

## Allergic bronchopulmonary aspergillosis in cystic fibrosis. A European epidemiological study

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**ABSTRACT:** Allergic bronchopulmonary aspergillosis (ABPA) is a disease resulting from a hypersensitivity response to *Aspergillus fumigatus*, although the pathogenesis of the disease is unknown and its prevalence in cystic fibrosis (CF) is still poorly defined.

Data from the Epidemiologic Registry of Cystic Fibrosis (ERCF) on 12,447 CF patients gathered from 224 CF centres in nine European countries were analysed. The ERCF definition of ABPA diagnosis is a positive skin test and serum precipitins to *A. fumigatus*, together with serum immunoglobulin (Ig)E levels >1,000 U·mL<sup>-1</sup> and additional clinical or laboratory parameters.

The overall prevalence of ABPA in the ERCF population was 7.8% (range: 2.1% in Sweden to 13.6% in Belgium). Prevalence was low <6 yrs of age but was almost constant ~10% thereafter. No sex differences were observed. ABPA affected 8.0% of patients with a  $\Delta F508/\Delta F508$  genotype and 5–6% with  $\Delta F508/G551D$ ,  $\Delta F508/G542X$  and  $\Delta F508/N1303K$  genotypes. ABPA patients presented a lower forced expiratory volume in one second (FEV<sub>1</sub>) than those without ABPA at any age and the prevalence ranged from 6.6% in patients with FEV<sub>1</sub>  $\geq 20$ –12.9% in those with FEV<sub>1</sub> <40%. ABPA was associated with higher rates of microbial colonization, pneumothorax and massive haemoptysis, and with higher IgG serum levels and poorer nutritional status. A mixed model regression analysis of lung function showed that FEV<sub>1</sub> decline during the follow-up period was not substantially different in ABPA patients compared with non-ABPA patients for any subgroups based on age or disease severity at enrolment.

To conclude, allergic bronchopulmonary aspergillosis is a frequent complication in cystic fibrosis patients, particularly after the age of 6 yrs, and it is generally associated with a poorer clinical condition. However, any clear independent influence of allergic bronchopulmonary aspergillosis on the rate of lung function decline in the short term was not shown.

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Allergic bronchopulmonary aspergillosis (ABPA) is a disease resulting from a hypersensitivity response to *Aspergillus fumigatus*, an ubiquitous fungal pathogen that can colonise the bronchial tree [1]. In reality, although *A. fumigatus* is the primary causative pathogen in ABPA, the disease can be caused by several other members of the *Aspergillus* spp, including *A. flavus*, *A. nidulans*, *A. niger*, as well as other fungi [2].

Patients with asthma are predisposed to developing ABPA, with a prevalence of 1–2% [3], and the pulmonary status of cystic fibrosis (CF) patients represents a favourable environment for the development of ABPA. The pathogenesis of the disease is unknown, although it is postulated that *A. fumigatus* spores are inhaled, trapped in the mucus of the large segmental bronchi, and subsequently germinate to form hyphae, which release antigens to elicit an immune response [3].

The hypersensitivity reaction is characterized by the production of immunoglobulin (Ig)G and IgE antibodies specific for *A. fumigatus*, as well as a rise in total IgE [4, 5]. This reaction, in combination with the generation of cytotoxic metabolites such as proteolytic enzymes by *A. fumigatus*, causes local immunosuppression, inhibition of phagocytosis and epithelial cell detachment [6]. Together these effects enhance *A. fumigatus* colonization and facilitate its persistence in the respiratory tract, encouraging the accumulation of pulmonary infiltrates, tissue damage, and eventually destruction of lung tissue [3–6].

The prevalence of ABPA in CF is still poorly defined. Available literature suggests a wide range of frequencies from 0–25% [7–14], which may in part be due to the use of different diagnostic criteria. Validated criteria for the diagnosis of ABPA have still not been established in CF and the situation has been critically discussed in a recent

epidemiological study [12]. In CF, diagnosis relies principally on immunological evidence (in particular a positive, immediate skin reactivity to *A. fumigatus* and on the *in vitro* demonstration of specific serum IgE, IgG and precipitating antibodies). Asthma, total serum IgE and peripheral eosinophilia are important additional criteria. Pulmonary infiltrates and central bronchiectasis on chest radiographs, usually suggestive of ABPA [1, 15], are commonly encountered in CF as a result of chronic bacterial infection and should be viewed with caution as selective criteria for diagnosis. The recommended combination of immunological evidence and clinical manifestations is important. Positive immune parameters and elevated total serum IgE levels in a patient experiencing increased cough or wheezing, pulmonary infiltrates, or a decrease in pulmonary function that are unresponsive to aggressive therapy (increased antibiotics and bronchodilators) may suggest a diagnosis of ABPA [13, 14, 16, 20]. In reality, sensitization to *A. fumigatus* is very common in CF, occurring in  $\geq 50\%$  of patients [10, 13, 14, 21–23]. In a prospective study of 222 consecutive CF patients >6 yrs of age, 56% were sensitized to *A. fumigatus* and 23% of these patients had ABPA [10]. In contrast, in a study of 51 adult CF patients, the immune response to *A. fumigatus* was present in 66% of patients, but only one patient was considered to have ABPA [12]. Therefore, *A. fumigatus* sensitization, in the absence of clinical features of ABPA, does not suggest an ABPA diagnosis.

One of the most relevant problems is the contribution of ABPA to disease progression in CF. This is a poorly documented and little understood issue for which no epidemiological studies have been performed to date. In spite of this, ABPA is considered to be a serious complication in patients with CF [8, 16, 17–19].

The data collated from a large European CF population (the Epidemiologic Registry of Cystic Fibrosis (ERCF)) permitted the present study. The main aim of this study was to establish the prevalence of ABPA in CF. Secondary aims were to describe the clinical conditions that can be associated with ABPA and to explore the possible influence of ABPA on the progression of lung disease. In the ERCF the diagnosis of ABPA is defined according to the above criteria and other recommendations.

## Patients and methods

### *Data ascertainment and quality control*

The ERCF is an international observational database initiated in Europe by F. Hoffmann-La Roche Ltd in 1994. Countries were eligible to participate if the local company affiliate had sufficient available manpower and financial resources to support initiation of the Registry. Clinics in these countries were eligible to participate if they delivered specialized care to >10 CF patients and were willing to submit defined clinical data collected from each of their patients at normal clinic visits.

Initially, data were collected on paper forms that were sent to Quintiles GmbH (Neu Isenburg, Germany) for data entry, quality control and assignment of preferred terms (coding) of CFTR mutations, medications, adverse events and causes of death. Quintiles subjected the data to >400 context and consistency checks and issued queries to the

submitting centre where necessary to clarify inconsistencies. In 1997 a transition was made to remote data entry (RDE) using Internet technology. The data entry forms were recreated in Java script and the pre-existing quality assurance checks (including longitudinal checks) and most of the coding were built into the RDE system to ensure high data quality. Participating centres were given controlled access to the data entry screens at [www.ercf.org](http://www.ercf.org) and the locally entered data were submitted in encrypted form to Spider Network AG (Otelfingen, Switzerland) for subsequent transfer to Quintiles.

Data analysis was under the control of an Advisory Board comprised of the authors of this manuscript and at no time were personal patient data available to Hoffmann-La Roche Ltd.

### *Patients and cystic fibrosis centres*

A total of 12,447 CF patients were enrolled in the ERCF by 13 December 1997 (5,967 females (48%), median age 12 yrs, age range from 0–62 yrs). Patients were enrolled in the ERCF from 224 CF centres in nine European countries (Austria, Belgium, Denmark, France, Germany, Ireland, Sweden, The Netherlands and the UK). Seventy-three centres enrolled  $\geq 50$  patients.

### *Allergic bronchopulmonary aspergillosis diagnosis*

The ERCF requires that the diagnosis of ABPA be reported, as defined by the following criteria: a positive immediate (type 1) skin test reaction to *A. fumigatus*, multiple serum precipitins to *A. fumigatus*, a total serum IgE level  $>1,000 \text{ U}\cdot\text{mL}^{-1}$ , and a physician's suspicion that the patient has ABPA, based in part on one or more of the following: reversible bronchoconstriction or asthma, pulmonary infiltrates, elevated serum IgE and/or IgG specific for *A. fumigatus*, peripheral eosinophilia ( $>1,000 \text{ mL}^{-1}$ ), recovery of *A. fumigatus* from sputum or hyphae present on smear, and response to steroids. In the absence of validated diagnostic criteria in CF, the physician's suspicion of ABPA based on multiple clinical, radiological and additional immunological conditions was considered a reasonable compromise to overcome the problem that the usual clinical and radiological criteria of ABPA are not selective in CF [2, 3].

### *Data handling criteria*

ABPA was studied in relation to the specific care centre, country, sex, CFTR genotype, age at enrolment, forced expiratory volume in one second (FEV<sub>1</sub>) and forced vital capacity (FVC) at enrolment and during the follow-up period, as well as body weight, serum IgG values, bacterial isolates from sputum, pulmonary complications (massive haemoptysis, pneumothorax, lung transplantation) and routine respiratory therapies, including antibiotics. The routine respiratory therapies were the usual therapeutic strategies adopted for the individual patients during the observation period, as recorded by the participating centres.

All data entered in the ERCF database from 28 September 1993 to 13 December 1997 were used for this

analysis, independent of the patients' clinical status at the time of each reported visit. The ERCF population was stratified by the absence/presence of ABPA. The ABPA positive subpopulation was selected using information from all visits. Individual events and medical conditions were considered present if they were recorded at least once during the observation period for each patient.

Spirometric tests, performed using either a bell-spirometer or a pneumotachograph following the ERS criteria, were recorded at follow-up visits, whether patients were in stable clinical situation or not. FEV<sub>1</sub> and FVC were calculated as percentages of the predicted values, according to KNUDSON et al. [24]. This standardization algorithm was adopted to make the results comparable with those of the United States Cystic Fibrosis Foundation and other epidemiologic registries which are in progress in USA and Canada [12]. The initial FEV<sub>1</sub> was used to determine the severity of the disease in patients  $\geq 6$  yrs of age.

The valid ERCF population was defined according to the particular analysis performed. For the genotype correlation, only patients with a known genotype were analysed; for the microbiological analysis, only patients with at least one valid sputum culture were included; for lung function analysis, the availability of FEV<sub>1</sub> or FVC values in patients  $\leq 6$  yrs of age were critical, however, valid lung function data were only available in a few patients. For IgG, the mean of all valid values for a subject was used in the analysis. For the weight z-scores (the standardized weight for age and sex: the z-score corresponds to the standard deviation from the mean of the reference population), the age in months was completed using the date of each visit and the date of birth, and was used together with actual weight to derive the z-score from sex-specific reference values published by the World Health Organization (WHO) [25]. The mean value was computed using all valid values for a subject. The antibiotic treatment analysis was performed by classifying the antibiotic regimens on the basis of their intensity, *i.e.* in order of decreasing intensity: prophylaxis, continuous use; prophylaxis, intermittent use; only for respiratory tract infection exacerbations. Any patient with more than one antibiotic regimen was considered to belong to the most intense of the reported classes. Anti-*Aspergillus* antibiotics were itraconazole or amphotericin.

#### Statistical analysis

The mixed model analysis [26, 27], which has been used to study the influence of ABPA on decline of lung function, is an extension of multiple regression analysis. Although it is a complex computation, it enables the estimation of individual and population regression parameters even in the presence of unevenly spaced repeated measures.

The intention was to model the FEV<sub>1</sub> percent predicted value at a given age using an initial severity score (severe: FEV<sub>1</sub> <40%; moderate: FEV<sub>1</sub> 40–69%; mild: FEV<sub>1</sub>  $\geq 70\%$ ), age groups at the start of the observation period (<6 yrs, 6–12 yrs, 13–17 yrs or  $\geq 18$  yrs), age at the time of observation and presence/absence of ABPA as fixed effects, and age and an intercept (the intersection point between the regression line and the y-axis) as random effects. All ERCF patients with at least two FEV<sub>1</sub> measurements after the initial one (which was not used in the

analysis) were included. However, due to the very large number of observations, the full database could not be analysed and the model was fitted independently to twelve subpopulations, defined by the initial severity and age subgroups. This involved the presence/absence of ABPA and age (as fixed effects), and an intercept and age (as random effects) only. The goal of this analysis was purely descriptive, and the results of statistical tests are reported only for formal reasons. In fact, due to the unusually large number of patients in each of the analyses, statistical significance can be reached in cases where the results are of no clinical relevance.

Duration of follow-up for the mixed model analysis ranged from 1–45 months, with a median of 25 months for both the ABPA and non-ABPA groups. The number of FEV<sub>1</sub> measurements per person ranged from 3–45, with median values of 7 and 6 for the ABPA and non-ABPA groups, respectively.

## Results

### *Allergic bronchopulmonary aspergillosis prevalence*

The diagnosis of ABPA was reported for 967 of the 12,447 CF patients; an overall ABPA prevalence of 7.8% (95% confidence interval (CI) 7.3–8.3). However, prevalence varied greatly between countries and individual centres. Prevalence of ABPA ranged from 2.1% in Sweden to 13.6% in Belgium (table 1), and from 0–27% for the bigger centres (*i.e.* those with  $\geq 50$  patients;  $n=73$ ). Similar variability was also apparent within individual countries.

ABPA was almost equally prevalent in males (509/6480, 7.9%) and females (458/5967, 7.7%). It was little represented in patients <6 yrs old, but was reported in ~10% of older patients (<6 yrs 56/3150, 1.7%; 6–12 yrs 320/3223, 9.0%; 13–18 yrs 237/1879, 11.2%;  $\geq 18$  yrs 354/3228, 9.9%). ABPA did not show a significant association with a particular CFTR genotype. Of the patients in the ERCF who had a known genotype ( $n=9584$ ), a comparable prevalence of ABPA was demonstrated in individuals with all of the most common genotypes ( $\Delta F508/\Delta F508$ , 412/4765, 8.0%;  $\Delta F508/G551D$ , 11/205, 5.1%;  $\Delta F508/G542X$ , 11/185, 5.6%;  $\Delta F508/N1303K$ , 7/107, 6.1%;  $\Delta F508$ /other, 216/2627, 7.6%; other/other, 66/972, 6.4%).

### *Allergic bronchopulmonary aspergillosis associated with clinical conditions*

Patient body weight, calculated in terms of z-score, was generally lower in ABPA patients than in those without ABPA (mean values of  $-0.9 \pm 1.2$  and  $-0.7 \pm 1.4$ , respectively). In addition, ABPA patients showed elevated serum IgG levels compared with those without ABPA (mean values of  $14.3 \pm 6.5$  g·L<sup>-1</sup> ( $n=626$ ) and  $12.4 \pm 6.3$  g·L<sup>-1</sup> ( $n=5693$ ), respectively).

Patients with ABPA had, on average, a 10% lower FEV<sub>1</sub> value than those without (table 2). A similar difference was found for FVC (mean values of 75.5% ( $n=906$ ) and 81.8% ( $n=8721$ ), respectively). FEV<sub>1</sub> percentage values were lower in the ABPA patients of all age classes compared with those without ABPA. Stratification of the patients by age and FEV<sub>1</sub> at enrolment demonstrated an ABPA prevalence in individuals with severe or moderate

Table 1. – Prevalence of allergic bronchopulmonary aspergillosis (ABPA) by country

Country	CF population	ABPA%	95% CI
Austria	169	5.3	2.5–9.9
Belgium	44	13.6	5.2–27.4
Denmark	320	3.4	1.7–6.1
France	2935	7.4	6.5–8.4
Germany	3070	11.5	10.4–12.7
Ireland	621	11.0	8.6–13.7
Netherlands	353	6.8	4.4–9.9
Sweden	424	2.1	1.0–4.0
United Kingdom	4511	6.0	5.3–6.7
All countries	12447	7.8	7.3–8.3

CF: cystic fibrosis; 95% CI: 95% confidence interval.

lung disease (FEV<sub>1</sub> <40% and 40–70%, respectively), which was twice that observed in patients with mild lung disease (FEV<sub>1</sub> ≥70%). Furthermore, adolescents (patients aged 13–17 yrs) had a slightly higher, although not significant prevalence of ABPA overall and in the severe and mild classes, while patients aged 6–12 yrs had the highest prevalence among the class with moderate lung disease.

The association of specific microbial infections with ABPA is shown in table 3. Notably, only 45% of patients with ABPA reported positive cultures for *A. fumigatus*, while ABPA prevalence among the *A. fumigatus* positives was 429/2193 (19.6%) compared with an overall prevalence of 8.1% in the patient population with microbiological data available. The colonization rates of some pathogens (*Pseudomonas aeruginosa*, *Candida albicans*, *Stenotrophomonas maltophilia* and *Burkholderia cepacia*) were higher in ABPA patients than in those without ABPA. No association was evident in the proportion of patients with cultures positive for *Staphylococcus aureus*, or normal flora/negative cultures (other pathogens are extremely rare), whereas *Haemophilus influenzae* was reported more frequently in the non-ABPA population.

The most common respiratory complications/events found were massive haemoptysis and pneumothorax. These clinical conditions were clearly associated with ABPA. In

contrast, ABPA was no more prevalent in patients who underwent lung transplantation than those in the general CF population (table 4).

Patients with ABPA received more respiratory therapies than those without ABPA, in particular, oral and inhaled corticosteroids, but also bronchodilators, dornase alfa (Pulmozyme®) and continuous antibiotic therapy (table 5). Conversely, ABPA was more prevalent in patients treated routinely with continuous antibiotic therapy (804/8191; 11.0%) than in those receiving intermittent antibiotics (5.7%) or antibiotics for exacerbations only (3.6%). Only 26.2% of ABPA patients (253/967) were treated with the anti-*Aspergillus* antibiotics, itraconazole or amphotericin (1.4% of those without ABPA were also treated with these antibiotics).

*Influence of allergic bronchopulmonary aspergillosis on lung function decline*

Regression analysis of percent pred FEV<sub>1</sub> in ABPA and non-ABPA patients was performed using the mixed model analysis, assuming linearity, for the three severity groups stratified for age. In subgroups of patients <6 yrs of age, analysis was not possible due to the small number of patients with valid values. The results demonstrated a marginally more rapid decline in FEV<sub>1</sub> by age in the non-ABPA patients than in the ABPA patients in several of the subgroups analysed (fig. 1; table 6). Regression analysis of percent pred FEV<sub>1</sub> in the ABPA and non-ABPA patients after the introduction of a quadratic term for age was able to summarize the general trends in FEV<sub>1</sub> over the follow-up period of the study (table 6), but did not identify important differences between ABPA and non-ABPA patients with respect to FEV<sub>1</sub> decline in any severity group. Only differences for the intercept values were demonstrated, which supported the poorer lung function levels in the ABPA subpopulation already identified by cross-sectional analysis.

**Discussion**

This is the second extensive epidemiological study of ABPA in CF to be published recently [12]. The large

Table 2. – Prevalence of allergic bronchopulmonary aspergillosis (ABPA) by initial forced expiratory volume in one second (FEV<sub>1</sub>) and age

Age yrs	CF Population n		Mean FEV <sub>1</sub> % (95% CI)		FEV <sub>1</sub> <40%			FEV <sub>1</sub> 40–70%			FEV <sub>1</sub> ≥70%					
	All	ABPA	No ABPA	ABPA	No ABPA	ABPA	No ABPA	ABPA	No ABPA	ABPA	No ABPA	ABPA				
			Yes	%	95% CI	Yes	%	95% CI	Yes	%	95% CI					
<6	1015	42	91	78.7	7	0	0	0–41.0	141	15	8.4	4.6–14.0	825	29	3.4	2.3–4.8
6–12	3284	306	79.8	70.1	136	16	10.5	6.1–16.5	731	132	15.3	13.0–17.9	2111	158	7.0	6.0–8.1
13–17	2004	228	68.5	60.1	272	52	16.1	12.2–20.5	633	92	12.7	10.4–15.3	871	84	8.8	7.1–10.8
≥18	3326	331	54.3	48.3	1001	141	12.4	10.5–14.4	1196	135	10.1	8.6–11.9	798	55	6.5	4.9–8.3
Total	9629	907	70	60	1416	209	12.9	11.3–14.6	2701	372	12.1	11.0–13.3	4605	326	6.6	5.9–7.3

Only patients with initial FEV<sub>1</sub> available are included. CF: cystic fibrosis; CI: confidence interval.

Table 3. – The association of specific microbial infections with allergic bronchopulmonary aspergillosis (ABPA)

Organisms	n/N	n/N %	95% CI
<i>P. aeruginosa</i>			
No ABPA	6975/10886	64.1	63.2–65.0
ABPA	806/954	84.5	82.0–86.7
<i>S. aureus</i>			
No ABPA	6551/10886	60.2	59.3–61.1
ABPA	602/954	63.1	60.0–66.2
<i>Candida</i>			
No ABPA	4062/10886	37.3	36.4–38.2
ABPA	483/954	50.6	47.4–53.8
<i>Aspergillus</i>			
No ABPA	1764/10886	16.2	15.5–16.9
ABPA	429/954	45.0	41.8–48.2
<i>H. influenzae</i>			
No ABPA	4425/10886	40.6	39.7–41.6
ABPA	295/954	30.9	28.0–34.0
<i>S. maltophilia</i>			
No ABPA	798/10886	7.3	6.8–7.8
ABPA	159/954	16.7	14.4–19.2
<i>B. cepacia</i>			
No ABPA	620/10886	5.7	5.3–6.1
ABPA	75/954	7.9	6.2–9.8
Other/normal flora			
No ABPA	5329/10886	49.0	48.0–49.9
ABPA	459/954	48.1	51.3

Other/normal flora refers to all patients with at least one sputum culture from which rare pathogens, normal flora or no bacteria were isolated. n/N: number of ABPA positive patients where bacteria were isolated/number of patients where bacteriology cultures were performed; n/N %: proportion of n/N expressed as per cent; 95% CI: 95% confidence interval; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. aureus*: *Staphylococcus aureus*; *H. influenzae*: *Haemophilus influenzae*; *S. maltophilia*: *Stenotrophomonas maltophilia*; *B. cepacia*: *Burkholderia cepacia*.

number of patients included in this study (>12,000) permitted the objective description of some epidemiological and clinical aspects of ABPA, without the need for statistical tests to evaluate the results of the descriptive analysis. Although the reliability of the parameters obtained (mean or frequencies) has been quoted as the 95% confidence intervals for each variable analysed, the breadth of these confidence intervals is largely dependent on the number of observations per group.

#### Allergic bronchopulmonary aspergillosis prevalence

An overall sex-independent prevalence of ABPA in CF of 7.8% was found. Previous reports of prevalence were highly variable [7–11, 13, 14], however, the present results cannot be compared with those of others due to the small population samples examined in these previous studies. A very low prevalence (2%) was reported in a North American study [12], which is comparable to the present study in population size (14,210). However, that prevalence was based only on medical conditions that were present within 6 months of the enrolment date. The authors of that study expressed their reservations about accepting such a low prevalence as the true prevalence of ABPA in CF and suspect that this condition is under-diagnosed for many reasons in North America.

Table 4. – Prevalence of allergic bronchopulmonary aspergillosis (ABPA) by respiratory complications

Medical history	n/N	n/N %	95% CI
Haemoptysis	105/684	15.4	12.7–18.3
Pneumothorax	33/256	12.9	9.0–17.6
Lung transplant	19/216	8.8	5.4–13.4

n/N: the number of patients with ABPA affected by the specified complication/number of patients in the given class; n/N %: proportion of n/N expressed as percent; 95% CI: extremes of the 95% confidence interval expressed as per cent.

The problem of variability clearly arose in the present study, as well as in the parallel epidemiological study carried out in North America [12], with prevalence ranging from 2–13% among countries and from 0–27% among CF centres. This wide variability may be partly due to the different number of cases enrolled by different centres or countries, as shown by the differences in the confidence intervals among them. Another explanation might be different levels of air-borne spores of *Aspergillus* spp., in different environments or geographical areas. However, it is recognized that *Aspergillus* spores are distributed ubiquitously in the environment [28] and that *A. fumigatus* is similarly found in all domestic environments, including hospital ventilation systems, mattress dust, between false ceilings and on plants [29]. Furthermore, it is not *Aspergillus* spores but soluble *Aspergillus* allergens that are responsible for disease: *Asp f 1* antigen is considered the most common inciting agent in ABPA and is produced only in the mycelial phase of *Aspergillus* growth. While germination of spores occurs in the bronchial mucus of CF patients [30], it is unlikely in the inanimate environment. Thus, this explanation appears invalid despite the absence of evidence to the contrary.

Another reason for variability could be different CF management practices and differences in the overall clinical condition of patients among centres, which may lead to different levels of colonization, germination of *Aspergillus* spores and *Aspergillus* sensitization. Indeed, differences in management strategy seem to be of great importance for overall clinical condition (H.K. Harms, Childrens Hospital, Munich University, Munich, Germany, personal communication based on ERCF data). If treatment policy and disease severity correlate with ABPA development, inter-centre differences in ABPA prevalence could be partly attributed to different approaches to disease management and the resulting clinical condition of patients in their care. However, it remains possible that variable interpretation of clinical and laboratory criteria in the definition of ABPA could explain, at least in part, the wide variability observed in the present study, given that ABPA diagnosis defined by the ERCF is partially based on the physician's suspicion that the condition is present.

The diagnosis of ABPA in CF remains difficult due to the commonly reported sensitization to *Aspergillus* in CF [10, 13, 14, 21, 23], and the poor specificity of the clinical manifestations such as bronchoconstriction, central bronchiectasis and pulmonary infiltrates [31]. The suggested combination of specific immunological criteria and a four-fold rise in total IgE, in conjunction with clinical deterioration and the newly-reported appearance of radiological shadowing in patients poorly responding

Table 5. – Routine respiratory therapy rate in allergic bronchopulmonary aspergillosis (ABPA) and non-ABPA patients

	n/N	n/N %	95% CI
<b>Bronchodilators</b>			
No ABPA	7726/11480	67.3	66.4–68.2
ABPA	876/967	90.6	88.6–92.4
<b>Corticosteroid (inhaled)</b>			
No ABPA	4432/11480	38.6	37.7–39.5
ABPA	725/967	75.0	72.1–77.7
<b>Corticosteroid (oral)</b>			
No ABPA	1693/11480	14.7	14.1–15.4
ABPA	543/967	56.2	53.0–59.3
<b>Dornase alfa</b>			
No ABPA	4523/11480	39.4	38.5–40.3
ABPA	628/967	64.9	61.8–68.0
<b>Antibiotics (continuous)</b>			
No ABPA	7387/11480	64.3	63.5–65.2
ABPA	804/967	83.1	80.6–85.5
<b>Antibiotics (intermittent)</b>			
No ABPA	1316/11480	11.5	10.9–12.1
ABPA	79/967	8.2	6.5–10.1
<b>Antibiotics (exacerbations)</b>			
No ABPA	1558/11480	13.6	12.9–14.2
ABPA	58/967	6.0	4.6–7.7

n/N: the number of patients using the given treatment/the number of patients in the given class; n/N %: proportion of n/N expressed as per cent; 95% CI: 95% confidence interval of the proportion n/N expressed as per cent.

to antibiotics [20], may improve the diagnosis of ABPA in CF. Elevated serum IgE in *A. fumigatus*-sensitized patients has been claimed by others to be clinically relevant [23]. At present, one of the major problems in diagnosing ABPA arises from the lack of standardized fungal extracts [32]. A new diagnostic method has been proposed based on particular disease-specific recombinant *A. fumigatus* allergens [32, 33]. Two categories of *A. fumigatus* allergens have been identified: secreted and cytoplasmic proteins. Secreted allergens may be recognized by serum IgE of *A. fumigatus*-sensitized individuals with or without ABPA, whereas nonsecreted cytoplasmic allergens may be recognized only by serum IgE from ABPA patients [32]. By using such allergens, distinct IgE responses to *A. fumigatus* have been found in CF patients with ABPA (against *rAspf4* and *rAspf6* antigens) or simple *A. fumigatus* allergy (against *rAsp F 1* and *rAsp F 3* antigens) [22, 34]. Differentiation of the two conditions is fundamental because of different therapeutic implications.

There are only a limited number of reports in the literature that have addressed the prevalence of ABPA at an early age in CF, despite evidence that an immune response to *A. fumigatus* usually develops very early in these patients [10, 12, 21]. Our data show that ABPA can affect even patients <6 yrs of age (56/3150 patients). In patients  $\geq 6$  yrs of age, prevalence is consistently  $\sim 10\%$ .

An attempt to correlate the prevalence of ABPA phenotype with CFTR genotype was performed by grouping patients with a known genotype into the four most common groups found among the ERCF patients ( $\Delta F508/\Delta F508$ ,  $\Delta F508/G551D$ ,  $\Delta F508/G542X$ ,  $\Delta F508/N1303K$ ). The remaining patients were grouped into two classes:  $\Delta F508$ /other and other/other. No significant differences were observed between genotypes.

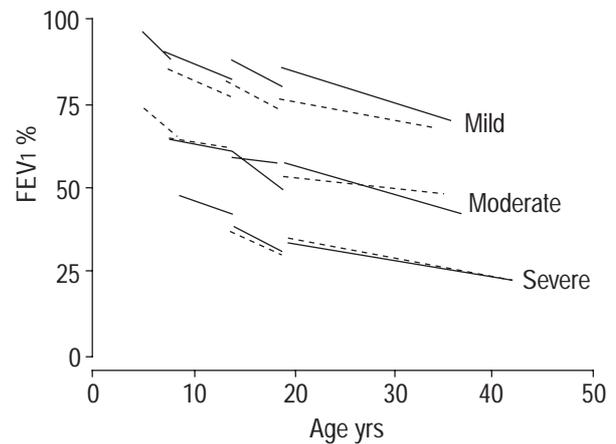


Fig. 1. – Mixed model regression lines of forced expiratory volume in one second (FEV1) (%) versus age (yrs) for ABPA (---) and non-ABPA (—) patients. The data are divided into three disease severity categories determined on the basis of FEV1 at enrolment, and each severity category is subdivided into four age classes at enrolment (from left to right: <6 yrs, 6–12 yrs, 13–17 yrs,  $\geq 18$  yrs.)

#### Allergic bronchopulmonary aspergillosis associations with clinical conditions

ABPA was generally associated with a poorer nutritional status (lower body weight z-score), slightly higher concentrations of serum IgG (an indication of greater inflammation), a poorer respiratory function (lower FEV1 and FVC) and a higher rate of respiratory tract colonization by pathogens.

Association with impaired lung function is of interest. Patients with ABPA had, on average, a 10% lower FEV1 % pred than those without ABPA. In addition, patients with FEV1  $\geq 70\%$  of the predicted value were less frequently affected by ABPA (7%) than the others, while in patients with FEV1 <70% of the predicted value ABPA can reach peak frequencies of 13–16% in adolescents (table 2). Poorer lung function in CF patients with ABPA or sensitization to *A. fumigatus* has been reported [10, 23]. In CF it is unclear whether ABPA is responsible for the deterioration, more severe lung impairment predisposes patients to ABPA, or some other clinical feature underlies both conditions. The absence of complete clinical and immunological patient histories prior to their enrolment in the ERCF means that these data cannot answer this question.

A greater frequency of pathogenic bacterial colonization of the bronchial tree in CF patients with ABPA is a novel observation. ABPA patients are more frequently colonized by *P. aeruginosa*, *S. maltophilia*, *B. cepacia* and *C. albicans* as well as *A. fumigatus* than those without ABPA. Again the causal relationship between these observations is unclear. In contrast, colonization by *S. aureus* was comparable between subgroups, probably due to its colonization of the youngest patients. The young age could also account for the greater colonization by *H. influenzae* of the non-ABPA subjects. It is important to reiterate that only a proportion of ABPA patients show a sputum culture positive for *A. fumigatus*.

The observation that ABPA patients have more severe pulmonary disease than those without ABPA is consistent with the higher incidence of lung complications such as

Table 6. – Forced expiratory volume in one second (FEV<sub>1</sub>) percentage decline by age according to initial severity groups independent of the age at enrolment (mixed model analysis)

Severity group	Population n	Slopes FEV <sub>1</sub> %			p-values <sup>#</sup>		
		Intercept	Linear	Quadratic	Intercept	Linear	Quadratic
Severe							
non-ABPA	1072	69.3±2.7	-2.7±0.3	0.04±0.01			
ABPA	167	57.5±5.5	-1.6±0.5	0.02±0.01	0.06	0.06	0.08
Moderate							
non-ABPA	2035	79.8±1.4	-1.8±0.2	0.03±0.00			
ABPA	306	71.1±3.7	-0.9±0.4	0.01±0.01	0.03	0.06	0.13
Mild							
non-ABPA	3249	101.6±1.1	-1.5±0.2	0.02±0.00			
ABPA	258	91.6±4.8	-1.0±0.6	0.01±0.02	0.04	0.43	0.46

Data represents mean±SEM. ABPA: allergic bronchopulmonary aspergillosis; <sup>#</sup>: ABPA versus non-ABPA.

massive haemoptysis and pneumothorax in those with ABPA. Lung transplantation was performed in a similar proportion of patients in the two subpopulations.

The poorer pulmonary status of ABPA patients is also consistent with the more intensive and aggressive therapy administered. All of the common therapies used to treat lung disease in CF were administered to considerably more ABPA patients than those without ABPA. Orally-administered corticosteroids are generally recommended for ABPA in non-CF patients [27] as well as in CF patients [11, 16, 18, 19] in order to control acute exacerbations and prevent permanent lung damage. Notably, only 56% of the ABPA patients received oral corticosteroids while 75% of them received inhaled corticosteroids. However, the latter route of administration was also commonly used in the non-ABPA patients (39%). There has been no evidence to date of the effectiveness of inhaled corticosteroids in controlling ABPA in CF. The greater use of continuous antibiotics and dornase alfa in patients with ABPA probably confirms the greater severity of lung disease in these patients, but one could also argue that continuous exposure to antibiotics may predispose to fungal colonization.

#### *Influence of allergic bronchopulmonary aspergillosis on lung function decline*

Despite the relatively short period of follow-up, the longitudinal aspect of this study has provided some interesting insight into independent effects of ABPA on the progression of pulmonary disease in CF. In the cross-sectional part of the present study, it has been demonstrated that ABPA is generally associated with a poorer pulmonary status, although a significant causal relationship is yet to be established. This is partly supported by other studies [10, 12, 23].

The mixed model regression analysis was unable to demonstrate substantial or clinically relevant differences in the rates of FEV<sub>1</sub> decline between ABPA and non-ABPA patients for any of the age by severity subgroups considered. In some subgroups, typically in the adult group with severe impairment, the regression lines are indistinguishable, whereas in other subgroups, including the adolescent and adult groups with moderate lung function impairment, FEV<sub>1</sub> decline seems to be more rapid in the patients without ABPA. The results of this analysis are not conclusive and a longer period of observation, combined

with the evaluation of other variables involved in the decline of pulmonary function, is necessary.

In conclusion, this study enabled the authors to estimate the variable prevalence of allergic bronchopulmonary aspergillosis in cystic fibrosis in countries of western, central and northern Europe. It has been demonstrated that this complication is associated with a poorer clinical condition. A longitudinal approach using a mixed model multiple regression analysis was unable to confirm an independent contribution of allergic bronchopulmonary aspergillosis to the progress of lung disease in the short term.

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