

Risk Factors for *Aspergillus* Colonization and Allergic Bronchopulmonary Aspergillosis in Children With Cystic Fibrosis

Virginie Jubin, MD,^{1*} Stéphane Ranque, MD, PhD,^{2,3} Nathalie Stremmer Le bel, MD,¹
Jacques Sarles, MD, PhD,¹ and Jean-Christophe Dubus, MD, PhD^{1,3}

Summary. Background: The annual prevalence of *Aspergillus* colonization (AC) and allergic bronchopulmonary aspergillosis (ABPA) has recently increased in pediatric patients with cystic fibrosis (CF). The reasons remain unclear although a number of factors have been suggested to be involved. This study was set up to investigate the association between potential predisposing factors, including new therapies recommended in CF, and the occurrence of AC or ABPA in children with CF. Methods: The medical records of 85 children monitored regularly in the Pediatric Reference Centre for Cystic Fibrosis Care (RCCFC) of the University Hospital of Marseille (France) were analyzed from the first time they attended the RCCFC until either the occurrence of an end event, or their last visit to the RCCFC. Risk factors for AC or ABPA were analyzed by univariate and multivariate logistic regression. Results: Eight children developed ABPA and 18 had AC. In univariate analysis, ABPA was significantly associated with RhDNase therapy, sensitization to *Alternaria* and *Candida*, and a low body mass index (BMI). Multivariate analysis identified an independent association between low BMI and ABPA (OR = 10.6, 95% CI [2.2–51.8], $P=0.004$), and for the first time, between long-term azithromycin therapy and AC (OR = 6.4, 95% CI [2.1–19.5], $P=0.001$). This latter association might be explained by the inhibitory effect of azithromycin on both the recruitment and the activation of neutrophils, which represent the first-line defenses against *Aspergillus*. Conclusions: The risk factors associated with AC and ABPA in children with CF identified in this comprehensive exploratory study now need to be confirmed in further prospective studies. **Pediatr Pulmonol.** 2010; 45:764–771. © 2010 Wiley-Liss, Inc.

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INTRODUCTION

Fungal infections, in particular those related to *Aspergillus* species, are relatively common in patients with cystic fibrosis (CF). In these patients, inhalation of *Aspergillus conidia* can chiefly result in two clinical scenarios, either *Aspergillus* colonization (AC) of the bronchial tree or allergic bronchopulmonary aspergillosis (ABPA).^{1–3}

AC is defined as either the presence of *Aspergillus* species in sputum^{4,5} or, more stringently, the isolation of *Aspergillus* from at least two out of four distinct sputum samples per year.² Colonization may be restricted to *Aspergillus* species (without differentiation of the species)^{4,5} or more specifically to *A. fumigatus*.^{6,7} The prevalence of AC ranges from 12% to 25% in CF populations including both children and adults.^{4–6} Variability in the definition of AC and laboratory techniques may account for this wide range of prevalence as the age of the patients too. Indeed, isolation of *Aspergillus* species from respiratory secretions has been found to be significantly more frequent in older children and young adults than in

younger children with CF.⁴ Recent studies have identified that *A. fumigatus* may be of clinical relevance in some individuals with CF that do not exhibit typical manifestations of ABPA. Shoseyov et al.⁸ have identified that *A. fumigatus* bronchitis only improves with antifungal

¹Pediatric Reference Centre for Cystic Fibrosis Care (RCCFC), Timone Children's Hospital, Marseille, France.

²Parasitology and Mycology Unit, Timone Hospital, Marseille, France.

³CNRS, URMITE 6236, Faculty of Medicine, Université de la Méditerranée, Marseille, France.

*Correspondence to: Virginie Jubin, MD, Pediatric Reference Centre for Cystic Fibrosis Care (RCCFC), Timone Children's Hospital, 13005 Marseille, France. E-mail: virginie-jubin@wanadoo.fr

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therapy. Moreover, in a retrospective cohort study including 230 CF children, Amin et al.⁹ have shown a deleterious effect of AC with a trend toward significance, after adjusting for baseline pulmonary function, of the association of chronic *A. fumigatus* infection (defined as the presence of at least two sputum or bronchoalveolar cultures positive for *A. fumigatus* in a given year) with pulmonary exacerbation requiring hospitalization.

ABPA is characterized by a clinical presentation of asthma with infiltrates and proximal bronchiectasis on chest radiography. Diagnosis is confirmed by the demonstration of an immune response to *Aspergillus*.¹⁰ ABPA is a complicating factor in 1–2% of patients with allergic asthma and a wide range from 1% to 15% of patients with CF, reflecting differences in ABPA definition and populations studied.³ Indeed, the prevalence of ABPA in patients with CF was lower in children under 6 years old in the ERCF study.¹¹ The diagnosis of ABPA is difficult in patients with CF because the clinical, imaging, and functional signs required for the diagnosis of ABPA are usual symptoms of CF. A consensus conference, held in 2001, proposed diagnostic criteria for ABPA that accounted for the underlying manifestations of CF.³ As ABPA can be treated effectively with a combination of corticosteroids and systemic antifungal drugs, failure to diagnose ABPA may result in earlier deterioration of pulmonary function and faster progression of CF lung disease.^{12,13}

An increase in the annual prevalence of aspergillosis in children with CF has been described in CF patients over the last decade. Between 1998 and 2002, the annual prevalence of AC increased from 7.4% to 18.8% and ABPA increased from 0.3% to 4% in children and teenagers with CF.⁵ The reasons for these increases are unclear. A few studies have evaluated the prevalence of ABPA and have investigated various risk factors but mainly in adult populations^{3,5,6,11,14,15} including a single study that evaluated the risk factors for both ABPA and AC.⁶ Besides, in children with CF, only Ritz et al.¹⁵ have investigated the association between a number of variables and ABPA or *Aspergillus* sensitization.

The present study was set up to investigate the association between AC or ABPA and a large number of possible predisposing factors, including those related to CF therapy, in children with CF.

METHODS

Study Setting

The medical records of 110 children regularly monitored in the Pediatric Reference Centre for Cystic Fibrosis Care (RCCFC) at the University Hospital of Marseille, France, were reviewed retrospectively. In the annual 2006 follow-up, this population had 5.3 consultations/patient/year and was characterized by a sex ratio equal to 1, an

average age of 8.5 (± 9) years and average CVF and forced expiratory volume in 1 sec (FEV1), respectively, equal to 88% ($\pm 20.8\%$) and 83.2% ($\pm 24.9\%$) of the predicted value, and a bacterial flora composed principally with *Staphylococcus aureus* (methicillin-S: 56.6%; methicillin-R: 10.1%), *Pseudomonas aeruginosa* (44.4%) and *Aspergillus* sp. (45.5%) (data of our center obtained from the French Register of CF). We have considered children with *Pseudomonas* colonization, FEV1 < 70% of the predicted value, or BMI < 3rd percentile to having a severe disease.

In order to be able to collect adequate informative data for each patient, inclusion criteria were: (1) at least four medical visits to our center per year during all the period of observation; (2) at least four sputum cultures per year; and (3) at least one check-up including chest X-rays, blood tests, particularly *Aspergillus*-related tests, and, when possible according to their age, functional respiratory tests per year. For these children, all medical records, from the beginning of aftercare until March the 31, 2006, were reviewed from the first time the patient attended the RCCFC to an end point. End points were either the occurrence of an *Aspergillus* event (either AC or ABPA as defined below) or, in the absence of any *Aspergillus* event, the last consultation at the RCCFC.

Definition of Events

Patients could display three types of events (or end points) and concomitant variables were recorded at each end point: (i) no diagnosis of AC or ABPA: In this case the end point was the last consultation at the RCCFC during the observation period; (ii) AC: The criteria for AC diagnosis were those described by Bargon et al.² The reference date was the date of the second positive culture; (iii) ABPA: The diagnosis of ABPA was based on the criteria from the 2001 consensus conference.^{3,7} In order to adapt these criteria to a pediatric population, we defined an increase in total IgE as an IgE level exceeding twice the upper limit of the age corrected normal range.^{3,15} Presence of serum IgE to *A. fumigatus* was retained if RAST were equal or greater than class 2.^{3,5} The reference date was the diagnosis and beginning of treatment. As ABPA and AC are related diseases, patients who fulfilled the diagnosis criteria for both ABPA and AC were considered to have ABPA. A patient might fulfill the criteria for different *Aspergillus* events during the observation period.

Variables of Interest

For each patient the following variables were recorded: *Patient characteristics*: (i) Gender; (ii) functional class of Cystic Fibrosis Transmembrane conductance Regulator (CFTR) genotype according to a classification of mutations of CFTR, which correlate with disease severity. Severe mutations correspond to classes I–III, whereas

mild mutations are classes IV and V.¹⁶ Three groups of patients were defined: A = patient with two severe mutations; B = patient with one severe and one mild mutation; and C = patient with two mild mutations; (iii) presence of atopy (excluding asthma): allergic rhinoconjunctivitis, atopic dermatitis, urticaria (drug-related urticaria was considered as an atopy criteria only if it was associated with other suggestive elements of atopy in order to cope with frequent non-allergic cutaneous adverse drug reaction), food allergy or positive skin test and/or serum-specific IgE against food or aeroallergens other than *A. fumigatus* (anti-*Candida* or anti-*Alternaria* specific blood IgE antibody levels, just before or at the time of the event, were mainly analyzed but were not measured in all patients); (iv) presence of asthma, defined as a familial or personal history of atopy in a child with symptoms of asthma and bronchial hyperresponsiveness in the metacholine test and/or a positive response to inhaled bronchodilators or corticosteroids.

At the end point. (i) Age; (ii) time to end point; (iii) nutritional status as assessed by the age- and gender-adjusted percentile of the body mass index (BMI) and analyzed if recorded in the 6 months before or 1 month after the end event; (iv) the baseline FEV1 value defined as the best FEV1 one year before the event,⁹ when available; (v) the cumulative dose of inhaled corticosteroids received by the patient (g of budesonide equivalent); (vi) continuous RhdNase therapy and its total duration; (vii) administration of tobramycin or colimycin aerosols during the previous 12 months. Short-term regimens and continuous administration (defined as a duration of prescription >6 months) were distinguished; (viii) continuous administration of oral azithromycin and its duration in months; (ix) number of antibiotic courses (oral or intravenous) for bronchopulmonary infection during the previous 5 years and the previous year; (x) presence of *S. aureus* and/or *P. aeruginosa* in sputum cultures within 1 year before the end point were distinguished as (i) primary infection, (ii) intermittent colonization, or (iii) chronic colonization (defined as growth of the pathogen over at least 6 months, demonstrated by three positive cultures at least 1 month apart); (xi) the involvement of *Stenotrophomonas maltophilia*, *Alcaligenes xylosoxydans*, *Burkholderia cepacia*, and *Candida* species was also determined; (xii) frequency of isolation of *Candida* species in the 12 months before the event and its temporal relationship with the *Aspergillus* event. Variations in the denominator are due to missing data because of a follow-up duration <1 or 5 years, or incomplete information for some children.

Statistical Analysis

The data for AC and ABPA were presented and analyzed separately. Quantitative variables were

described using the mean, standard deviation (SD), and range. Qualitative variables were described by total number and percentage. The association of either AC or ABPA with each potential risk factor was first tested by univariate analysis using ANOVA for quantitative variables and Fisher's exact test for qualitative variables. Factors found to have a level of significance $P \leq 0.20$ in univariate analysis were included in multivariate unconditional logistic regression analysis using a stepwise selection procedure to build a parsimonious model that included only those factors that remained significant ($P < 0.05$) in the presence of other factors. Two-sided tests were used throughout. Statistical analysis was performed using SAS 9.1.3 SP4 statistical software (SAS, Inc., Cary, NC).

RESULTS

Patients

The files of 110 children were screened. Twenty-five patients were excluded because they lacked sufficient information to be adequately analyzed. Eighty-five patients who were followed-up regularly in the RCCFC (the mean follow-up duration was 6 years) fulfilled the inclusion criteria. Of these 85 children, 63 (74%) did not present any *Aspergillus* event during the observation period and 22 (26%) had at least one *Aspergillus* event: 13 developed AC, 5 developed ABPA, 3 had an AC episode and a temporally unrelated ABPA episode, and 1 developed two temporally unrelated AC episodes. Overall, 26 *Aspergillus* events were analyzed, including 18 (21%) cases of AC and 8 (9%) ABPA, which represent, respectively, 69% and 31% of the *Aspergillus* events.

Aspergillus Colonization

Among the 18 children with AC, 12 (67%) were colonized exclusively with *A. fumigatus*. The others were colonized with various *Aspergillus* species: *A. fumigatus* (n = 4), *A. flavus* (n = 4), and *A. niger* (n = 3).

In the univariate analysis, the general characteristics of the children with AC did not significantly differ from the others (Table 1). Long-term azithromycin therapy was significantly associated with AC (67% vs. 24%, respectively) (Table 2). Patients treated with long-term azithromycin were more frequently colonized with *P. aeruginosa* than untreated patients (60% vs. 28.6%, $P = 0.01$), but had as much bronchial obstruction and denutrition as the other children (22.7% vs. 20.8% and 10.7% vs. 12.3%, respectively). Neither intravenous, oral, or inhaled drug therapy nor the composition of bronchial flora (Tables 2 and 3) was significantly associated with AC. However, there was a statistically non-significant trend for children with AC to be more frequently treated with RhdNase than the others. The multivariate analysis

TABLE 1—Relationship Between Patient Characteristics and the Occurrence of *Aspergillus* Colonization or Allergic Bronchopulmonary Aspergillosis (ABPA)

	<i>Aspergillus</i> colonization			ABPA		
	Yes	No	<i>P</i> -value	Yes	No	<i>P</i> -value
Time to end point (months), mean (SD) [range]	89.3 ± 53.7 [2.5–172.9] (n = 18)	64.6 ± 52.9 [0.3–203.6] (n = 71)	0.08	67.6 ± 57.0 [0.3–151.6] (n = 8)	71.5 ± 54.9 [2.5–203.6] (n = 81)	0.91
Age (years), mean (SD) [range]	10.6 ± 3.5 [5.8–16.9] (n = 18)	8.3 ± 5.4 [0.5–17.6] (n = 71)	0.09	11.3 ± 3.4 [6.6–15.6] (n = 8)	8.5 ± 5.2 [0.5–17.6] (n = 81)	0.14
Male (%)	55.6 (n = 18)	50.7 (n = 71)	0.19	50.0 (n = 8)	51.9 (n = 81)	0.29
CFTR genotype (%)	Group A: 100 (n = 17)	Group A: 93.9, Group B: 6.1 (n = 66)	0.39	Group A: 100 (n = 7)	Group A: 94.7, Group B: 5.3 (n = 76)	0.70
Atopy (%)	27.8 (n = 18)	21.1 (n = 71)	0.20	22.2 (n = 8)	25 (n = 71)	0.32
Asthma (%)	66.7 (n = 18)	53.5 (n = 71)	0.13	50.0 (n = 8)	56.8 (n = 81)	0.27
BMI <3rd percentile (%)	5.6 (n = 18)	14.1 (n = 71)	0.23	50.0 (n = 8)	8.6 (n = 7)	0.007
FEV1 <70% (%)	31.2 (n = 5)	21.2 (n = 7)	0.72	33.3 (n = 2)	22.7 (n = 10)	0.37

n, number of patients evaluated for this characteristic.

CFTR genotype groups: A = patient with two severe mutations; B = patient with one severe and one mild mutation; and C = patient with two mild mutations.

confirmed that long-term azithromycin therapy was the only measured variable independently associated with AC (OR = 6.4, 95% CI [2.1–19.5], *P* = 0.001). The selected criteria of severe disease were not correlated to the occurrence of AC.

Allergic Bronchopulmonary Aspergillosis

ABPA was diagnosed in eight children. In six cases, *Aspergillus* species were isolated from sputum samples. *A. fumigatus* was present in all cases, either alone

TABLE 2—Influence of Cystic Fibrosis Treatment on the Development of *Aspergillus* Colonization or Allergic Bronchopulmonary Aspergillosis (ABPA)

	<i>Aspergillus</i> colonization			ABPA		
	Yes (n = 18)	No (n = 71)	<i>P</i> -value	Yes (n = 8)	No (n = 81)	<i>P</i> -value
Cumulative dose of inhaled corticosteroids (g), mean ± SD [range]	1.4 ± 1.03 [0.2–3.5] (n = 15)	1.1 ± 1.2 [0–4.4] (n = 66)	0.34	1.4 ± 1.3 [0.3–3.5] (n = 5)	1.1 ± 1.2 [0–4.4] (n = 76)	0.67
RhDNase (%)	77.8	50.7	0.06	100	51.9	0.008
Cumulative duration (months), mean ± SD [range]	36.0 ± 38.9 [0–128]	18.9 ± 33.5 [0–134]	0.07	47.3 ± 43 [5–121]	20.2 ± 33.8 [0–134]	0.05
Inhaled antibiotics (%)	55.6	45.1	0.44	37.5	48.2	0.71
Tobramycin (%)	44.4	38	0.79	37.5	39.5	1.0
Short/long-term regimen	50/50	59.3/40.7	0.70	33.3/66.7	59.4/40.6	0.56
Colimycin (%)	38.9	25.4	0.26	37.5	27.2	0.68
Short/long-term regimen	62.5/37.5	77.8/22.2	0.64	33.3/66.7	78.3/21.7	0.17
Azithromycin (%)	66.7	23.9	0.001	37.5	32.1	0.71
Cumulative duration (months), mean ± SD [range]	10.4 ± 13.0 [0–41]	5 ± 12.9 [0–61]	0.12	7 ± 11.6 [0–32]	6 ± 13.2 [0–61]	0.84
Number of antibiotic courses						
Oral route/past 5 years, mean ± SD [range]	10.2 ± 6.5 [0–23]	10.7 ± 6 [0–21]	0.81	16 ± 4.2 [10–20]	10.0 ± 6.0 [0–23]	0.06
Intravenous route past 5 years, mean ± SD [range]	1.6 ± 2.1 [0–6] (n = 11)	1.0 ± 2.1 [0–8] (n = 34)	0.40	0.8 ± 1.0 [0–2] (n = 4)	1.2 ± 2.2 [0–8] (n = 41)	0.67
Oral route past year, mean ± SD [range]	2.4 ± 2.0 [0–7]	2.2 ± 1.7 [0–6]	0.82	3.3 ± 1.6 [1–5]	2.2 ± 1.8 [0–7]	0.11
Intravenous route past year, mean ± SD [range]	0.6 ± 0.9 [0–3] (n = 17)	0.4 ± 0.8 [0–4] (n = 63)	0.26	0.7 ± 0.8 [0–2] (n = 7)	0.4 ± 0.9 [0–4] (n = 73)	0.37

n, number of patients evaluated for a given characteristic.

TABLE 3—Influence of Bronchial Flora on the Development of *Aspergillus* Colonization and Allergic Bronchopulmonary Aspergillosis (ABPA)

	<i>Aspergillus</i> colonization			ABPA		
	Yes (n = 15)	No (n = 52)	<i>P</i> -value	Yes (n = 7)	No (n = 60)	<i>P</i> -value
<i>Staphylococcus aureus</i> (%)	60	76.9	0.20	71.4	73.3	1.0
Chronic colonization	40.0	33.8	0.77	57.1	44.1	0.69
<i>Pseudomonas aeruginosa</i> (%)	60	44.2	0.38	71.4	45.0	0.25
Chronic colonization	33.3	13.7	0.12	28.6	17.0	0.60
<i>Stenotrophomonas maltophilia</i> (%)	20	3.9	0.07	0	8.3	1.0
<i>Alcaligenes xylosoxydans</i> (%)	13.3	1.9	0.12	0	5	1.0
<i>Candida</i> species (%)	53.3	62.8	0.56	71.4	59.3	0.69

n, number of patients evaluated for a given characteristic.

(n = 3) or associated with at least another *Aspergillus* species (n = 3) (*A. niger*, *A. nidulans*, *A. terreus*, *A. flavus*).

In the univariate analysis, a BMI <3rd percentile was the only general characteristic covariate (Table 1) significantly associated with ABPA (50.0% vs. 9%, respectively; $P = 0.007$). When CF therapy was considered, RhDNase treatment was statistically more frequent in children with ABPA (100% vs. 52%, $P = 0.008$). Moreover, children with ABPA had a significantly longer cumulative duration of RhDNase therapy than the others (47.3 vs. 20.2 months, respectively; $P = 0.05$). Although it was statistically non-significant, there was a trend for children with ABPA to have received more courses of oral antibiotics during the preceding 5 years than the others (16 vs. 10; $P = 0.06$) (Table 2). No association between ABPA and bronchial flora (Table 3) could be established. However, our data showed that ABPA, in contrast to AC, was significantly associated with *Candida* sensitization (60% vs. 4.6%, respectively; $P = 0.003$) and *Alternaria* sensitization (100% vs. 13%; $P < 0.001$) (Table 4). The multivariate analysis showed that only a BMI <3rd percentile was independently associated with ABPA (OR = 10.6, 95% CI [2.2–51.8], $P = 0.004$). Because of the low number of ABPA cases and missing data, the effect of both *Candida* and *Alternaria* sensitization could not be tested in multivariate analysis.

DISCUSSION

To our knowledge this is the first study to analyze a comprehensive range of risk factors for both AC and

ABPA in children with CF. Previous studies mostly involved adults with CF and focused either on ABPA or on AC. Our major findings were a strong association in multivariate analysis between long-term azithromycin therapy and AC, whereas poor nutritional status was associated with ABPA. The main limitations to our study are its retrospective design, the relatively small sample size, and multiple statistical tests, which might inflate type I error. All patients were followed-up in one center and the data are thus relatively standardized. However, it is difficult to generalize our findings to other patient populations, which may present distinct characteristics. Despite this drawback, our study has the main advantage of evaluating, simultaneously, the impact of a large number of putative risk factors for both AC and ABPA in children with CF, who are particularly susceptible to *Aspergillus* events.

In our study we paid attention to consider AC not as a single positive culture for *A. fumigatus*. Indeed, *Aspergillus* species can occasionally contaminate sterile sputum cultures and *A. fumigatus* infection can be transient and in this case might not be associated with clinical significance. This has been shown in a recent study where lung function deterioration was associated with persistent multi-resistant *S. aureus* (MRSA) colonization but not with any MRSA positivity.¹⁷ In the current study, the prevalence of AC was 21%, which is close to the 19% reported in children⁵ but much lower than the 41% reported in adults.² Previous studies evaluating AC have either been restricted to *A. fumigatus* colonization^{6,7} or have made no distinction between the different *Aspergillus* species.^{4,5} In our study, *A. fumigatus* was involved in

TABLE 4—Influence of *Candida* and *Alternaria* Sensitization on the Development of *Aspergillus* Colonization and Allergic Bronchopulmonary Aspergillosis (ABPA)

%	<i>Aspergillus</i> colonization			ABPA		
	Yes	No	<i>P</i> -value	Yes	No	<i>P</i> -value
Positive <i>Candida</i> RAST	0 (n = 15)	10.9 (n = 55)	0.33	60 (n = 5)	4.6 (n = 65)	0.003
Positive <i>Alternaria</i> RAST	25 (n = 12)	17.0 (n = 47)	0.68	100 (n = 4)	12.7 (n = 55)	<10 ⁻³

n, number of patients evaluated for a given characteristic.

16 out of the 18 cases of AC. This predominance of *A. fumigatus* might be partially explained by its higher virulence, as demonstrated in vitro,¹⁸ compared to other *Aspergillus* species. Some previous studies have reported that the frequency of AC increases with age^{4,6} and correlates with increased disease severity, as assessed by clinical and radiographic scores.⁶ We did not observe any significant influence of age or other variables usually associated with more severe disease (lower nutritional status, lower lung function, and chronic colonization with *P. aeruginosa*) on the occurrence of AC. This might be because our study did not include younger adult in contrast with these previous studies.

Prophylactic oral and inhaled antibiotics against *S. aureus* or *P. aeruginosa* have been associated with an increased risk of AC in CF.^{2,19} As inhaled antibiotics and number of antibiotic courses did not appear to increase the risk of AC in our CF patients, low-dose continuous (or long-term) therapy with the macrolide antibiotic azithromycin was independently associated with AC. Although patients on azithromycin could have a more severe disease, the result obtained in multivariate analysis suggests that the association between azithromycin therapy and AC cannot be explained by an increased severity of the illness but could rather be explained by the mechanism of the drug itself. It is well known that azithromycin therapy triggers immunomodulatory effects that are associated with enhanced lung function, decreased frequency of pulmonary exacerbations, and lower CRP levels.²⁰ However, the effect of azithromycin on neutrophils, which are essential in the host defense against *Aspergillus* species,^{21–24} may facilitate the occurrence of AC. It has been demonstrated that early neutrophil recruitment and production of oxidative-active aggregates are pivotal and sufficient^{23,25} in preventing germination of *A. fumigatus* conidia²⁵ and that neutrophils mediate *Aspergillus* hyphal killing.²² Macrolide antibiotics have the following effects: They inhibit the chemotaxis and infiltration of neutrophils into the airways^{26–28} and also decrease the production of IL-8 and LTB₄, both known to be chemotactic factors for neutrophils.^{26,28} They block the formation of adhesion molecules necessary for neutrophil migration and inhibit the release of superoxide anions by neutrophils.²⁶ Thus, this increased risk of AC in patients treated with long-term azithromycin might result from an inhibition of neutrophil infiltration and activity in the airways with a consecutive down-regulation of host defenses against *Aspergillus*.

The prevalence of ABPA in our CF children was 9%. This is higher than the 4–7% reported in previous pediatric studies.^{5,15} Differences in the diagnostic criteria for ABPA may, in part, explain this variation. However, as suggested by Skov et al.,⁵ it might also indicate an increase in the prevalence of ABPA. Likewise AC, *A. fumigatus* was the most frequent *Aspergillus* species isolated from

ABPA patients' sputum samples. In previous studies, ABPA occurred preferentially in older and male CF patients with atopic symptoms and deteriorated lung function.^{6,11,14,29} Moreover, in a large European study, Mastella et al.¹¹ found that ABPA was associated with a poor nutritional status. A higher rate of microbial colonization with either *P. aeruginosa*¹⁴ or *S. maltophilia*¹⁵ has also been described in patients with ABPA. Furthermore, higher cumulative doses of inhaled corticosteroids were associated with either ABPA¹¹ or *A. fumigatus* sensitization.¹⁵ Finally, the effect of genetic polymorphisms including HLADR2 and DR5,³⁰ IL-4Ralpha,³¹ and IL-10³² has been associated with ABPA.

In our study, a poor nutritional status was the only independent risk factor of ABPA. A previous study³³ provided evidence that immune function in CF patients is impaired as nutritional status deteriorates. In CF patients, poor nutritional status may favor a Th2 response against *Aspergillus* species that might facilitate the switch from AC to ABPA. Poor nutritional status could affect the lymphocytic compartment of specific regulatory T cells (T_{reg}). Indeed, in CF patients, low body weight is correlated with decrease of lymphocytes subsets and lymphocyte proliferation in vitro but non-specific suppressor cell activity appears normal in vitro.³³ Moreover, T_{reg} have been implicated in immunity and tolerance to *Aspergillus*, particularly through the inhibition of Th2 cells by tolerogenic T_{reg} producing IL-10 and TGF-β.³⁴

RhDNase therapy was more frequently administered to children with AC than to the others and we observed a trend toward significance between AC and RhDNase therapy. In keeping with the findings of Mastella et al.,¹¹ prolonged RhDNase therapy was found to be significantly associated with ABPA. The lack of association between AC and other nebulized therapies does not support the hypothesis of nebulizer contamination with *Aspergillus*. This association between RhDNase therapy and AC and ABPA could be partly due to the effect of prolonged (≥6 months) RhDNase therapy on pulmonary *Aspergillus* defenses, in particular by inhibiting the usual increase of neutrophils and neutrophil elastase activities and by reducing IL-8 concentrations in the bronchoalveolar lavage³⁵ and the sputum³⁶ of CF patients.

Another observation was the significant association of ABPA with prior or concomitant sensitization to *Candida* and *Alternaria* species. These findings are in line with those of Maiz et al.³⁷ who found that 50% of their CF patients sensitized to *C. albicans* had confirmed ABPA and the other 50% had some immunological characteristics of ABPA. Sensitization to *Alternaria* has, to our knowledge, never been described as a risk factor for ABPA. This sensitization may result from an increase in total serum IgE concentration or may be due to allergenic cross-reactivity between products from *Alternaria* and *Aspergillus* species as it has been demonstrated between

A. fumigatus and *C. boydii*.³⁸ Shared epitopes³⁹ could explain a similar mechanism of sensitization with a contributory role of IgE directed against *Candida* sp.⁴⁰ and *Alternaria* sp. in the pathogenesis of ABPA by exacerbating pulmonary eosinophil-mediated inflammatory reactions.

This study reveals the pattern of AC and ABPA in children with CF attending the RCCFC in Marseille. Conclusions regarding ABPA need to be put in the context of the small sample size. However, in line with previous studies, poor nutritional status (BMI <3rd percentile) was associated with an increased risk of ABPA. We hypothesize that an alteration of the lymphocytic compartment of some regulatory T cells related to poor nutritional status is pivotal for the development of ABPA. The most significant finding in this study is the association of long-term azithromycin therapy with an increased risk of AC that might be explained by the inhibitory effect of azithromycin on both the recruitment and the activation of neutrophils, which are the first-line defenses against *Aspergillus*. Although no association with long-term azithromycin and ABPA was found in this study, this could be a result of an inadequate sample size. Further prospective and fundamental studies are needed to confirm these risk factors for AC and ABPA in children with CF. They may also provide more insight into the pathophysiologic pathways of each syndrome, the relevance of AC to long-term outcome in CF and whether AC is a distinct clinical entity from ABPA or part of a continuum of *Aspergillus* disease in CF.

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