Prevalence of allergic bronchopulmonary aspergillosis in cystic fibrosis in an area with a high frequency of atopy

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Summary  Background: Lower airway colonisation with \textit{Aspergillus fumigatus} and the complicating hypersensitivity reaction allergic bronchopulmonary aspergillosis (ABPA) is well recognised in patients with cystic fibrosis (CF). There is a wide range in reported prevalence of ABPA in CF. Differences in predisposing factors such as atopy and climatic humidity, but also differences in reporting may in part explain this observation. In the Australian population there is a high frequency of atopy and the climate is relatively humid.

Patients and methods: Children and adolescents with CF ($n = 277$) from the CF Clinic, Children's Hospital at Westmead, Sydney, Australia were included in a retrospectively conducted study of \textit{Aspergillus} colonisation and ABPA (1998–2003).

Results: The prevalence of \textit{Aspergillus} colonised patients increased significantly from 7.4% in 1998 to 18.8% in 2002. No seasonal variation in initial positive \textit{Aspergillus} culture or in humidity was observed. A total of 13 patients (4.7%) were diagnosed with ABPA over the study period, with a significant increase in prevalence from 0.3% in 1998 to 4.0% in 2002. In addition, the criteria used for reporting ABPA in the study population were in agreement with the recently published diagnostic criteria for ABPA in CF.

Conclusions: In spite of a high frequency of atopy and a relatively humid climate in the Sydney area, \textit{Aspergillus} colonisation and ABPA in CF patients was not disproportionate. Moreover, criteria for reporting of ABPA in this setting was not different from that in the Northern Hemisphere.

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ABPA; Atopy; CF; Diagnosis; Humidity; Prevalence
Introduction

Colonisation with Aspergillus fumigatus (A. fumigatus) in the lower airways is prevalent in <5% and up to 57% of patients with cystic fibrosis (CF). Factors predisposing to ABPA have been considered to be climate humidity, resulting in higher Aspergillus spore counts, and atopy. Differences in these factors between populations may partly explain the wide range in prevalence. Since diagnostic criteria for ABPA have primarily been based upon studies performed in patients with asthma and since symptoms in ABPA overlap with other common features in CF, the diagnosis of ABPA is difficult to establish. Thus, the diagnosis and reporting of ABPA may often rely upon a variety of diagnostic criteria. A set of diagnostic criteria for ABPA in patients with CF, as well as a screening program, has recently been proposed by a CF Foundation sponsored Consensus Conference panel.

In an attempt to investigate some of the possible factors responsible for differences in the reported prevalence of ABPA, we performed a 5-year retrospective study of patients with CF at The Children’s Hospital at Westmead, Sydney, Australia to assess (a) Aspergillus colonisation and presence of ABPA in an area with a high frequency of atopy, (b) the influence of humidity on Aspergillus colonisation and (c) the agreement between clinically used ABPA criteria in a southern hemisphere centre and the recently proposed CF Foundation Consensus criteria for ABPA in CF. Data obtained in this setting were compared to data previously reported for the USA and Europe.

Patients and methods

Residents of the state New South Wales (NSW) represents 33.6% of the patients in the Australasian CF Data Registry (Australia and New Zealand). Approximately 58% of children and adolescents with CF in NSW attend The Children’s Hospital at Westmead in Western Sydney. Thirty-seven of the children attend a twice-yearly CF clinic conducted by the hospital in Canberra, 300 km from Sydney, but they were excluded from this study due to differences in climatic conditions (drier year round as well as hotter in summer and colder in winter). The remaining 277 patients attending the hospital for their CF care in 2002 were the subjects of this study. According to the data submitted to the Australasian CF Registry by the hospital in 2000, patients with CF have 3–5 visits per year in the centre, and the number of sputum samples per patient sent for microbiological culture per year is 1.14 in the age group 0–4 years and 1.9 cultures in the age group ≥5 years. Serological screening for ABPA is performed once-a-year, primarily with measurement of total IgE and eosinophil count. Patients who are suspected of having ABPA on clinical grounds have cutaneous skin prick to Aspergillus, precipitating antibodies to Aspergillus and occasionally specific IgE antibodies to Aspergillus measured. Within the study period from January 1998 to April 2003 (5.25 years) a total of 109 patients (56 females/53 males, mean age 12.2 years) had positive Aspergillus cultures. Data pertained to these patients were retrospectively reviewed and, where relevant, compared with that from patients with no Aspergillus found on culture sputum samples (n = 168).

The data for this study were obtained from a number of sources including the electronic patient medical and pathology record (Powerchart, Cerner Corp; version 2002.03), the patient pulmonary function record (Vmax Spectra, SensorMedics Corp; version 10-1A) as well as the patient’s record in the Australasian CF Registry. The following data were recorded:

(a) Total IgE as determined using the UniCap system with results expressed as kIU/L with a normal range less than 200 kIU/L.
(b) Specific IgE antibodies (RAST) to Aspergillus as measured by the CAP system according to the manufacturers instructions (Pharmacia, Uppsala, Sweden). Results were expressed as negative or positive, and positive results were further subdivided into high and very high levels.
(c) Eosinophil count as measured by automated electronic detection system. Results were expressed as 10³/L. Eosinophilia was defined as eosinophil counts >0.3 x 10³/L.
(d) Precipitating antibodies to Aspergillus as measured by radial immunodiffusion. Results were expressed as positive or negative relative to two somatic extracts, 1#: 20 mg/mL and 2#: 2 mg/mL and two filtrate antigens, 3#: 20 mg/mL and 4#: 2 mg/mL.
(e) Chest X-ray and chest CT-scan results.
(f) Lung function measured using spirometry and expressed as a percentage of that predicted for gender and height. Data from 1999 to 2003 were included.
Immediate cutaneous reactivity—prick tests (skin prick tests—SPT) using commercial preparations (Bayer, Leverkusen, Germany) including controls (histamine dihydrochloride and glycerol), *Dermatophagoides pteronyssinus* (*D. pteronyssinus*), grass mix, rye grass, *A. fumigatus*, *Aspergillus* mix and *Alternaria* species (*Alternaria* sp.). A positive reaction was regarded as a 3 mm wheal to one or more of the allergens. Some of the SPT had been performed prior to the study period.

Microbiological culture results—lower respiratory tract secretions are obtained by expectoration or endolaryngeal suction, and plated on blood agar.

Data on humidity in NSW were obtained from ADAM, the Australian Commonwealth Bureau of Meteorology’s climate archive. Data on humidity in Denmark were obtained from the Danish Meteorological Institute, Copenhagen (2002).

Ethical approval was obtained from the Children’s Hospital at Westmead Human Ethics Committee.

The Kruskall–Wallis test (KW) for non-parametric unpaired data was used to compare more than two groups. Differences were considered significant when the *P* values were less than 0.05. The values are presented as medians (range) or means as indicated. Changes in prevalence and incidence were tested using multiple regression analysis. The squared correlation coefficient (*r*²) is given. The statistical analyses were performed using StatView 4.5.

**Results**

The number of patients decreased slightly within the observation period from 299 in 1998 to 277 in 2002, mainly due to patients transferred to the adult clinics at the age of 18 years and natural variation in patient numbers. The annual number of sputum samples cultured increased significantly from 33 in 1998 (1.5 cultures per patient) to 104 in 2002 (2.0 per patient) (linear regression analysis, *r*² = 0.994, *P* < 0.01).

A significant increase was observed (linear regression analysis, *r*² = 0.924, *P* < 0.01) in the prevalence of patients with positive *Aspergillus* sputum cultures from 22 patients in 1998 (7.4%) to 52 patients in 2002 (18.8%) (Fig. 1). *A. fumigatus* represented the majority (93%) of *Aspergillus* species in the cultures. The other species identified were *A. flavus* 3%, *A. terreus* 2%, *A. niger* 1%, *A. nidulans* 0.3%, and *A. species* 0.7%. The incidence of patients with positive *Aspergillus* cultures, based upon the prevalence in the first year of the observation period, remained unchanged (linear regression analysis, *r*² = 0.185, *P* > 0.5). The number of years over which each patient had a positive *Aspergillus* culture (maximum 6 years within the study period) was 2.0 years (mean, range 1–6 years). The initial positive *Aspergillus* culture was identified at a median age of 9 years (range 1–18 years).

**Aspergillus allergy and ABPA**

*Aspergillus* allergy characterised by increase in total and specific anti-*Aspergillus* IgE antibody responses (SPT or RAST) was found in 12 (7%) of the patients with positive *Aspergillus* sputum cultures. Thirteen patients (4.7% of the entire study sample) were clinically diagnosed with ABPA within the period (8 females/5 males, mean age 13 years, range 7–17). All the patients fulfilled the recently recommended diagnostic criteria for ABPA in CF. An additional two patients had ABPA according to the paraclinical parameters, but have not yet had clinical symptoms of ABPA. The 1-year prevalence of ABPA increased significantly from 0.3% in 1998 to 4.0% in 2002 (linear regression analysis: *r*² = 0.997, *P* < 0.01) (Fig. 2).

**Seasonal variation**

No seasonal variation in the incidence of the initial positive *Aspergillus* culture for each patient was observed (KW, *P* > 0.4) (Fig. 3). The mean annual humidity in NSW was 64%. The relative humidity ranged from 55% (so ± 7) in October to 70% (so ± 9) in February, but no significant difference in mean monthly humidity was observed (1998–2002).
Immediate cutaneous reactivity to *Aspergillus* and in vitro presence of *Aspergillus*-specific serum IgE antibodies (SPT and RAST)

SPTs were performed in 88 (81%) of the patients with positive *Aspergillus* cultures. Of these 88 patients, 8% were positive to *D. pteronyssinus*, 7% to *Alternaria* sp. and 6% were positive to all allergens in the panel. Thirty-four percent had positive reactions to *Aspergillus*. In patients with a positive SPT to *Aspergillus*, there was also a positive reaction to *D. pteronyssinus* in 18% and in 10% to grass or rye/or both as well. Two of the 13 ABPA patients failed to have a positive SPT to *Aspergillus* within the study period; 5 patients were positive to *Alternaria* sp. and 3 patients to all allergens in the panel. Additional 4 patients had positive reaction to *D. pteronyssinus*. None of the ABPA patients had isolated positive reaction to grass or rye.

Specific IgE to *Aspergillus* (RAST) was measured in seven of those with positive *Aspergillus* sputum cultures. Two patients had a negative RAST. Five patients, one with *Aspergillus* allergy and four with ABPA, had a positive RAST; all of them had very high levels.

**Total IgE**

The majority (75%) of *Aspergillus*-colonised patients had IgE levels <100 kIU/L, but some had levels up to 500 kIU/L. When the patients were further categorised as *Aspergillus* allergic (i.e. without ABPA but positive to *Aspergillus* on SPT), or having ABPA, a relationship with total IgE was observed. In patients with *Aspergillus* allergy, IgE levels were within the range of 100–1000 kIU/L, except for one patient who had a level of 1466 kIU/L. The IgE levels were above 1000 kIU/L in all but one ABPA patients (mean 4353 kIU/L, range 861–9140 kIU/L).

**Eosinophil counts**

Eosinophil counts in patients with positive *Aspergillus* sputum cultures but without *Aspergillus* allergy, or ABPA were generally <0.3 × 10⁹/L. In some patients, levels as high as 0.9 × 10⁹/L were occasionally seen. In patients with *Aspergillus* allergy, eosinophil counts ranged up to 1.6 × 10⁹/L. The mean eosinophil count in ABPA patients at the time of diagnosis was 1.0 × 10⁹/L (range 0.1–2.6 × 10⁹/L).

**Precipitating antibodies to *A. fumigatus***

The precipitating antibodies to *Aspergillus* were measured in 51 patients with positive *Aspergillus* sputum cultures. Of 30 patients with positive precipitating antibodies, 13 were diagnosed with ABPA. The precipitating antibodies were in general positive in all four solutions in these patients (see Patients and methods).

**Lung function and *P. aeruginosa* colonisation**

The mean FEV₁% was 84% predicted (range 27–165%) in patients with positive *Aspergillus* sputum cultures and 74% (range 29–116) in ABPA patients. The ABPA patient with an FEV₁ of 29% had a lung transplant within the study period as a result of sequelae from chronic PA infection. The percentage of *Aspergillus* colonised patients also
colonised with \textit{P. aeruginosa} was 66% and of ABPA patients 69%.

**Chest X-rays and CT scans**

In all ABPA patients, chest X-rays showed opacities especially in the right upper and mid lobe prior to and during acute ABPA. Nine patients had bronchial thickening and bronchiectasis. One patient had aspergillomas and another rounded areas of atelectasis. On a chest CT scan, one patient had cylindrical bronchiectasis and another two had bronchiectasis primarily in the upper right lobe.

**Aspergillus culture negative CF patients**

Screening of the 168 CF patients who had negative \textit{Aspergillus} cultures showed that 111 (66%) had IgE levels <100 kIU/L, 15 (9%) had IgE levels between 100 and 200 kIU/L, 21 (12.5%) had IgE levels between 200 and 500 kIU/L and 21 (12.5%) had IgE levels >500 kIU/L. Three patients with IgE levels above 500 kIU/L had positive precipitating antibodies, and one of them may have had an episode of ABPA previously. Two patients with IgE levels between 100 and 500 kIU/L had positive precipitating antibodies as well. None of these patients were tested for IgE-Af. (RAST).

**Discussion**

In NSW, Australia a high-atopy frequency of 32.5% in children\(^1\) and the average humidity of 64%, would be expected to pave the way for colonisation with \textit{Aspergillus} and development of ABPA in CF patients. We found a prevalence of 18.8% colonisation with \textit{Aspergillus} in the study population (2002), which is lower than the recently reported prevalence of approximately 25% from a US multicentre study,\(^1\) and the 42% in Copenhagen, Denmark.\(^9\) Atopic symptoms are present in 23% of Danish children and adolescents.\(^1\) According to the 2000 report of the Australasian CF Data Registry which includes 2195 patients in Australia and 311 in New Zealand (27 centres), the prevalence of \textit{Aspergillus} colonisation was 20%. Data from Europe and USA include children as well as adults, but were nevertheless used for comparison since prevalences of \textit{Aspergillus} colonisation and ABPA seem to plateau in late adolescence.\(^9\)

The presently observed tendencies to humidity changes during the year, although not significant, combined with findings in other studies indicate that humidity variation does not affect the incidence of initial \textit{Aspergillus} culture.\(^15,16\) In spite of the higher mean monthly relative humidity in Denmark, 2002 (83%, range 78–91%) the Danish climate is not considered to be a major contributor to the higher prevalence of \textit{Aspergillus} colonisation,\(^9\) since other North European centres with a similar humidity report lower prevalences.\(^5\) Furthermore, it has recently been shown that \textit{Aspergillus} spore counts do not correlate with \textit{Aspergillus}-related disease.\(^16\)

The increase in prevalence of \textit{Aspergillus} colonisation from 7.4% in 1998 to 18.8% in 2002 that we report in this study is in accordance with the general tendency towards increasing prevalence over the last decades, e.g. in Denmark from 2% in 1975 to 42% in 1995.\(^9\) In 1980, relatively high and comparable prevalences of \textit{Aspergillus} colonisation of 8.6% in Sydney (a prospective study)\(^17\) and 12.5% in Copenhagen\(^9\) were found based on studies including an average of 9.3 and 10.2 sputums per patient per year, respectively.\(^18\) These results reflect the relationship between prevalence and frequency of sampling. Different culturing methods may also add to the range in various reports. Since \textit{Aspergillus} colonisation and ABPA is rare in patients <6 years, the increase in mean and median age of patients observed in Danish CF population\(^18\) may also influence the increasing prevalence. A liberal use of prophylactically inhaled antibiotics has been suggested to increase the risk of colonisation. Burns et al.\(^13\) found that colonisation increased from 25% to 30% in patients who received inhaled tobramycin. Previous studies of colistin inhalation did not show an increase.\(^9,19\) The importance of inhaled antibiotics was not addressed in the present study, but needs further investigation.

The ABPA prevalence varies from 1% to 14% in different studies.\(^4,5,7,20-23\) We found a ABPA prevalence of 4% in 2002. According to the Australasian Registry (2000), the ABPA prevalence was 0.7% for the age group 0–4 years and 4.2% for the age group 5–17 years, indicating that the results obtained for this centre in this study are consistent with those reported across for Australia. This prevalence is however twice as high as the prevalence reported from the multicentre Epidemiologic Study of Cystic Fibrosis from USA (ESCF)\(^4\) and from the US Cystic Fibrosis Foundation (CFF) Registry.\(^24\) However, it has recently been estimated that the most likely prevalence of ABPA patients in CF in USA is 8–12%.\(^3\) The prevalence of ABPA reported from the European Registry of Cystic Fibrosis (ERCf) is two-fold higher than the presently observed Australian prevalence (mean 7.8%, range: 2.1% in Sweden to 13.6% in Belgium).\(^3\)
High total IgE levels, far exceeding 1000 kIU/L (mean 4353 lIU/L) were found in patients with ABPA in the present study. The reason for this remains unclear, although a generally higher sensitivity to allergens in the population may be suggested. The diagnosis was supported by changes in chest X-rays in all patients prior to the development of ABPA. As previously suggested, eosinophil counts were of less utility for the diagnosis of ABPA in patients with CF, and severity of lung function as assessed by FEV1 or chronic P. aeruginosa colonisation were not associated with the presence of Aspergillus colonisation in the present study. This is in agreement with other studies. Patients with ABPA were more likely to have positive cutaneous reactivity to D. pteronyssinus and, as previously observed in Danish patients, to the other mould Alternaria sp., but not to the other allergens in the panel.

Differences in screening and reporting within centres may explain some of the variation in ABPA prevalence. The prevalence of ABPA according to the US CFF Registry relies on reporting from the centres without strictly specified criteria. Criteria are required in the ESCF and ERCF, but may be applied to different degrees in the various centres. From UK it has recently been reported that major criteria for ABPA; such as IgE-Af, wheeze and cough or total IgE > 420 kIU/L were only present in approximately 50% of the time. All the patients diagnosed with ABPA in the present study fulfilled the recently suggested criteria for ABPA in CF. Furthermore, annual screening of total IgE along with a clinical suspicion of ABPA in patients > 6 years was a safe way of identifying patients with CF at risk of ABPA.

Thus, we conclude that a high background level of atopy in a population and a humid climate do not seem to have a major impact on the prevalence of Aspergillus colonisation and ABPA in CF patients. Recently suggested genetic factors such as HLA-DR subtypes, MBL, GM haplotypes and CFTR may be more likely involved in the susceptibility to ABPA.

References