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Risk factors for allergic bronchopulmonary aspergillosis and sensitisation to *Aspergillus fumigatus* in patients with cystic fibrosis

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Abstract An increasing incidence of allergic bronchopulmonary aspergillosis (ABPA) as a complication in patients with cystic fibrosis (CF) is reported. The objective of this retrospective case-control study was to assess potential risk factors for ABPA and for *Aspergillus fumigatus* sensitisation (AFS). In a group of 160 CF patients, 11 (7%) fulfilled the diagnostic criteria for ABPA and 20 (13%) had evidence of AFS. They were compared to 62 control CF patients (25 for ABPA and 37 for AFS group) without evidence of ABPA or AFS using extended matching for sex, age and weight. AFS patients had received significantly higher cumulative doses of inhaled corticosteroids than their respective controls (OR 8.0; 95% CI 1.74–63). Bronchial colonisation with *Stenotrophomonas maltophilia* was strongly and independently associated with ABPA (OR 20; 95% CI 2.8– infinity). A longer duration of *Pseudomonas aeruginosa* colonisation was independently associated with AFS (OR per year 1.50; 95% CI 1.12– infinity). *Conclusion:* Cystic fibrosis patients with allergic bronchopulmonary aspergillosis have a more frequent isolation of *S. maltophilia* in their sputum than their controls. Longer duration of colonisation with *P. aeruginosa* is a risk factor for *Aspergillus fumigatus* sensitisation. Higher cumulative doses of inhaled corticosteroids are associated with *Aspergillus fumigatus* sensitisation and their role as a risk factor needs to be clarified.

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Abbreviations *ABPA*: allergic bronchopulmonary aspergillosis · *AFS*: *Aspergillus fumigatus* sensitisation · *BMI*: body mass index · *CF*: cystic fibrosis · *CFTR*: cystic fibrosis transmembrane regulator · *IQR*: interquartile range · *rAsp f*: recombinant *Aspergillus fumigatus* antigen

Introduction

Aspergillus fumigatus is a ubiquitous fungus growing in humid environments and on decaying organic waste. Inhalation of spores may result in invasive aspergillosis, pulmonary aspergilloma, allergic bronchopulmonary aspergillosis (ABPA), sensitisation to *A. fumigatus* (AFS) or asymptomatic colonisation of the bronchial tree. Predisposing factors such as immunodeficiency (e.g. prolonged severe neutropenia, congenital immunodeficiency syndromes), lung cavities, impaired mucociliary clearance, asthma and cystic fibrosis (CF) influence the clinical course. CF is an autosomal recessive inherited disease with a defect in the cystic fibrosis transmembrane regulator (CFTR) gene. The CFTR has a central role in regulation of salt and water translocation and mutations in the *CFTR* gene lead to an altered mucus composition in affected lungs causing impaired mucociliary clearance. In patients with CF, an increasing prevalence of ABPA has been reported and can vary between 0.9% and 13% [1, 8, 9, 13, 14, 16, 18, 21, 27, 30, 31,35]. In two recent analyses of epidemiological registries, the reported prevalence of ABPA was 2.0% in North America among 14210 eligible patients older than 5 years of age [10] and 7.8% in nine European countries among 12447 patients [19].

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Clinical presentation, findings on chest X-ray films or CT scans and laboratory data are combined for the diagnosis of ABPA and AFS. There have been many proposals for a set of diagnostic criteria for ABPA with marked nonconformity between different authors [1, 10, 14, 16, 18, 19, 21,26]. Therefore a consensus conference held in 2001, proposed the following diagnostic criteria for ABPA [33]: (1) clinical deterioration (cough, wheeze, exercise intolerance, exercise-induced asthma, decline in pulmonary function, increased sputum), (2) serum total IgE concentration of >1000 IU/ml, (3) immediate cutaneous reactivity to *A. fumigatus* in prick skin test or in vitro presence of serum IgE antibody to *A. fumigatus*, (4) precipitating antibodies to *A. fumigatus* or serum IgG antibody to *A. fumigatus* by an in vitro test, and (5) new or recent abnormalities on chest radiography or chest CT that have not cleared with antibiotics and standard physiotherapy.

In patients with CF, the clinical, imaging and laboratory criteria are constantly influenced by the disease itself and the underlying lung pathology, therefore the criteria are difficult to apply. Fluctuations in clinical presentation due to viral or bacterial pulmonary infections further limit the accurate diagnosis [8,30]. Recently, the IgE response towards recombinant *A. fumigatus* allergens (rAsp f) has been proposed to improve diagnosis of allergic diseases related to *A. fumigatus* in CF patients. Serological investigations involving a panel of recombinant allergens (Asp f1, Asp f3, Asp f4, Asp f6) show a high sensitivity and specificity in the detection of ABPA and AFS [5,12]. The distinction between ABPA and AFS is crucial to determine therapy. In the case of ABPA, therapy consists of oral corticosteroids and a systemic antifungal agent (i.e. itraconazole), and some groups recommend inhaled corticosteroids [19, 21,22]. Specific recommendations for treating patients with AFS do not exist.

The presence of atopy, the use of inhaled antibiotics, decreased forced expiratory volume (FEV 1) and *Pseudomonas aeruginosa* in sputum have been suggested as risk factors for the development of ABPA in patients with CF [3, 10, 22].

The impact on CF is not yet clear, but ABPA is suspected to cause a more rapid decline in pulmonary function and a more rapid progression of CF lung disease [8, 24,34]. Early recognition or even prevention is important and knowledge of risk factors will change the management of CF patients. To evaluate putative risk factors for CF patients to acquire either ABPA or AFS, we performed a case-control study on the basis of our CF registry.

Subjects and methods

Subjects

The CF registry of the Department of Paediatrics at the University of Berne contains data of CF patients followed at our clinic and was started in 1994. After written informed consent of the patients or their next of kin is obtained, patient's data are entered continuously into the registry. All patients entering the database have a diagnosis of CF confirmed by a positive sweat test with increased sweat chloride concentration (>60 mmol/l) and DNA analysis of a CF specific genotype. The patients are seen regularly depending on their clinical course but at least once a year. All clinical, anthropometric, radiological and laboratory data are available from the time of initial diagnosis. The collection of these data was approved by the ethics committee of the Medical Faculty of the University of Berne.

Selection of cases

In December 2000, the CF database was searched to identify patients with ABPA or AFS fulfilling all of the following criteria.

Patients with ABPA: (1) clinical signs, i.e. more coughing, wheezing, increasing expectorations; (2) increased (≥ 2SD of normal) total serum IgE concentration based on normal age corrected values (ImmunoCAP-FEIA-System); age 3–4 years >20 IU/ml; 4–7 years >160 IU/ml; 10–14 years >570 IU/ml; older than 14 years >195 IU/ml); (3) positive specific IgE in serum against *A. fumigatus* with RAST class ≥2 (Pharmacia; corresponding to >3.5 kU/l); (4) increased specific IgG in serum against *A. fumigatus* >20 kU/l (reference values in [12]); (5) increased specific IgE in serum against rAsp f1 >9.6 EU/ml and rAsp f4 >8.4 EU/ml and rAsp f6 >7.2 EU/ml (reference values in [12]) and (6) at least one positive culture result from sputum or pharyngeal swab for *A. fumigatus*.

Patients with AFS: (1) positive specific IgE in serum against *A. fumigatus* (RAST class ≥2) and/or increased specific IgE in serum against rAsp f1 >9.6 EU/ml, with normal values for rAsp f4 (≤ 8.4 EU/ml) and rAsp f6 (≤ 7.2 EU/ml) and (2) the remaining above mentioned criteria for ABPA not fulfilled.

Selection of controls

For each patient of the ABPA and AFS groups, the CF registry was searched for at least one matched control patient. More than one control was taken per case when available (extended matching) in order to enhance the power of comparison [2, 20,32]. The following matching criteria were used: (1) same sex as the case patient, (2) age ±18 months to the case patient, (3) weight ±5 kg and height ±20 cm compared to the case patient, (4) negative sputum and/or pharyngeal swab culture for *A. fumigatus*, (5) total serum IgE as well as IgE RAST against *A. fumigatus* in the normal (age corrected) range and (6) normal serum values for specific IgG against

A. fumigatus and specific IgE against rAsp f1, rAsp f4 and rAsp f6.

Variables

Data of patients who were seen at least twice a year during outpatient follow-up were analysed. The following variables, which were obtained from the database and before 1994 from medical records, were included in this case-control study: (1) CF genotype; (2) dose, duration and substance of prescribed inhaled corticosteroids; (3) chronic colonisation with *P. aeruginosa*, i.e. positive microbiological assays of the sputum or throat swab at least twice a year; (4) presence of *S. maltophilia* in sputum or throat swab.

Body mass index (BMI) was calculated as weight/height^2 (kg/m^2) and standard deviation score (z-score) was calculated relative to a Swiss reference population [29].

Statistical analysis

Because of the small sample sizes, exact methods were used throughout. Risk factors for ABPA or AFS were analysed with univariate and multivariate stratified logistic regression (exact conditional type, with the matched-set identifier as stratification variable). This kind of analysis is adapted to the case-control data with extended matching. Exact 95% CI were calculated for OR and proportions [4]. LogXact-4.1 and StatXact-5.0.3 software was used (Cytel Software, Cambridge, USA).

Results

Of 160 patients in the CF registry, 11 (7%; 95% CI 4%–12%), fulfilled the criteria for ABPA and 20 (12%; 95% CI 8%–19%) for AFS. A total of 25 CF controls (1 to 5 per case) were matched to the ABPA group and 37 (1 to 5 per case) to the AFS group. Table 1 gives information on gender, age, weight and height of cases and controls.

The proportions of *CFTR* mutations in the different groups were comparable (Table 2).

The associations of specific variables with the presence of ABPA and AFS given in Table 3 and Table 4 respectively. Before a diagnosis of AFS was made, the median cumulative dose of inhaled steroids in AFS cases was significantly higher than in their controls (0.57 g; interquartile range (IQR) 0.27–0.86 g vs. 0.00 g; IQR 0.00–0.43 g; $P = 0.004$). Multivariate analysis showed that this association was independent of other factors. The median cumulative dose of inhaled steroids in ABPA cases, however, was not significantly higher compared to their controls (0.81 g; IQR 0.46–1.34 g vs. 0.52 g; IQR, 0.00–1.02 g; $P = 0.055$). The median duration of *P. aeruginosa* colonisation was comparable in ABPA patients and their controls (5 years; IQR 2–10 years vs. 6 years; IQR 2–9 years; $P = 1.00$), but significantly higher in AFS cases than in their controls (7 years; IQR 5–8 years vs. 2 years; IQR 1–4 years; $P < 0.001$), corresponding to a per-year OR of 2.0 (95% CI 1.3–3.9). Multivariate analysis showed that this association was again independent of other factors.

S. maltophilia was isolated in sputum or throat swab at least once in a significantly higher proportion of ABPA patients than in their controls (9 of 11 = 82%; 95% CI 50%–97% vs. 1 of 25 = 4%; 95% CI 0.2%–19%; $P < 0.001$) and its presence was independently associated with ABPA. AFS patients did not differ significantly from their controls in that variable (2 of 20 = 10%; 95% CI 2%–32% vs. 3 of 37 = 8%; 95% CI 2%–21%; $P = 1.00$).

Table 1 Sex, age, weight, height, BMI and z-scores in the different groups

	ABPA ($n = 11$)	ABPA controls ($n = 25$)	AFS ($n = 20$)	AFS controls ($n = 37$)
Female	6	12	8	13
Male	5	13	12	24
Median age (years)	12	14	12	9
Median weight (kg)	27	36	28	28
Median height (m)	1.35	1.49	1.34	1.34
Median BMI	15.8	13.9	15.6	16.6
Median BMI z-score	-0.90*	-1.28	-0.53	-0.15

* $P = 0.007$ significantly different from ABPA controls; all other differences were not significant

Discussion

This is the first case-control study of CF patients which analyses risk factors for ABPA and AFS. The preva-

Table 2 Distribution of *CFTR* gene mutations in cases with ABPA, AFS and their control groups. (Pearsons $\chi^2 = 9.3$, exact $P = 0.41$)

CFTR mutation	ABPA ($n = 2\times11$)	ABPA controls ($n = 2\times25$)	AFS ($n = 2\times20$)	AFS controls ($n = 2\times37$)
ΔF508	18 (82%)	41 (82%)	31 (78%)	49 (66%)
3905insT	0 (0%)	1 (2%)	4 (10%)	7 (9%)
R553X	1 (5%)	2 (4%)	1 (3%)	7 (9%)
Others	3 (14%)	6 (12%)	4 (10%)	11 (15%)
	1717–1G + 17 1199DelG	R347P	2176insC	G126D
	R560S	2347delG	G542X	G542X
		Q523X	Q525X	E585X
		3732delA	N1303K	4005 + 1G- > A
				M1101K
				K710X
				Q525X
				3732delA
				N1303K
				2789 + 5G- > A

Table 3 Cases with ABPA versus controls

Univariate exact logistic regression	OR (exact 95% CI)	*P*
Inhaled steroids (cumulative dose in g)	4.8 (0.97–38)	Not significant
Years of *P. aeruginosa* colonisation	1.0 (0.88–1.13)	Not significant
Ever colonised with *S. maltophilia*	20 (2.8– infinity)	< 0.001
BMI z-score	0.13 (0.02–0.7)	0.007
Multivariate exact logistic regression	**OR (exact 95% CI)**	***P***
Ever colonised with *S. maltophilia*	20.1 (2.8– infinity)	< 0.001
All the other variables		Not significant

lence of ABPA in our centre is 7%, compared to 0.9% for Denmark [30], 2.0% for North America [10], 10% for Canada [9] and 13.6% for Belgium [19]. The reported variation of ABPA prevalence in CF patients is rather a consequence based on difficulties in diagnosis using different criteria than due to epidemiological differences. The diagnosis of ABPA in our study was based on the criteria of the Cystic Fibrosis Foundation consensus conference [33] with the following specifying modifications: increased IgE was defined for age corrected normal values, imaging was not taken into account and *A. fumigatus* was cultured at least once in the sputum. Additionally, specific IgE against rAsp f were used. The development of antibodies to recombinant allergens has recently been reported [5, 12,15] and shows an improvement in diagnosis as well as the capacity to distinguish between ABPA patients (even during remission) and AFS patients.

Table 4 Cases with AFS versus controls

Univariate exact logistic regression	OR (exact 95% CI)	*P*
Inhaled steroids (cumulative dose in g)	8.0 (1.7–63)	0.004
Years of *P. aeruginosa* colonisation	2.0 (1.3–3.9)	< 0.001
Ever colonised with *S. maltophilia*	1.2 (0.1–17)	Not significant
BMI z-score	0.58 (0.2–1.3)	Not significant
Multivariate exact logistic regression	**OR (exact 95% CI)**	***P***
Inhaled steroids (cumulative dose in g)	26.6 (2.1–3588)	0.007
Years of *P. aeruginosa* colonisation	1.50 (1.12– infinity)	0.001
All the other variables		Not significant

The prevalence of AFS in our centre is 13%. Usually skin prick tests and/or specific IgE (RAST) against *A. fumigatus* are used for this diagnosis. Using either positive skin test or elevated specific IgE titre (RAST > 2), Wojnarowski et al. [34] found 26% AFS patients in their clinic in Vienna and Nikolaizik et al. [25,26] described 105 CF patients in the UK of whom 30% had positive skin tests to *A. fumigatus* and 23% had positive IgE antibody to *A. fumigatus*. The reliability of skin tests is limited as they depend on the composition of antigen preparation [12,26] and therefore skin tests are not routinely done, as was the case in our patients. Instead of skin testing we used a combination of specific IgE (RAST) against *A. fumigatus* and specific IgE against recombinant allergens (rAsp f) to define AFS [12]. Due to these more specific criteria, our rate of AFS patients is lower than in other cohorts.

AFS was significantly and independently (from other factors) associated with higher doses of inhaled corticosteroids. Postulating inhaled corticosteroids as a risk factor, a possible pathway for sensitisation might be a depressed immune function by steroids (especially macrophages ingesting and killing spores), leading to enhanced germination of *A. fumigatus* and increased release of allergens. Moreover, in vitro pharmacological doses of hydrocortisone increase the growth rate of *A. fumigatus* via a possible presence of a ligand/receptor system [28]. An association of inhaled corticosteroids with AFS has not been previously found and the increasing use of inhaled corticosteroids for inflammation control in CF patients is therefore of major concern [23].

Whether sensitisation is a preliminary state for patients who develop ABPA later has never been shown and few data are available showing whether ABPA patients are able to return to AFS (Fig. 1). Hutcheson and coworkers [13], in their longitudinal study of ABPA and AFS patients, showed that reactivity of the skin prick test, specific IgE and IgG against *A. fumigatus* as well as increased total IgE were sometimes lost spontaneously. Because we concentrated in our study on the time before diagnosis, we are not able to answer conclusively the question as to what happens with ABPA patients after treatment. We followed up our ABPA patients (data not shown) with determinations of antibodies to recombinant allergens and we occasionally saw that they can return to an AFS state.

The duration of colonisation with *P. aeruginosa* in ABPA patients was similar to their controls. These findings correspond to those of Simmonds et al. [31] who reported that only 3 of their 11 ABPA patients had chronic *P. aeruginosa* colonisation; on the other hand, this was not seen in a survey of 16 ABPA patients in the United States [22].

In AFS patients, however, a significantly longer colonisation of *P. aeruginosa* was seen. An association between *P. aeruginosa* and positive skin test as well as positive specific IgE against *A. fumigatus* in serum has been shown previously [25]. In vitro it seems that *P. aeruginosa* can alter growth and phenotype of

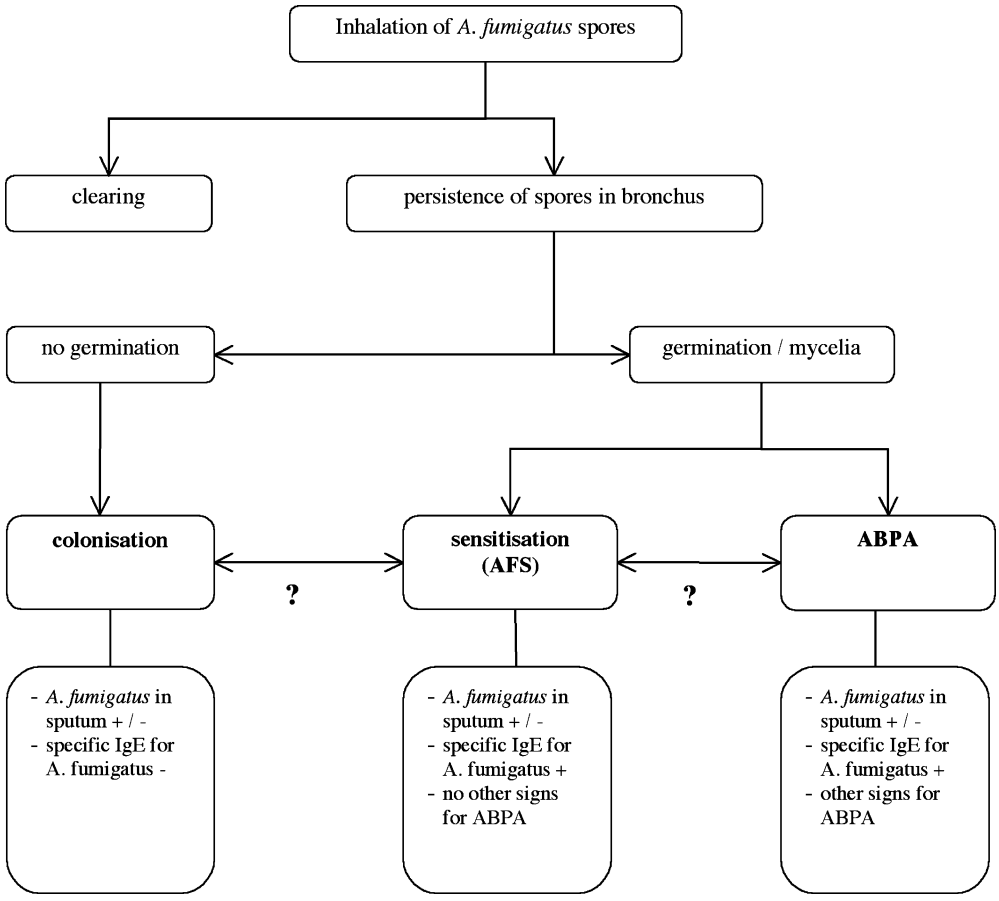

Fig. 1 Hypothesis for the pathogenesis of colonisation, AFS and of ABPA

A. fumigatus [22], increase the release of protein structures of the fungus and hence cause sensitisation [34]. Of course we are unable to distinguish between association and cause, but it seems plausible that a longer *P. aeruginosa* presence in the bronchial tree is a risk factor for AFS.

Of 11 ABPA patients, 9 harboured *S. maltophilia* at least once, whereas in all the other groups this was a rare observation. The association between the isolation of *A. fumigatus* and subsequent *S. maltophilia* infection has been shown recently [17]. The study design of this group made no distinction between AFS, ABPA and non-AFS/nonABPA groups. Our data suggest that the association is true for the ABPA but not for the sensitised group. The reason for the susceptibility in CF patients might be a more advanced lung disease, but both lung function before and after acquisition of *S. maltophila* have been studied and no significant deterioration was reported [6,17]. In our study we refrained from the analysis of lung function, as there were too many missing values due to children under 6 years of age and the number of lung function tests that were done varied much depending on patients clinical state.

The significance of *S. maltophilia* in ABPA patients remains unclear, but it is possibly a marker for a different response to microorganisms, without or before changes in lung function occur. In our study, the presence of *S. maltophilia* in ABPA patients was independent of the use of inhaled corticosteroids. Two previous studies looked at the influence of systemic and/or inhaled corticosteroids on *S. maltophilia* isolation and both failed to show an association [11,17].

The question whether *CFTR* gene mutations play a role in the aetiology of ABPA has been raised by the finding that atopic asthmatics showed a higher than expected prevalence of carriers with *CFTR* mutations [7]. We did not find an association of type of *CFTR* gene mutation with ABPA or AFS.

By choosing a matched case-control design, we were not able to evaluate if sex or age are risk factors. The fact that mean age was different in cases compared to controls was due to extended matching, where more than one control was selected per case. The mode of analysis, i.e. stratified logistic regression, avoided an influence of this difference on the results. In the univariate analysis of BMI z-scores we realised that the groups differed more from each other than expected after being matched. Multivariate analysis corrected this problem, demonstrating that a lower BMI was not independently associated with ABPA.

The use of extended instead of the usual one-to-one matching was chosen to increase statistical power. Furthermore, exact nonparametric statistical methods were applied because of small sample sizes and non-normal distributed variables. The application of the exact type of analysis will not yield incorrect results even in small samples, thus allowing multivariate analyses even with

few patients [2, 20,32]. Both the study type and the corresponding type of analysis are scientifically well-founded but not widely known methods to find associations between a disease entity with only a few patients and a number of risk factors. Of course, the problem of generalisation of results observed in small patient samples remains and cautious interpretation is implicated. Large prospective studies are needed to further understand and evaluate the differences between ABPA, AFS and CF patients with no allergic reaction to *A. fumigatus*.

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