

Effect of Allergic Bronchopulmonary Aspergillosis on Lung Function in Children with Cystic Fibrosis

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Rationale: The relationship between sensitization to *Aspergillus fumigatus* and progression of pulmonary function is not yet established in cystic fibrosis (CF).

Objectives: We aimed to evaluate onset of *A. fumigatus* sensitization and development of allergic bronchopulmonary aspergillosis (ABPA), as well as to determine the physiologic factors of lung function influencing these mechanisms in CF.

Methods: Serial annual lung function tests performed in 122 children with CF (62 males; 60 females; age: 6–18 yr) provided data pertaining to FRC measured by plethysmography, lung clearance index, volume of trapped gas, effective specific airway resistance, and forced expiratory indices (FEV₁, FEF at 50% VC). Specific IgE to recombinant *A. fumigatus* allergens, rAspf1 and rAspf3, served as marker for sensitization, and to rAspf4 and rAspf6 as indications for a serologic ABPA, were clinically diagnosed (Nelson criteria). By linear mixed-effect model analysis, five patient groups, (1) not sensitized and free from *Pseudomonas aeruginosa*, (2) intermittently *P. aeruginosa* colonized, (3) chronically *P. aeruginosa* infected, (4) sensitized, and (5) with ABPA, were retrospectively evaluated.

Measurements and Main Results: *A. fumigatus* sensitization was best reflected by increased rAspf1 + 3-specific IgE levels, whereas, in most patients, sensitization was preceded by *P. aeruginosa* infection. Patients with ABPA demonstrated the most severe progression in all lung function parameters, and FEF at 50% VC, volume of trapped gas, and effective specific airway resistance were the best predictors ($p < 0.0001$). However, regarding distinction between sensitization to *A. fumigatus* and development of ABPA in the course of CF, chronic *P. aeruginosa* infection has to be taken into account.

Conclusions: Airway narrowing, gas trapping, and small airway disease are the major targets for functional derangement in ABPA.

Keywords: allergic bronchopulmonary aspergillosis; *Aspergillus fumigatus* sensitization; cystic fibrosis; lung function; *Pseudomonas aeruginosa* infection

Allergic bronchopulmonary aspergillosis (ABPA) is a lung disease resulting from a hypersensitivity to *Aspergillus fumigatus*, clinically characterized by impaired mucociliary clearance, mucoid impactions, episodic bronchial obstruction, and pulmonary infiltrates. Immunologically, ABPA is associated with recurrent episodes of eosinophilic airways inflammation, peripheral blood eosinophilia, high total serum IgE levels, and increased circulating specific IgE and IgG against *A. fumigatus* antigen (1). ABPA

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

The relationship between sensitization to *Aspergillus fumigatus* and progression of pulmonary function in patients with cystic fibrosis is yet unclear.

What This Study Adds to the Field

We demonstrate how lung function deteriorates over time in relation to *A. fumigatus* sensitization and allergic bronchopulmonary aspergillosis based on extended pulmonary function characterization.

may afflict patients with asthma (2), and an increasing prevalence of ABPA in patients with cystic fibrosis (CF), varying between 0.9 and 13%, has been reported. Extended epidemiologic studies, including more than 12,000 patients, reported a prevalence in North America of 2% (3) and 7.8% in Europe (4). Impaired mucus clearance and airway obstruction may favor germination of spores and release of antigens, resulting in a complex immune response by the host, which is detrimental for patients with CF (5, 6). The pathophysiology of ABPA still remains largely speculative. Familial occurrence has been reported, suggesting a possible genetic contribution to the disease in CF (7–9), whereas patients with ABPA without CF have a higher frequency of CF transmembrane conductance regulator (*CFTR*) mutations than that found among subjects with bronchitis or in the normal population (10). However, the significance of heterozygous *CFTR* mutations with regard to the properties of mucus is unclear. It has been suggested that HLA-DR molecules DR2, DR5 and possibly DR4 or DR7 contribute to susceptibility, whereas HLA-DQ2 contributes to resistance to ABPA development (9, 10).

In CF, the abnormal mucus might promote the trapping of *A. fumigatus* spores within the bronchial airway, presumably promoting growth of *A. fumigatus* mycelia, thus contributing to an increased *A. fumigatus* colonization of the lung (11), which may stimulate T-helper cell type (Th) 2-biased responses favoring development of ABPA (12). The inflammatory process initiated by Th2 immune responses to fungal colonization is related to the production of specific IgE and IgG antibodies against *A. fumigatus* (13–15). The atopic status associated with susceptibility to developing ABPA was supposed to be the cause for airway changes seen in ABPA in CF, and may be an example of allergen-induced airway remodeling (15). In CF, the consequences of such an airway inflammatory process may be a progressive course of development of pulmonary hyperinflation, ventilation inhomogeneities, chronic obstructive lung disease, trapped gases, and gas exchange disturbances.

The relationship between *A. fumigatus* sensitization and progression of pulmonary function is not yet well established. Nicolai and colleagues found a correlation between positive RAST

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against *A. fumigatus* and lung function parameters, if age and weight were excluded from multiple regression analyses (16). The relationship between *A. fumigatus* sensitization and rapid decline in pulmonary function for *A. fumigatus*-sensitized patients with CF with or without ABPA was studied by different groups, with conflicting results (3, 4, 17, 18). Therefore, there is still a need for adequate longitudinal studies aimed at establishing the effect of *A. fumigatus* sensitization and development of ABPA on the decline in pulmonary function in CF. The objectives of this study were (1) to evaluate onset of *A. fumigatus* sensitization in relation to onset of chronic *P. aeruginosa* infection and in relation to development of ABPA and (2) to evaluate the lung function decline in subgroups of *A. fumigatus*-sensitized patients with and without ABPA in comparison with progression of lung function in patients with CF without *A. fumigatus* sensitization. The study offered the occasion to (3) search and define potential predictors for *A. fumigatus* sensitization and development of ABPA and (4) to evaluate potential associations between *A. fumigatus* sensitization, ABPA, and *CFTR* genotypes. Some of the results of this study have been previously reported in the form of an abstract (19).

METHODS

Database and Selection of Patient Cohort

For the present retrospective study, a total of 122 children and adolescents (62 males, 60 females) with CF born between 1978 and 1999, and examined regularly between 1983 and 2005, were enrolled. The inclusion and exclusion criteria related to the Bernese CF database were the same as previously reported (20), and methodologic details are given in the online supplement (21–25). The study protocols have been approved by the departmental ethics committee of the Children's Hospital and by the Governmental Ethics Committee of the State of Bern, Switzerland.

Diagnosis of ABPA and Analysis of Allergen-specific IgE and Recombinant *A. fumigatus* Allergens

The diagnostic criteria for ABPA proposed by Nelson (26) were used as a "gold standard." Details on how ABPA was assessed over the years are given in the online supplement. Sensitization to *A. fumigatus* was defined as positive for specific IgE RAST class 2 or higher in serum and/or for increased specific IgE to recombinant allergens in serum and increased *A. fumigatus*-specific IgG in serum, as determined by ELISA (27). Based on the concept presented by Hemmann and colleagues, rAspf1 and rAspf3 (rAspf1+3) elicit strong IgE responses in both sensitized patients with CF against *A. fumigatus* and patients with CF with ABPA (22). Onset of rAspf1+3 over cut-off (rAspf1 > 9.6 EU/ml; rAspf3 > 13.2 EU/ml) was defined as a first event of sensitization. We have also been interested in the question of whether the onset of rAspf4 (> 8.4 EU/ml) and rAspf6 (> 7.2 EU/ml), termed rAspf4+6, supposed to be specifically increased in sera of patients with CF suffering from ABPA (22), is different from rAspf1+3. We defined onset of rAspf4+6 as a second event of sensitization, in so far as specific IgE to recombinant *A. fumigatus* allergens rAspf1 and rAspf3 served as marker for sensitization, and to rAspf4 and rAspf6 as indicative for serologic ABPA (22, 23, 28). Methodologic details concerning allergen-specific IgE detection are given in the online supplement.

Pulmonary Function Measurements

Whole-body plethysmography and the multibreath nitrogen washout technique provided data pertaining to FRC measured by plethysmography (FRC_{pleth}), lung clearance index (LCI), volume of trapped gas (VTG), effective specific airway resistance (sReff), and forced expiratory indices (FEV₁, FEF at 50% VC [FEF₅₀]). All values were expressed as an SD score based on sex- and age-specific regression equations (29–31). More details are given in the online supplement.

Genotype Analysis

The entire coding sequence of the *CFTR* gene was screened in each patient, allowing detection of 97 to 98% of both known and novel

CF mutations (21, 32). According to the genotype frequencies of our population-specific genotypes, patients were classified into four groups (Table 1). More details are given in the online supplement.

Data Computation and Statistical Evaluation

Statistical methods included calculation of descriptive statistics, including Kaplan-Meier estimates of event occurrence. Linear mixed-effect model (LMM) analysis (4, 33, 34) was used to evaluate the annual measurements of lung function, and prediction of ABPA by several immunoserologic parameters was assessed by binary logistic regression analysis (SPSS, version 11; SPSS, Inc., Chicago, IL). Results with a *p* value of less than 0.05 were considered statistically significant.

RESULTS

Patient Cohort, Database Characteristics, and *CFTR* Genotype Distribution

The baseline characteristics of the study cohort fulfilling the inclusion criteria are presented in Table 1. Sex distribution was equal in these 122 patients with CF (62 males and 60 females), spanning an observation period of 28 yr. There were 1,400 lung function tests, with a median of 10 tests per child (4–15), corresponding to a median of 79 (23–111) tests per year. The age ranges were covered to 80% between ages 6 and 10 yr, to 71% between ages 11 and 15 yr, and to 39% between ages 16 and 20 yr. Regarding *CFTR* stratification, 72 (59.0%) were homozygous for $\Delta F508(2)$, 9 were compound heterozygous for the frame shift Swiss-type mutation 3905insT/ ΔF , 12 (9.8%) were compound heterozygous for the missense mutation R553X/ ΔF , and 29 (23.8%) had other miscellaneous genotypes (Table 1).

Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, and Prediction of ABPA

Distinction between patients with CF with ABPA (*n* = 16) and those without sensitization or ABPA (control subjects: *n* = 36) was made using several diagnostic cut-off levels of IgE, specific IgG antibodies, and rAspf. The sensitivity (% of patients with ABPA with a positive test), specificity (% of patients without ABPA with a negative test), positive predictive value (PPV, % of patients with a positive test with ABPA), and negative predictive value (NPV, % of patients with a negative test without ABPA) were calculated. Onset of elevated IgE levels depends on how that cut-off is defined. We tested three cut-off levels as potential diagnostic tools for ABPA: (1) IgE greater than 500 IU/ml; (2) IgE greater than 800 IU/ml; and (3) IgE greater than 1,000 IU/ml (Table 2). Sensitivity of the three levels was calculated to be 81, 81, and 75%, respectively; specificity, however, was 83, 86, and 89%. Accordingly, PPVs of 68, 72, 75%, and NPVs of 91, 91, and 89%, respectively, were obtained. It follows that the highest prediction for the development of ABPA can be made with a cut off-level of IgE less than 800 IU/ml (81%), and ABPA can best excluded by IgE less than 1,000 IU/ml (89%).

For specific IgG, two cut-off levels were compared: specific IgG greater than 50 kU/L and IgG greater than 100 kU/L. Both presented with a sensitivity of 100%, where specificity was 86%. It follows that both parameters (IgG > 50 kU/L and IgG > 100 kU/L) could be considered as suitable parameter for detection of ABPA. Moreover, the number of patients in whom IgG was determined was rather low (*n* = 21). In relation to rAspf values and their potential to detect ABPA, the highest specificity was found for rAspf4 (100%) and rAspf4+6 (100%).

Prediction of ABPA by several immunoserologic parameters was also evaluated by binary logistic regression analysis (Table 3). Either by forward or backward stepwise analysis, the highest significance within the groups IgE, specific IgG, or rAspf was achieved by IgE greater than 1,000 IU/ml (*p* < 0.0001). Specific

TABLE 1. PATIENT COHORT, DATA BASE CHARACTERISTICS, AND DISTRIBUTION OF *CFTR* MUTATIONS IN STUDY PATIENTS WITH CYSTIC FIBROSIS

	n (%)	No. of Test Sessions	Age Range Covered, yr (%)
Patient cohort			
Total, n	122		
Males, n (%)	62 (50.8)		
Females, n (%)	60 (49.2)		
Database follow-up			
Total		1,400	6–10 (80)
Per child		10 (4–15)	11–15 (71)
Per yr		79 (23–111)	16–20 (39)
<i>CFTR</i> mutation stratification*			
ΔF508(2)	72 (59.0)		
3905insT/ΔF	9 (7.4)		
R553X/ΔF	12 (9.8)		
Miscellaneous	29 (23.8)		

Definition of abbreviation: *CFTR* = cystic fibrosis transmembrane conductance regulator.

* Miscellaneous—numbers in brackets: ΔF508 and 1717-1G>A(3), W1282X(2), 2347delG (1), 621+1G>T(1), Q525X(2), N1303K(1), 2176insC (1), 394delTT (1), 4005+1G-A(1), 420DEL9 (1), E585X(1), G126D(1), G85E(1), R347P(1); 3905insT and 1717-1G>A(1), K710X(1), M1101K(1), Q39X(1); R553X and R553X(1), 3905insT (1); G542X and T5(3), G542X(1); Q542X and K1200E(2); N1303K and 2347delG (1), 2789+5G>A(1); 1199delG and R560S(1).

IgG seems not to be of major value. Comparing rAspf1, rAspf3, rAspf4, and rAspf6, the highest significance was achieved by rAspf4 ($p < 0.02$). In the comparison between rAspf1+3 and rAspf4+6, rAspf4+6 was superior ($p < 0.0001$). Finally, taking all group parameters together in the model (IgE, specific IgG, and rAspf), by stepwise elimination, the model of prediction was most significant for rAspf4 ($p = 0.002$) combined with IgE greater than 1,000 IU/ml ($p < 0.005$).

Immunoserology in Its Time–Event Relations

In the assessment and definition of parameter characteristics for prediction of disease milestones, the time–event relation of each parameter has to be considered. Event occurrence can be defined as the age at which a specific parameter presents with a value beyond a certain cut-off level (usually $2 \times \text{SD}$). These event occurrences were evaluated by Kaplan–Meier plots, depicted in Figure 1, showing the evaluation of parameters with their specific time events as the age at which the parameter had exceeded the cut-off level. A comparison between each rAspf is given in the left-hand panel, and, in the right-hand panel, sensitization against *A. fumigatus* (rAspf1+3, rAspf4+6) is compared with the onset of chronic *P. aeruginosa* infection, as well as with the onset of clinically established ABPA. The grouping

of rAspf1 with rAspf3 (rAspf1+3) and the grouping rAspf4 and rAspf6 (rAspf4+6) was previously shown to be helpful in CF (22). The mean (95% confidence interval [CI]) at onset of abnormal values (event) of rAspf for rAspf1, rAspf3, rAspf4, and rAspf6 (left-hand panel of Figure 1) were calculated to be 13.5 (12.5–14.5), 14.2 (11.4–13.4), 16.9 (15.7–18.4), and 16.0 (14.7–17.3) yr, respectively. Onset of abnormal rAspf3 was significantly earlier than onset of rAspf4 (log rank statistic, 30.13; $p < 0.0001$) and rAspf6 (log rank statistic, 22.99; $p < 0.0001$), but onset of abnormal rAspf1 was also significantly earlier than onset of rAspf4 (log rank statistic, 17.47; $p < 0.0001$) and rAspf6 (log rank statistic, 12.23; $p < 0.0005$). Therefore, it seems to be reasonable to define sensitization against *A. fumigatus* by grouping rAspf1 with rAspf3 (rAspf1+3), and grouping rAspf4 with rAspf6 (rAspf4+6).

As shown in the right-hand panel of Figure 1, onset of chronic *P. aeruginosa* infection presented with a mean (95% CI) of 11.6 (9.5–13.7) yr as the earliest event occurrence, followed by sensitization against rAspf1+3 with a mean of 13.1 (12.1–14.2) yr. Sensitization against rAspf4+6 at 17.8 (16.3–19.3) yr was significantly later. The latest onset of event was found for ABPA by a calculated mean of 20.5 (19.2–21.7). Differences between

TABLE 2. SENSITIVITY, SPECIFICITY, AND POSITIVE AND NEGATIVE PREDICTIVE VALUES OF SEVERAL IMMUNOLOGIC PARAMETERS ADDRESSING WHETHER PATIENTS WITH CYSTIC FIBROSIS DEVELOPED ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

Parameter	Target	ABPA/Above Parameter Level (n/n)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	p Value*	OR (95% CI)
IgE > 500 IU/ml	ABPA	13/52	81	83	68	91	< 0.0001	21.667 (4.686–100)
IgE > 800 IU/ml	ABPA	13/52	81	86	72	91	< 0.0001	26.867 (5.584–129)
IgE > 1,000 IU/ml	ABPA	12/52	75	89	75	89	< 0.0001	24.000 (5.163–111)
Specific IgG > 50 kU/L	ABPA	14/21	100	86	93	100	< 0.0001	NA
Specific IgG > 100 kU/L	ABPA	14/21	100	86	93	100	< 0.0001	NA
rAspf1 (> 9.6 EU/ml)	ABPA	14/21	100	86	93	100	< 0.0001	0.143 (0.023–0.877)
rAspf3 (> 13.2 EU/ml)	ABPA	14/21	100	86	93	100	< 0.0001	0.143 (0.023–0.877)
rAspf4 (> 8.4 EU/ml)	ABPA	12/21	86	100	100	78	< 0.0001	7.000 (1.940–25.255)
rAspf6 (> 7.2 EU/ml)	ABPA	13/21	92	86	93	76	< 0.001	78.000 (4.141–1469)
rAspf _{1,3}	ABPA	14/21	100	100	100	100	< 0.0001	0.167 (0.028–0.997)
rAspf _{4,6}	ABPA	11/21	79	100	100	70	< 0.001	5.333 (1.923–14.790)

Definition of abbreviations: ABPA = allergic bronchopulmonary aspergillosis; NA = not applicable; NPV = negative predictive value; PPV = positive predictive value.

* Fisher's exact test (one-sided).

TABLE 3. PREDICTION OF ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS BY SEVERAL IMMUNOSEROLOGIC PARAMETERS IN 122 PATIENTS WITH CYSTIC FIBROSIS, EVALUATED BY BINARY LOGISTIC REGRESSION ANALYSIS WITH BACKWARD (STEPWISE) ELIMINATION

	B (SE)	Wald Statistics	p Value	OR (95% CI)
IgE: 500, 800, 1,000 IU/ml				
IgE > 1,000 IU/ml	2.686 (0.633)	18.019	< 0.0001	14.667 (4.244–50.681)
Constant	–3.091 (0.511)	36.557	< 0.0001	
IgE > 800 IU/ml	2.806 (0.684)	16.839	< 0.0001	16.545 (4.331–63.202)
Constant	–3.332 (0.588)	32.162	< 0.0001	
Specific IgG: 50, 100 kU/L				
IgG > 100 kU/L	9.376 (25.348)	0.137	NS	
Constant	–10.203 (25.346)	0.162	NS	
rAspf1, rAspf3, rAspf4 and rAspf6				
rAspf4	1.928 (0.824)	5.482	< 0.02	6.878 (1.369–34.555)
rAspf6	2.438 (1.084)	5.061	< 0.05	11.449 (1.369–95.754)
Constant	–4.649 (1.160)	16.052	< 0.0001	
rAspf1+3 and rAspf4+6				
rAspf4+6*	2.654 (0.695)	14.585	< 0.0001	10.196 (2.571–40.432)
Constant	–2.979 (0.592)	25.334	< 0.0001	
Summary predictor combination				
IgE > 1,000 IU/ml	2.015 (0.694)	8.431	< 0.005	7.504 (1.925–29.251)
rAspf4+6	2.272 (0.734)	9.582	0.002	9.699 (2.301–40.876)
Constant	–3.731 (0.729)	26.220	< 0.0001	

Definition of abbreviations: B = slope; CI = confidence interval; NS = not significant; OR = odds ratio.

* As best selected also in combination rAspf1, f3, f4, rAspf1_3, and rAspf4_6.

all time–event curves were highly significant ($\chi^2 = 189.1$; $p < 0.0001$).

Having selected IgE, specific IgG, and the combined rAspf1+3, as well as rAspf4+6, as potential predictors of sensitization against *A. fumigatus* and, hence, infection of ABPA, the values of these parameters at the time of their specific event (i.e., age at which values exceeded cut-off) are presented in Figure 2 as box plots (line: mean; box: 68%; whisker: 95% CI). The best distinction in the event comparison between onset of ABPA and a group of control patients (patients not sensitized for *A. fumigatus*) was found for IgE greater than 1,000 IU/ml (mean difference between those with ABPA and control subjects: 2,763 IU/ml; $t = 5.669$; $p < 0.001$; mean difference between those sensitized against *A. fumigatus* and ABPA: 2,763 IU/ml, $t = 5.669$, $p < 0.001$), and for rAspf1 (mean difference between ABPA and control subjects: 757 EU/ml; $t = 4.013$; $p < 0.001$;

mean difference between those sensitized against *A. fumigatus* and ABPA: 684 EU/ml; $t = 5.729$; $p < 0.001$). However, with an onset of 13.1 (CI, 12.1–14.2) yr, the event for rAspf1+3 is reached significantly earlier than the event of IgE greater than 1,000 IU/ml with 19.9 (CI, 17.8–20.2) yr. It follows that rAspf1+3 is the earliest and most significant parameter indicating onset of sensitization against *A. fumigatus* and, therefore, the best predictor for ABPA.

Progression of Lung Function Decline

From Figure 1 it must be assumed that progression of lung function deterioration in ABPA is additionally influenced by the onset of *P. aeruginosa* infection. Therefore, stratification into five etiologic groups was performed. Apart from a control group, collecting patients with CF neither colonized with *P. aeruginosa* nor sensitized to *A. fumigatus* (control-CF: $n = 16$), patients

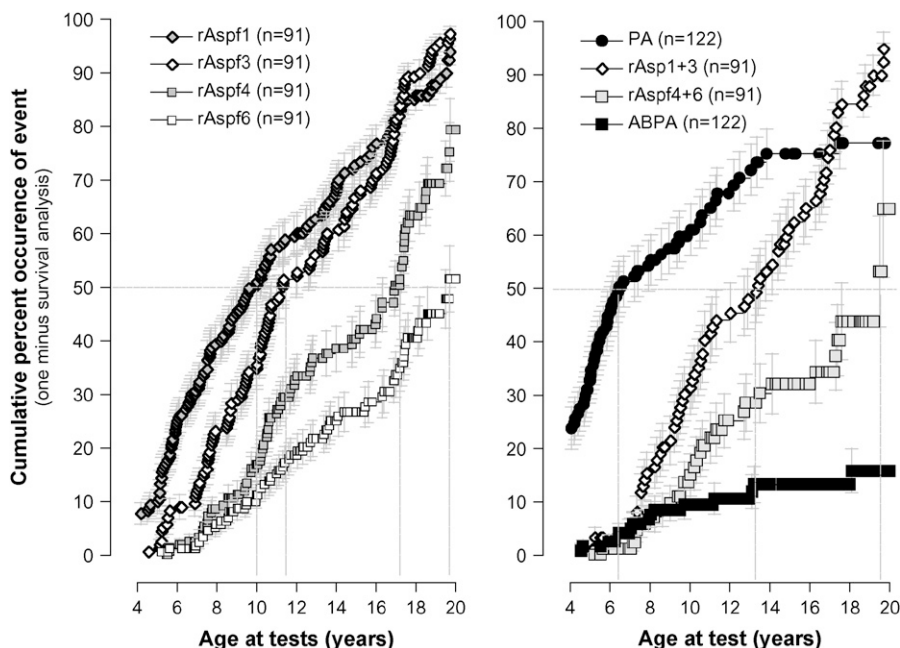


Figure 1. Time sequence comparing onset of specific IgE to recombinant allergens (rAspf 1, rAspf 3, rAspf 6) in the left panel and time sequence comparing the onset of chronic *Pseudomonas aeruginosa* infection, onset of *Aspergillus fumigatus* sensitization (Aspf1+3, Aspf4+6), and onset of allergic bronchopulmonary aspergillosis (ABPA) in the right panel in patients with cystic fibrosis (CF).

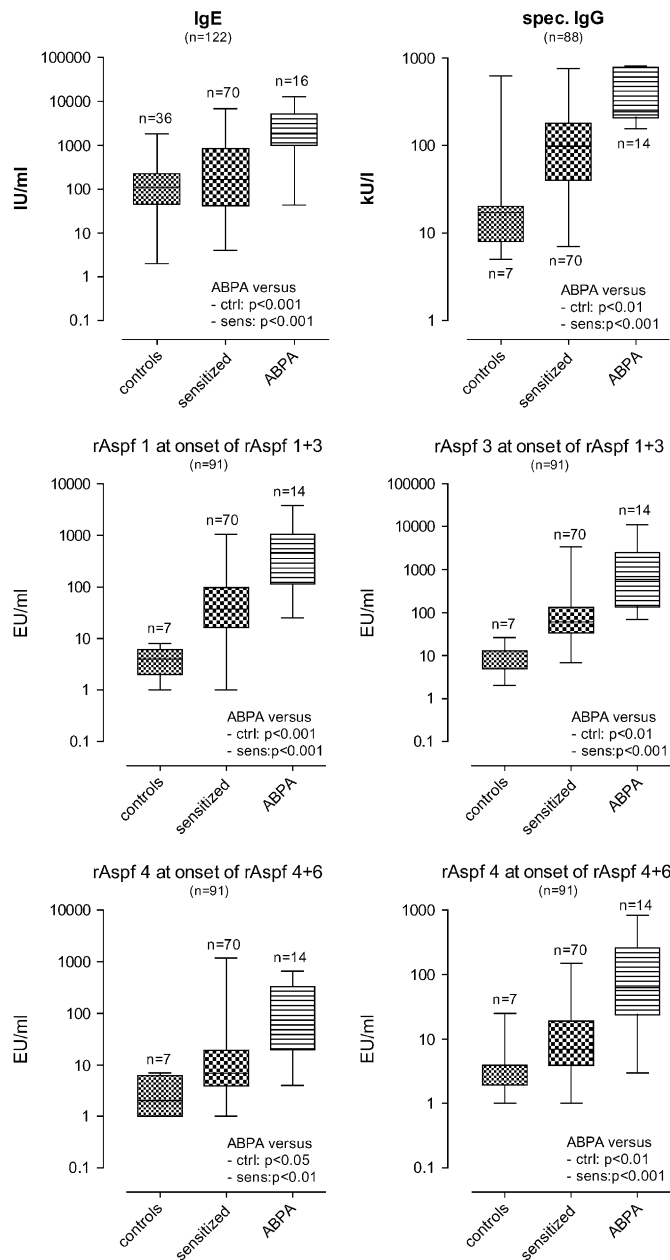


Figure 2. Immunoserology at time of their specific event (age at which values exceeded cut-off shown in Figure 1). Values are presented as box plots (line, mean; box, 68%; whisker, 95% confidence interval). Differences between values at onset of ABPA in relation to values obtained in the control group, as well as at time of sensitization against *Aspergillus fumigatus*, were calculated by one-way analysis of variance with Bonferroni's multiple comparison tests.

demonstrating *A. fumigatus* sensitization against rAspf1+3 (considered as *A. fumigatus* sensitized [$n = 45$]) were distinguished from those having developed ABPA ($n = 16$). Concerning *P. aeruginosa* infection, patients only intermittently colonized by *P. aeruginosa* (intermittent *P. aeruginosa*: $n = 21$) were differentiated from those chronically infected by *P. aeruginosa* (*P. aeruginosa* infection: $n = 24$). Progression with age (represented by the slopes) is given for each group, being most pronounced for FEF₅₀ (Table 4). LMM analysis demonstrated significant changes of all lung function parameters within all groups. A group effect (comparison of

means of lung function in relation to age) was noticed for LCI and FEV₁. Comparison of group slopes (group $\times$ age effect) was significantly demonstrated for all lung function parameters, most strongly for FEF₅₀ ($F = 10.074$; $p < 0.0001$).

The slopes of the five groups were computed from the fixed predicted values obtained by the LMM analysis (Table 4), and mean changes of lung function of all 122 patients with CF, aggregated for 6-mo intervals and restricted to the groups "controls," "*P. aeruginosa* infected," and "ABPA," are shown in Figures 3A and 3B. With the exception of the CF control subjects, VTG, sReff, FEV₁, FEF₅₀, and in the group intermittent for *P. aeruginosa*, LCI and FEF₅₀, all slopes were highly significant. The highest progression of *A. fumigatus* sensitization and ABPA was detected by FEF₅₀ (slope: -0.408 and -0.619 , respectively; $p < 0.0001$). The best differentiation between *A. fumigatus* sensitization and ABPA development was observed in FEF₅₀ ($t = -4.739$; $p < 0.0001$), VTG ($t = 3.712$, $p < 0.0001$), and sReff ($F = 3.623$; $p < 0.0001$).

The distinction regarding *A. fumigatus* sensitization and development of ABPA, taking into account intermittent colonization and chronic infection with *P. aeruginosa*, is given in Table 4. Except for LCI, all lung function parameters presented with high prediction for *P. aeruginosa* infection, highest for FEF₅₀, ($F = -5.763$, $p < 0.0001$), followed by FEV₁ ($F = -4.863$, $p < 0.0001$), as well as for ABPA, followed by VTG ($F = 3.712$, $p = 0.0001$) and sReff for ABPA ($F = 3.623$, $p < 0.0001$). VTG presented with similar distinction (*P. aeruginosa* infection: $F = 3.544$; $p < 0.0001$; ABPA: $F = 3.712$; $p = 0.0001$). As synoptically presented in Figure 4, LCI already presented with high z scores at age 5 yr, suggesting that most progression already occurred before that age. The slope comparison in Figure 4 indicates that patients developing ABPA show the worst progression in all lung function parameters. Patients with *A. fumigatus* sensitization had an intermediate position between CF control subjects and patients with ABPA. Patients with chronic *P. aeruginosa* infection showed the second-worst progression, and presented with slopes between *A. fumigatus*-sensitized patients and those with ABPA.

DISCUSSION

The diagnosis of ABPA in patients with CF is complicated by a number of characteristics shared by the two diseases, including pulmonary infiltrates, *A. fumigatus* sensitization, elevated specific IgE and IgG antibodies to *A. fumigatus* extract, as well as centrally located bronchiectasis (1, 6). Therefore, serologic findings should strongly contribute to confirming or excluding ABPA suspected on the basis of clinical signs (22, 24). Colonization of the lower respiratory tract with *A. fumigatus* is particularly frequent in patients with CF, with a reported incidence of 57% (26), and a prevalence of 40% (35). Interaction of *A. fumigatus*-specific IgG and IgE antibodies in the bronchial tree leads to activation of complement and mast cells, resulting in mediator release and cytokine production, contributing to lung damage (1). A sizeable minority, ranging from 1 to 11% might develop ABPA (3, 4). Demonstration of precipitating *A. fumigatus* antibodies is the most widely used method for serodiagnosis of ABPA. In various studies, patients with CF have been divided into groups according to increasing positivism to different *A. fumigatus*-specific tests (1, 36), but the relationship between *A. fumigatus* sensitization and progression of pulmonary function decline has not yet been age-dependently studied in a large number of patients.

The present study demonstrates differences in the onset of specific *A. fumigatus*-IgE responses to recombinant allergens in patients with CF with *A. fumigatus* sensitization and clinically proven ABPA. Moreover, based on extended, serial lung function

TABLE 4. GROUP COMPARISONS OF PROGRESSION WITH AGE IN RELATION TO SENSITIZATION TO *Aspergillus fumigatus* AND DEVELOPMENT OF ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS, INCLUDING *Pseudomonas aeruginosa* COLONIZATION AND INFECTION

		Group Comparisons				Within-Group Comparison of Progressions [§]	
		Group Means [†]		Group Slopes [‡]			
		Lung Function Progression with Age*	F Value	p Value	F Value	p Value	t Value
FRC _{pleth}							
CF controls	0.062						
Intermittent <i>Pseudomonas aeruginosa</i>	0.123					1.037	NS
<i>Aspergillus fumigatus</i> sensitized	0.172	0.866	NS	2,725	0.028	2.667	0.008
<i>P. aeruginosa</i> infection	0.140					2.342	0.019
ABPA	0.185					3.066	0.002
LCI							
CF controls	0.157						
Intermittent <i>P. aeruginosa</i>	0.098					−0.477	NS
<i>A. fumigatus</i> sensitized	0.185	2.881	0.023	3.635	0.006	0.319	NS
<i>P. aeruginosa</i> infection	0.329					2.326	0.020
ABPA	0.187					0.324	NS
VTG							
CF controls	0.052						
Intermittent <i>P. aeruginosa</i>	0.185					1.985	0.047
<i>A. fumigatus</i> sensitized	0.218	1.209	NS	4.160	0.002	3.476	0.001
<i>P. aeruginosa</i> infection	0.195					3.544	0.0001
ABPA	0.236					3.712	0.0001
sReff							
CF controls	0.034						
Intermittent <i>P. aeruginosa</i>	0.512					2.423	0.016
<i>A. fumigatus</i> sensitized	0.332	0.784	NS	4.209	0.002	2.171	0.030
<i>P. aeruginosa</i> infection	0.453					3.728	0.0001
ABPA	0.518					3.623	0.0001
FEV ₁							
CF controls	0.067						
Intermittent <i>P. aeruginosa</i>	−0.207					−2.434	0.015
<i>A. fumigatus</i> sensitized	−0.172	2.429	0.048	6.080	0.0001	−2.993	0.003
<i>P. aeruginosa</i> infection	−0.252					−4.863	0.0001
ABPA	−0.182					−3.192	0.001
FEF ₅₀							
CF controls	0.027						
Intermittent <i>P. aeruginosa</i>	−0.197					−1.138	NS
<i>A. fumigatus</i> sensitized	−0.408	1.391	NS	10.074	0.0001	−3.113	0.002
<i>P. aeruginosa</i> infection	−0.634					−5.753	0.0001
ABPA	−0.619					−4.739	0.0001

Definition of abbreviations: ABPA = allergic bronchopulmonary aspergillosis; CF = cystic fibrosis; FEF₅₀ = FEF at 50% VC; FRC_{pleth} = plethysmographic FRC; LCI = lung clearance index; NS = not significant; sReff = effective specific airway resistance; V_{TG} = volume of trapped gas.

P. aeruginosa stratified into those intermittently colonized or chronically infected; *A. fumigatus* sensitized (sensitization is defined as onset of specific IgE against both recombinant allergens, rAspf1+3).

* Sensitization to *A. fumigatus* and development of allergic bronchopulmonary aspergillosis, including *P. aeruginosa* colonization and infection, respectively; slopes within groups.

† Group comparison; fixed-effect estimates of lung function as dependent variables.

‡ Group × age effect.

§ Within-group comparison of progressions in relation to CF controls; differences of progression between groups and CF controls, evaluated by linear mixed-effect analysis with estimates of fixed effects evaluated by *t* test.

measurements, we are able to show how lung function deteriorates over time in relation to *A. fumigatus* sensitization and ABPA in comparison with a CF-control group. It is noteworthy that, in comparison with the nonsensitized, noncolonized patients with CF, both patients with CF sensitized to *A. fumigatus* and those with ABPA had significantly increased IgE responses to the four recombinant *A. fumigatus* strains tested. Thus, early onset of increased levels of allergen-specific IgE antibodies may well be an indicator for onset of ABPA (15). In combination with lung function parameters, they indicate specific pathophysiologic changes induced by *A. fumigatus* in the lung of patients with CF.

Study Limitations

After diagnosis of ABPA, our patients with CF were treated with oral corticosteroids in decreasing doses, and some also with itraconazole and/or inhaled corticosteroids. A study looking at

a 12-mo outcome was recently published, showing that under corticosteroid treatment, IgE antibodies to rAspf4 and/or rAspf6, and total serum IgE and IgG levels decreased, but did not normalize, and were followed by an increase in antibody titers after therapy cessation (24). Despite intensive treatment with oral and/or inhaled corticosteroids with or without itraconazole, no significant changes in lung function (restricted to FVC and FEV₁) could be observed (24). Having evaluated the course of disease based on (1) extended lung function testing, (2) a substantial period of time (10 yr), and (3) comparison with a CF-control group, we believe that the progression rates (slopes and Δslopes) were only marginally influenced by treatment.

Immunologic Considerations

The recombinant allergens rAspf1, rAspf2, rAspf3, rAspf4, and rAspf6 have been evaluated for their diagnostic performance in

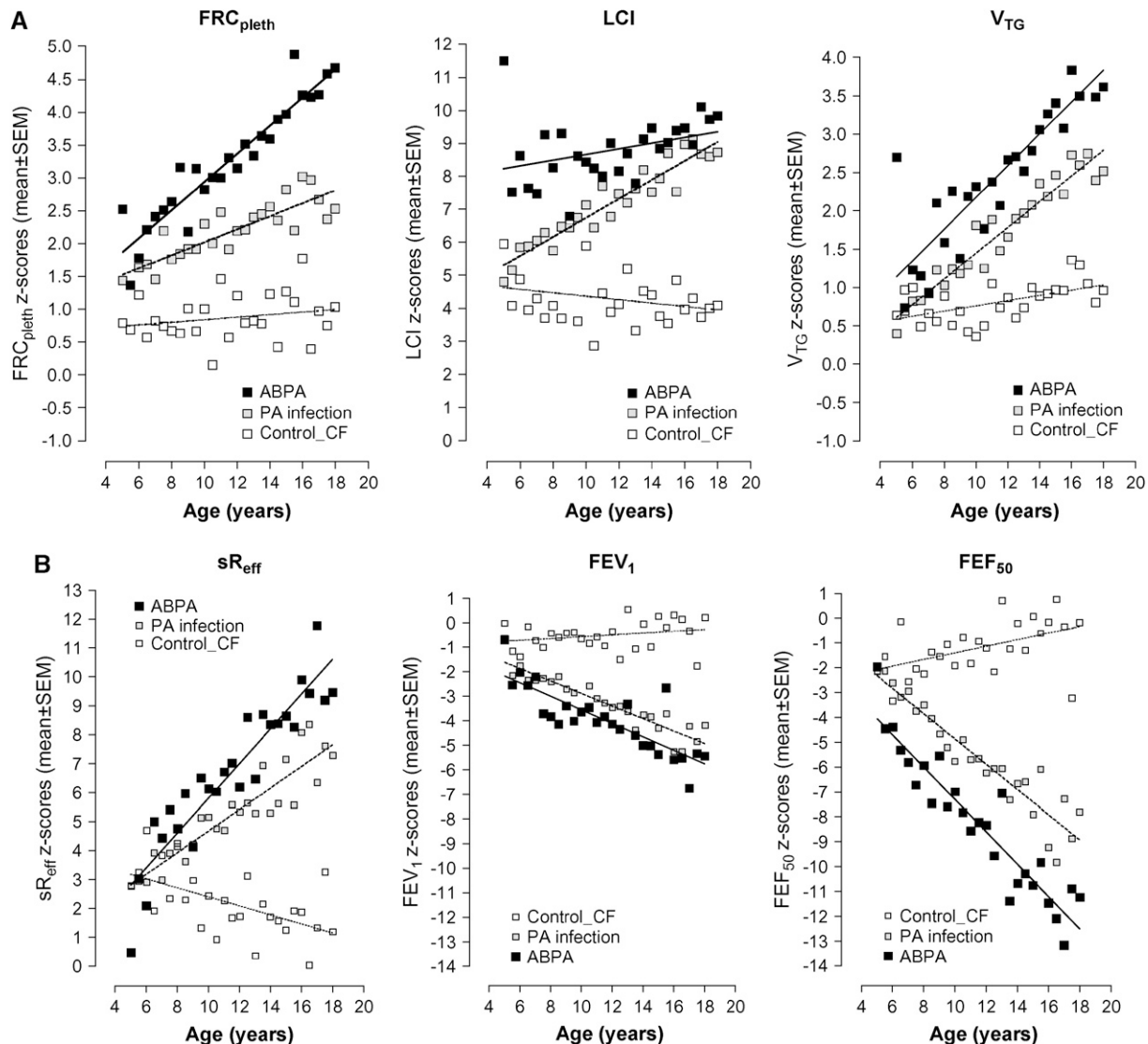


Figure 3. (A) Progression with age for specific lung function parameters, such as plethysmographic FRC (FRC_{pleth}), lung clearance index (LCI) obtained by multibreath nitrogen washout (20), and trapped gas (V_{TG}). Values are given as mean changes of lung function of all 122 patients with CF, aggregated for 6-mo intervals computed from the fixed predicted values obtained by the linear mixed-effect model (LMM) analysis and presented as regression lines of these mean values, the slope indicating the degree of progression. (B) Progression with age for specific lung function parameters, such as specific airway resistance (sR_{eff}) (31), FEV₁, and FEF at 50% VC (FEF₅₀). Values are given as mean changes of lung function of all 122 patients with CF, aggregated for 6-mo intervals, computed from the fixed predicted values obtained by the LMM analysis, and presented as regression lines of these mean values, the slope indicating the degree of progression.

several serologic studies in patients with asthma (23, 28, 37) and in patients with CF (22, 24, 27, 38), with or without ABPA, showing a high specificity for the detection of *A. fumigatus* sensitization as well as ABPA. These studies showed that rAspf1 and rAspf3 are recognized by antibodies of *A. fumigatus*-sensitized persons with asthma with 100% specificity, reaching a sensitivity of 88%, and positive and negative predictive values of 100 and 63% with respect to an overall diagnosis of *A. fumigatus* sensitization (23). Serologic investigations involving rAspf4 and rAspf6 showed that allergen-specific IgE raised against these proteins could be detected almost exclusively in sera of patients with ABPA (22, 23). A total of 80% of the subjects with ABPA elicited an IgE response to rAspf4, 55% to rAspf6, and 90% to at least one of these allergens (23, 28), in agreement with the present study. Serologic investigations of patients with CF yielded comparable results for the recombinant allergens tested (22, 24, 27, 38). In a recent study (39), a systematic investigation comparing two

distinct strategies, the criteria proposed by the Cystic Fibrosis Foundation (40) and the detection of IgE specific to recombinant allergens (rAspf1, rAspf2, rAspf3, rAspf4, and rAspf6), in a limited number of patients was employed. The authors conclude that specific IgE to recombinant antigens of *A. fumigatus* is a promising tool for early detection of sensitization to *A. fumigatus* and of ABPA itself, especially during an early phase, when clinical symptoms are lacking. These results are corroborated by those obtained in a further study (41), showing that determination of specific IgE to recombinant *A. fumigatus* allergens, either by ELISA or commercial ImmunoCAPs, allow a better discrimination between groups of patients with CF with or without ABPA than that obtained with crude *A. fumigatus* extracts. In terms of diagnostic specificity, the recombinant *A. fumigatus* allergens have proven superior to allergen extracts, suggesting the possibility of relying on serologic data to detect sensitization, thereby avoiding allergen challenges (37).

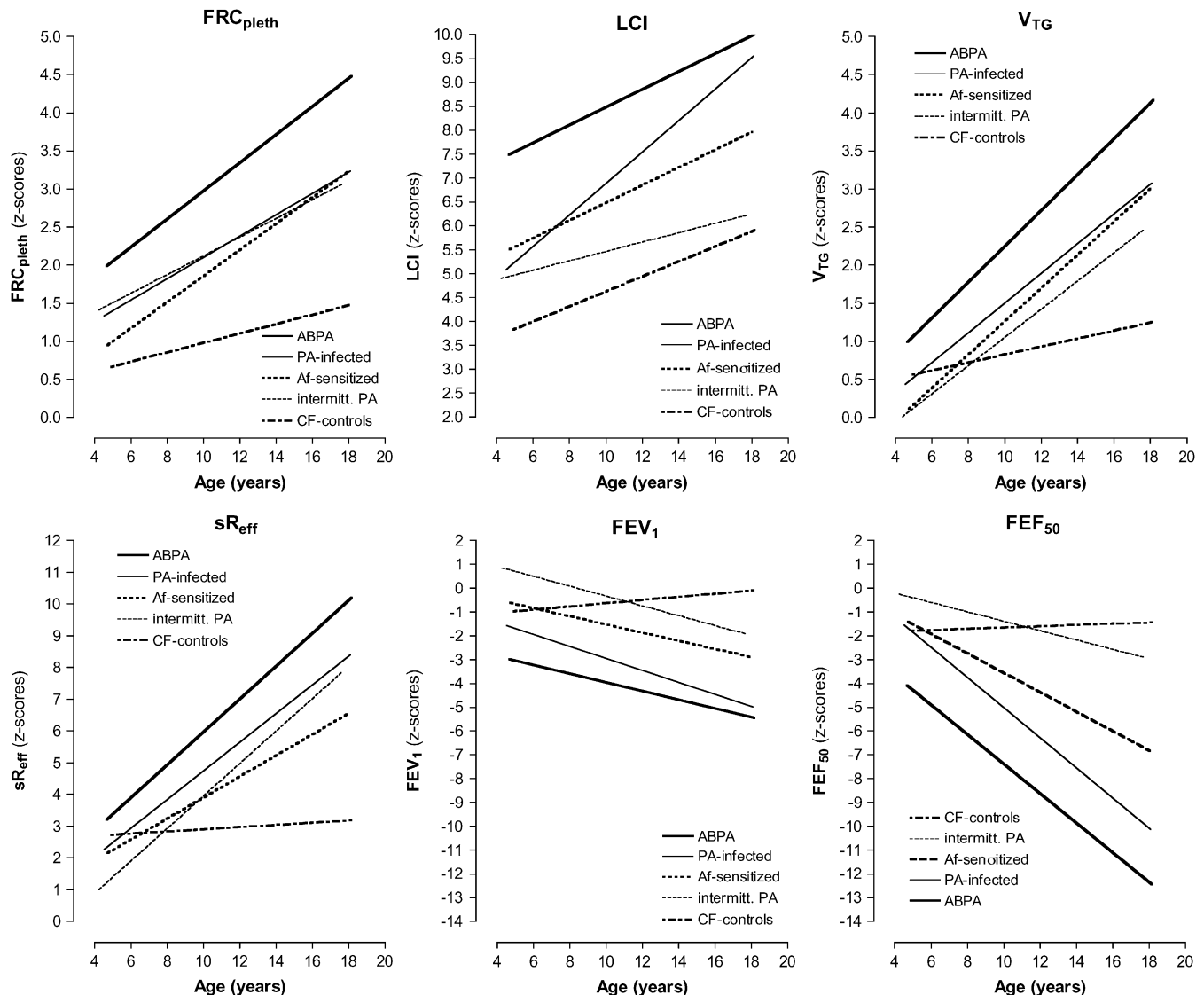


Figure 4. Distinction of lung progression within the five etiologic groups defined according the question of whether patients were *A. fumigatus* sensitized, have developed ABPA, or are colonized or chronically infected by *P. aeruginosa*, functionally assessed by the six lung function parameters. Values are given as means $\pm$ SEM of repeated annual measurements in terms of z scores, and presented as regression lines of these mean values, the slope indicating the degree of progression.

To overcome the difficulty of clinically characterizing ABPA, and considering the problems of inconsistency in commercial *A. fumigatus* extracts, production of highly pure recombinant *A. fumigatus* allergens has been considered as a major breakthrough in improving the serologic diagnosis of ABPA in patients with asthma (23, 28) or CF (27). rAspf1, rAspf3, rAspf4, and rAspf6 are available for routine diagnostic use. In a recent consensus report of the North American Cystic Fibrosis Foundation on defining diagnosis, diagnostic procedures, and therapy for ABPA in patients with CF, it is stated that recombinant *A. fumigatus* allergens should further contribute to improved accuracy in detecting sensitization to *A. fumigatus* and serologic ABPA (15).

Effect of ABPA on Lung Function

The relationship between *A. fumigatus* sensitization and progression of pulmonary function is not well established. Hartl and colleagues (42) studied 66 patients within various etiologic groups of children with asthma and patients with CF, and found an association between thymus and activation-regulated chemokine

and FEV₁ (expressed as % predicted), but not between macrophage-derived chemokine and lung function. Wojnarowski and colleagues (17) studied the relationship between *A. fumigatus* sensitization and pulmonary function tests in 118 children with CF, 31 (26%) being sensitized to *A. fumigatus*. With adjustment for sex, age, height, and weight, sensitization was associated with lower values in the flow-volume curve. There was evidence for a more rapid decline in pulmonary function test results for *A. fumigatus*-sensitized patients with elevated total IgE levels than for those with normal IgE levels. In a study by Nicolai and colleagues (16), independent negative correlations between *A. fumigatus*-RAST and indices of the flow-volume curve were found. Thus, ABPA appears to be associated with an increased decline in lung function.

The finding of the present study shows the following: (1) patients with ABPA demonstrated the most severe progression in all lung function parameters ($p < 0.0001$) in relation to colonization or chronic infection with *P. aeruginosa*; (2) sensitization to *A. fumigatus* was best reflected by FEF₅₀ (Table 4; Figure 4); (3)

for the assessment of the influence of sensitization to *A. fumigatus* and development of ABPA, and especially for the distinction of different etiologic groups, it is mandatory to take into account colonization and chronic infection with *P. aeruginosa*; and (4) distinction between sensitization to *A. fumigatus* and development of ABPA, including the effect of *P. aeruginosa* colonization or infection, was best shown by FEF₅₀, VTG, and sReff.

From the point of view of lung physiology, it has been shown that, in patients with CF, five mechanisms of functional deterioration have to be distinguished: (1) progression of pulmonary hyperinflation, represented by FRC_{pleth}; (2) progression of ventilation inhomogeneities given by the LCI; (3) development of trapped gas estimated by VTG; (4) airway narrowing given by sReff; and (5) small airway disease represented by FEV₁ and FEF₅₀. The statistical evaluation revealed that the process of *A. fumigatus* sensitization, *P. aeruginosa* colonization or infection, and the development of ABPA differently influence these five functional characteristics.

Therefore, progression in lung function decline was evaluated within five etiologic groups, specific for sensitization and/or infection, compared with the CF-control group. The most striking progression among all the lung function parameters was found for patients with ABPA, differing significantly from those with chronic *P. aeruginosa* infection, and from those sensitized against *A. fumigatus* (Figure 4). The most significant progression for *A. fumigatus* sensitization and for patients with ABPA was demonstrated by the FEF₅₀ value, followed by that for sReff, VTG, and LCI (Table 4 and Figure 4). The best differentiation between *A. fumigatus* sensitization and ABPA (taking into account intermittent colonization or chronic infection by *P. aeruginosa*) was reflected by LCI. Therefore, these four lung function parameters may be considered as major predictors of functional deterioration in the process of *A. fumigatus* sensitization and ABPA development. Based on that observation, it can be argued that these allergic responses predominantly affect intrapulmonary gas distribution (LCI), most impeded by bronchial obstruction and airway narrowing (sReff), and developing small airway disease (FEF₅₀). Pulmonary hyperinflation and the development of trapped gas are physiopathologic consequences of these disease processes. For all lung function parameters, a highly significant interaction between these two influencing phenomena could be found. Thus, airway narrowing, gas trapping, and small airway disease seem to be the major targets of functional derangement in ABPA, and, therefore, specific lung function parameters have to be taken as follow-up parameters, if the effect of sensitization to *A. fumigatus* and development of ABPA is the target of evaluation.

Association with Onset of Chronic *P. aeruginosa* Infection

Nikolaizik and colleagues (43) reported an association between *P. aeruginosa* colonization and the development of ABPA, in contrast to a previous report (36) showing that ABPA occurs independently of *P. aeruginosa* infection. The present study, however, demonstrates that interaction must be considered for each lung function parameter, except sReff. Moreover, because the onset of chronic *P. aeruginosa* infection is significantly earlier than the onset of *A. fumigatus* sensitization or ABPA development, it can be hypothesized that *P. aeruginosa* colonization favors the process of *A. fumigatus* sensitization.

Associations with *CFTR* Genotype

Previous work suggests that *CFTR* gene mutations may be implicated in the etiology of ABPA (9). Familial occurrence of ABPA has been reported, suggesting a possible genetic contribution to the disease in CF (9, 44), as indicated by a higher than expected frequency of CF mutations in ABPA (8). It has been suggested that HLA-DR molecules DR2, DR5, and possibly DR4 or DR7

contribute to susceptibility, whereas HLA-DQ2 contributes to resistance, and a combination of these may determine the outcome of ABPA in CF and asthma (7, 10). However, looking at the distribution of *CFTR* mutations within collectives of patients with CF, Mastella and colleagues could not demonstrate a significant association of ABPA with particular *CFTR* genotypes (4).

In our study, an attempt to correlate the prevalence of *A. fumigatus* sensitization and the development of an ABPA phenotype with the four most common *CFTR* genotypes in Switzerland ($\Delta F508/\Delta F508$, $\Delta F508/3905\text{insT}$ [Swiss type], $\Delta F508/R553X$, and miscellaneous $\Delta F508/N1303K$) failed to demonstrate a relevant association.

Conclusions

Our study shows that, in comparison with a control group of patients with CF not sensitized to *A. fumigatus*, progression of lung function deterioration is most strikingly pronounced in patients with ABPA. Therefore, early diagnosis and treatment of ABPA is of importance to prevent serious and potentially irreversible lung damage. Especially patients colonized or chronically infected with *P. aeruginosa* should be regularly tested for *A. fumigatus* sensitization. Lung function decline is best evaluated by FEF₅₀, VTG, and sReff, demonstrating that airway narrowing, gas trapping, and small airway disease are the major targets of functional assessment. Therefore, adequate longitudinal studies aimed at establishing the effect of *A. fumigatus* sensitization and later development of an ABPA on the decline in pulmonary function and, more generally, its influence on the final prognosis of CF, must be based on the follow-up of extended lung function testing.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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