ratios of 15.9, 14.9, and 10.7, respectively. This association was independent of other associated factors (*P. aeruginosa* transient detection, age, being underweight, and low FEV₁ at baseline). **Conclusions:** In addition to ABPA, sensitization and persistent carriage appear to have an impact on pulmonary function in CF patients.

Keywords: Cystic fibrosis, fungal infection, pulmonary function, epidemiology

Introduction

Cystic fibrosis (CF) is one of the most common life-shortening autosomal-recessive diseases in Caucasians, affecting 1 in 2500 individuals [1]. Chronic endobronchial infection with CF-related pathogens and the associated neutrophil-dominated airway inflammation contribute to progressive obstructive pulmonary disease. *Pseudomonas aeruginosa* is the most significant bacterial pathogen associated with CF, and based on immune responses in young children, infection appears to occur much earlier than

previously thought [2]. The presence of *P. aeruginosa* and other pathogens in the lower airway is hypothesized to increase the host inflammatory response and thereby accelerate the process of airway damage [3]. Infection has been identified as an independent risk factor for the rate of decline in the forced expiratory volume in 1 s (FEV₁) in children and adolescents presenting with CF [4,5].

In contrast to *P. aeruginosa*, the impact of *Aspergillus fumigatus* on pulmonary function is less well known. *Aspergillus* spp., most frequently *A. fumigatus*,

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are isolated from more than 25% of patients [2]. *A. fumigatus* is a fungal pathogen that causes a wide range of pulmonary diseases, which are not restricted to CF patients. One of these diseases, allergic bronchopulmonary aspergillosis (ABPA) is particularly frequent in CF patients, with a prevalence ranging from 2% to 25% [6–9]. Recommendations for the treatment of ABPA in patients with CF are now clearly established [10,11]. However, in the absence of ABPA, the pathogenic role of *A. fumigatus* [12–14] and the benefit of treatment [15,16] of an *A. fumigatus* bronchial infection, as diagnosed from a positive sputum culture, remain to be clarified. Nevertheless, CF patients presenting with *A. fumigatus* persistent positive cultures or sensitization to *A. fumigatus* often receive antifungal therapies because, among lung specialists, the presence of the fungus is commonly thought to impede lung function, even in the absence of ABPA. The aim of this study was therefore to assess the impact of persistent *A. fumigatus* carriage and sensitization as independent risk factors of a decline in pulmonary function in CF patients.

Materials and methods

From January 1995 to July 2007, details of patients with CF at the Toulouse Cystic Fibrosis Resources and Competence Centre of the Toulouse University Hospitals were entered into a database. The diagnosis of CF relied upon the detection of an iontophoretic sweat chloride level of 60 mEq/l or greater [17,18]. The database contained all data collected by physicians during each patient's visit, which occurred twice per year or more frequently if necessary. After the patients had received detailed information about the purpose of establishing this database, all of them gave their written consent for any electronic medical use of their information. Management of the database was compliant with French regulations concerning the protection of privacy (Commission Nationale Informatique et Libertés, Data Protection Statement No. 1203335). Patients lost to follow-up, those who died, and those who were transferred to another medical centre were evaluated based on our most recent information.

Demographic and medical characteristics

Date of birth, gender, diagnosis date, and cystic fibrosis transmembrane conductance regulator gene (CFTR) mutations were entered into the database. As previously reported [19–22], the 'severe/mild mutation' classification system [23] was used, and CFTR gene mutations were classified as mild mutations (classes IV and V, associated with residual

CFTR function) or severe mutations (classes I to III, equivalent to the 'null' mutation consequence, including patients with pancreatic insufficiency).

Body mass index (BMI) values and FEV₁ were recorded at each visit. BMI classes (underweight/normal weight) were determined using French age- and sex-specific curves if the age was under 18 y [24–26] and using international cut-off points for adults if the age was over 18 y. FEV₁ results were expressed as predicted percentages based on the accepted reference standard [27]. The result of the first FEV₁ yielded the FEV₁ baseline.

Laboratory investigations

Expectorated or induced sputum was collected at each visit and tested for the presence of *A. fumigatus* and *P. aeruginosa*. *A. fumigatus* was identified on the basis of macroscopic and microscopic examination of the culture [28] after the inoculation of vials containing Sabouraud–chloramphenicol–gentamicin agar medium (Bio-Rad, Marnes-la-Coquette, France). *P. aeruginosa* was identified according to the national recommendations for CF specimens [29]. Colonization by *P. aeruginosa* was defined as the presence of the pathogen in the bronchial tree for at least 6 months, based on at least 3 positive cultures taken at intervals of at least 1 month [30,31]. For *A. fumigatus*, the same definition was used but the term 'persistent positive culture' was chosen because it is impossible clinically to distinguish between colonization and infection. Positive detection without *A. fumigatus* persistent positive culture or *P. aeruginosa* colonization was defined as transient detection for *A. fumigatus* and *P. aeruginosa*, respectively. The Leeds criteria [32] could not be applied because approximately 50% of the patients made fewer than 4 visits a year. As a consequence, these patients would have remained unclassifiable.

The presence of precipitating antibodies to *A. fumigatus* (*A. fumigatus* precipitin) was detected using immunoelectrophoresis (IEP) (Beckman Instruments, CA, USA) against *A. fumigatus* antigenic extracts and metabolites (Bio-Rad, Marnes-la-Coquette, France). The levels of total IgE (t-IgE) and specific anti-*A. fumigatus* IgE (Af-IgE) were determined by fluoro-enzyme immunoassay using an ImmunoCap[®] automated system (Phadia, Montigny-le Bretonneux, France). All laboratory data were verified from the database handled by the management software in use in the laboratories of bacteriology, mycology, or immunology.

Patients were divided into groups according to the results of the laboratory investigations. The ABPA group included patients who displayed ≥ 3 precipitin lines found by IEP [33,34], a level of Af-IgE > 0.35

IU/ml, and a t-IgE dosage ≥ 500 IU/ml. The sensitization group displayed a level of Af-IgE > 0.35 IU/ml with a t-IgE dosage < 500 IU/ml. The characteristics of the persistent carriage group were the combination of an Af-IgE level ≤ 0.35 IU/ml with either an *A. fumigatus* persistent positive culture or the detection of *A. fumigatus*-precipitin ≥ 3 lines, provided this serological finding had been found associated with an *A. fumigatus* transient detection. The remaining patients represented the control group. At the end of the study, patients were ranked among the 3 groups. The 3 groups of patients were graded according to the patient's status from the worst to the best. This classification was based upon the following assumption: ABPA $>$ sensitization $>$ persistent carrier. Since the patient's status could worsen during the study period, some of them were finally allotted to a lower group, according to the definition of the groups.

Statistical analysis

The characteristics of the studied population were described using percentages and medians along with interquartile ranges (IQR) instead of means and standard deviations when the distributions were found to be non-Gaussian. A bivariate analysis was performed using the Kruskal-Wallis test (for non-normally distributed continuous variables), analysis of variance (ANOVA; for normally distributed continuous variables), or the Chi-square test (for dichotomous variables). Then, a multivariate analysis (linear mixed-effect model analysis [35]) was carried out with FEV₁ as the outcome variable, using a step-by-step descending method. The heterogeneity in the number of sputa and their spacing was managed by choosing the linear mixed-effect model analysis. This technique is convenient for the analysis of association between time and covariates from irregularly spaced serial data from individuals (i.e., repeated measurements), without being affected by missing data. Only variables found to be significant by bivariate analysis, with $p < 0.2$, were tested. The odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to describe associated factors (p -value of less than 0.05). All statistical tests and procedures were performed using the Intercooled Stata 9.2® statistical package (StataCorp, College Station, TX, USA).

Results

Two hundred and fifty-one patients were cumulatively evaluated 6242 times from 1 January 1995 to 3 July 2007. Among these patients, a complete set of data was available for 206 patients. The 45 patients for whom several parameters were missing were not

significantly different from the included patients (data not shown). Seventeen patients died during the study period, 16 were transferred to another medical centre, and 8 were lost to follow-up. The demographic and medical characteristics along with the results of the laboratory investigations are displayed in Table I.

One hundred and thirty-four patients exhibited CF associated with the characteristics of 1 of the 3 groups, namely ABPA ($n = 34$, 16%), sensitization to *A. fumigatus* ($n = 63$, 31%), or persistent carriage ($n = 37$, 18%); 72 (35%) represented the control group. Furthermore, when patients became sensitized, their Af-IgE levels remained positive until the end of the study.

Table II presents the results of the statistical analysis. The presence of ABPA, sensitization, or persistent carriage, being underweight, transient detection of *P. aeruginosa*, age, and a lower FEV₁ at baseline were significantly associated with an increased risk of having a change in the FEV₁. Colonization by *P. aeruginosa*, a high level of t-IgE, the severity of the mutation, and *A. fumigatus*-related abnormalities analyzed separately (transient detection of *A. fumigatus*, *A. fumigatus* persistent positive culture, or

Table I. Characteristics of the study population (number of patients = 251; number of visits = 6242); results are median (IQR), or n (%).

Age at the date of the CF diagnosis (months)	8.2 (1.6–38.4)
Age at the end of the study (y)	16.3 (9.8–23.6)
Follow-up duration (y)	3.6 (2.1–8.7)
Number of visits per patient per y	4.7 (3.4–6.7)
Number of FEV ₁ tests per patient	10 (2–23)
Baseline FEV ₁ (%)	83 (63–102)
Sex (male)	138 (55)
Severe mutation	175 (69.7)
CFTR mutation	
ΔF508/ΔF508	95 (37.8)
ΔF508/other	106 (42.2)
Other/other	29 (11.6)
Incomplete or unknown genotype	21 (8.4)
Known diagnosis at birth	18 (7.2)
At least 1 sputum examination positive for Pa	171 (68.1)
At least 1 sputum examination positive for Af	156 (62.1)
At least 1 episode of Pa colonization	54 (21.5)
At least 1 episode of Af persistent positive culture	68 (27.1)
At least 1 result showing Af IgE > 0.35 IU/ml	102 (39.5)
At least 1 result showing ≥ 3 precipitin lines with Af extracts	100 (39.8)
At least 1 result showing total IgE ≥ 500 IU/ml	36 (14.5)

IQR, interquartile range; CF, cystic fibrosis; FEV₁, forced expiratory volume in 1 s; CFTR, cystic fibrosis transmembrane conductance regulator gene; Pa, *Pseudomonas aeruginosa*; Af, *Aspergillus fumigatus*; IgE, immunoglobulin E.

Table II. Risk of a change in lung function (number of patients = 206; number of visits = 3249).

Variables	Bivariate analysis		Multivariate analysis
	FEV ₁ (%), median (IQR)	p-Value	Adjusted OR (95% CI)
Female		0.91	-
Male			-
Non-severe mutation		0.4	-
Severe mutation			-
Control group	92.5 (66–109)	< 0.01	1
Persistent carriage group	76 (60–98)		10.7 (3.0–18.3)
Sensitization group	76 (52–99)		14.9 (8.4–21.4)
ABPA group	81 (57–102)		15.9 (8.3–23.5)
Age at the visit (y)		< 0.01	1.9 (1.8–2.1)
Baseline FEV ₁ (%)		< 0.01	0.4 (0.3–0.5)
Normal weight	92 (70–108)	< 0.01	1
Underweight	56 (39–78)		7.7 (6.2–9.3)
No Pa detection	95 (71–111)	< 0.01	1
Transient Pa detection	68 (44–92)		8.0 (3.9–12.1)

FEV₁, forced expiratory volume in 1 s; IQR, interquartile range; OR, odds ratio; 95% CI, 95% confidence interval; ABPA, allergic bronchopulmonary aspergillosis; Pa, *Pseudomonas aeruginosa*.

presence of *A. fumigatus* precipitin) were not significantly associated with a worse FEV₁. So the model that included the variable 'group' was preferred to a model that considered each *A. fumigatus*-related parameter separately. As shown in Table II, according to the multivariate analysis, ABPA, sensitization, and persistent carriage were significantly associated with a larger FEV₁ decline compared with the control group, with ORs of 15.9, 14.9, and 10.7, respectively. Furthermore, this association was independent of other associated factors such as transient detection of *P. aeruginosa*, increasing age, being underweight, and low FEV₁ at baseline. Figure 1 presents the slopes of the change in FEV₁ according to the significant variables.

Discussion

As far as we know, this study presents the first evaluation of the impact of both the sensitization and the persistent carriage of *A. fumigatus* on the FEV₁ in paediatric and adult CF patients examined as part of a 12-y observational cohort maintained at the Toulouse University Hospital, France. Unlike the assessment of each *A. fumigatus*-related parameter independently, the 3 groups together, i.e., ABPA, sensitization, and persistent carriage, were significantly and independently associated with a larger FEV₁ decline compared with the control group. Furthermore, this relationship was independent of transient *P. aeruginosa* detection. These results strengthen our hypothesis that the presence of *A. fumigatus*-positive sputum must not be considered separately

but rather must be regarded as part of a clinical condition to assess its harmful influence.

Our cohort was similar to those found in the literature with respect to the sex ratio [5,6,36], age [4,6,36], $\Delta F508$ mutation ratio [6,36], frequency of ABPA [6–9], frequency of *A. fumigatus* persistent positive culture [37], frequency of *P. aeruginosa* colonization [12], and *P. aeruginosa* transient detection [5]. As shown in other studies, transient *P. aeruginosa* detection [4,5,36], low weight-for-age [5], and greater age [4] were also independently associated with a worse respiratory status. Regarding the effect of genetic mutations on FEV₁, in our study, unlike others [4,36], the severity of the CFTR genotype was not independently associated with changes in FEV₁. At the present time, the literature on the effect of genetic CF mutations remains conflicting [19,21,22]. The effect on the decline in lung function of *P. aeruginosa* transient detection – but not of *P. aeruginosa* colonization – was demonstrated in our study. The explanation for this finding could be that antibiotic prophylaxis of *P. aeruginosa* colonization was increasingly employed through the period of the study. Unfortunately, the data concerning treatment were not sufficiently reliable to be used in the statistical analysis. Because it was an observational cohort, the number of visits per patient was heterogeneous, depending on the severity of the pulmonary disease. When patients were clinically lightly affected, they attended consultations only twice a year. This aspect has been managed by the statistical methods used.

First, in concordance with Kraemer et al. [36], we found that a sensitized profile with or without ABPA

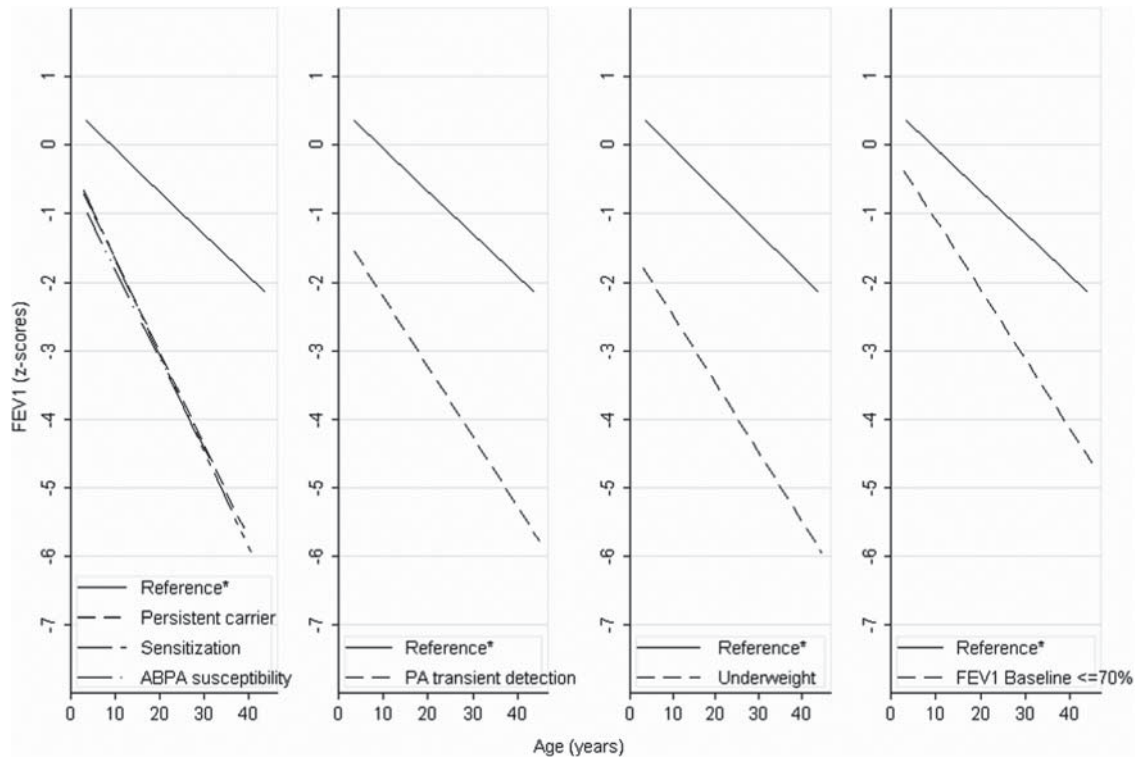


Figure 1. Progression of FEV₁ with age, according to the significant variables. Values are given as the mean changes in lung function computed from the fixed predicted values obtained by the linear mixed-effect model analysis, with the slope indicating the degree of progression.*The reference group represents a control group with 'no *Pseudomonas aeruginosa* transient detection', 'normal BMI', and 'FEV₁ baseline >70%'. (FEV₁, forced expiratory volume in 1 s; BMI, body mass index; ABPA, allergic bronchopulmonary aspergillosis; PA, *P. aeruginosa*.)

was associated with a larger FEV₁ decline in CF patients compared to the FEV₁ decline of the non sensitized CF patients. Unlike Wojnarowski et al. [14], we observed this association even when the t-IgE level was <500 IU/ml (sensitization group). Among the sensitized patients, some but not all experienced an ABPA event. These results are consistent with the results of Hutcheson et al. Some patients developed up to 5 parameters of sensitization to *A. fumigatus* without developing ABPA [38]. Moreover, our study clearly demonstrates that when sensitization to *A. fumigatus* occurred, it became permanent.

Second, we showed that persistent carriage of *A. fumigatus* led to a significant change in lung function. This result supports those of Amin et al., who showed that persistent *A. fumigatus* infection was an independent risk factor for hospital admission [12]. In contrast, de Vrankrijker [39] found no significant difference in the lung function decline. This result could be explained by the constitution of the group, which was not based on the presence or the absence of colonization but rather on the duration of colonization. The pathogenic role of *A. fumigatus* in the absence of ABPA was previously underlined by the study of Shoseyov et al. [15], in which lung function improved in patients after antifungal treatment. The authors presented 6 CF

patients with respiratory deterioration and *A. fumigatus*-positive sputum cultures who did not meet the ABPA criteria and did not respond to appropriate antibiotic therapy, at which point treatment with antifungal agents was followed by an improvement in the clinical condition.

In conclusion, in addition to ABPA, persistent *A. fumigatus* carriage and *A. fumigatus* sensitization appear to have an impact on pulmonary function. Therefore, in agreement with others [12,13,16], we suggest that in patients with CF, *A. fumigatus* should be considered a pathogen that may directly impair respiratory function. Despite the existence of some limitations, this study is relevant to the community of lung specialists in their practice and could form the basis of prospective clinical research on this topic, because the correct course of treatment for these *A. fumigatus*-induced diseases remains to be determined. Although the most recent clinical practice guidelines of the Infectious Diseases Society of America recommend itraconazole and corticosteroids for the treatment of ABPA [11], further studies are necessary to evaluate the efficacy of antifungal therapies in persistent carriers and in sensitized CF patients.

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