



Fungal atopy in adult cystic fibrosis

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This study set out to estimate the prevalence of atopy to a variety of common ubiquitous fungi, including *A. fumigatus*, in cystic fibrosis (CF), and to evaluate the investigations by which the diagnosis was made. Particular attention was paid to the usefulness of skin testing and immunoassays in detecting which patients had simple fungal atopy, and which patients were at high risk of developing allergic bronchopulmonary mycoses.

This cross-sectional study included 21 adult CF patients and 20 matched controls. Serum samples were taken for the measurement of total serum IgE and specific serum IgE to nine common fungi. Immediate hypersensitivity skin prick testing to each of the fungi was also performed. Simple fungal atopy was described in subjects fulfilling the following criteria: total serum IgE $> 100 \text{ KU l}^{-1}$ with specific radioimmunoassay $\geq$ grade 1 to at least one fungus and a positive skin prick test (SPT) $\geq 3 \text{ mm}$ to the same fungus. 'High risk' for developing allergic bronchopulmonary mycosis (ABPM) was described in subjects fulfilling the following criteria: total serum IgE $> 200 \text{ KU l}^{-1}$ with specific radioimmunoassay $\geq$ grade 2 to at least one fungus and a positive skin prick test (SPT) $\geq 6 \text{ mm}$ to the same fungus.

The adult CF group had a significantly higher total SPT score ($P=0.005$) and mean total serum IgE ($P < 0.05$) than controls. Forty-three percent of CF patients fulfilled the criteria for fungal atopy to at least a single fungus. Over half this group had an atopic tendency to more than one fungus. Nineteen percent of the CF group were at least 'high risk' of developing ABPM. Skin prick testing is a better marker of fungal atopy and a better predictor of those adult CF patients at higher risk of developing ABPM than specific radioimmunoassay serum testing.

There is a high prevalence of fungal atopy in the adult CF population. Total serum IgE and skin prick testing are good predictors of fungal atopy and help predict those at risk of developing ABPM in CF.

Key words: cystic fibrosis; fungal atopy; allergic bronchopulmonary mycosis (ABPM); skin prick test (SPT); specific serum IgE (RAST).

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Introduction

The incidence of *Aspergillus fumigatus* in the airways of cystic fibrosis (CF) patients has been reported to be as high as 57% (1). A minority (5–11%) (1–3) of these patients develop allergic bronchopulmonary aspergillosis (ABPA). To prevent lung damage, early and aggressive treatment with high dose corticosteroids may be important (4). The diagnosis of ABPA (bronchial obstruction, blood eosinophilia, immediate skin reactivity to *A. fumigatus*, precipitating antibodies against *A. fumigatus*, elevated serum IgE level, pulmonary infiltrates, central bronchiectasis, and elevated *A. fumigatus*-specific IgE or IgG serum antibodies) in CF patients may be more difficult to make than in non-CF patients because of the overlap between the cardinal clinical and paraclinical findings of ABPA (5,6) and those of CF, including pulmonary infiltrates, intermittent wheez-

ing and frequent colonization with *A. fumigatus* that leads to humoral immunity.

Because of the frequent colonization with *A. fumigatus* in CF, antibody reactivity to *A. fumigatus* proteins is frequently observed. Likewise, the presence of positive skin test to responses to *A. fumigatus* is common in CF patients. It is not clear however, to what degree this atopic tendency to *A. fumigatus* is a prognostic indicator of lung function decline or morbidity in CF. Neither is it clear when fungal atopy in CF merges into the clinical spectrum of full blown ABPA. It has been suggested that sensitization to *A. fumigatus* in patients with cystic fibrosis is, in itself, associated with decreased lung function and poorer clinical outcome (7,8). Both fungal atopy and ABPA are predominantly conditions affecting adult CF patients who tend to have more severe pulmonary disease (9). It has been suggested that while the incidence of ABPA is higher in older CF patients, this cohort of patients do not have significantly worse pulmonary disease than their counterparts who are colonized with *A. fumigatus*, without fulfilling the diagnostic criteria for ABPA (10). Both of these groups of patients had lower chest radiograph scores than CF patients not colonized with *A. fumigatus* and both

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groups benefited from treatment with systemic corticosteroids.

Diagnostic cut-off levels along with positive and negative predictive values of the various commonly used paraclinical markers of ABPA in CF have remained speculative. Specific IgE/IgG *A. fumigatus* antibodies detected by ELISA have been proposed as having the power to discriminate between patients with CF and ABPA and those CF patients colonized with *A. fumigatus*, having positive precipitins, but lacking the clinical and radiographic evidence of ABPA (11,12). More recently Skov *et al.* have described specific IgE antibodies, total serum IgE and specific *A. fumigatus* immediate hypersensitivity skin prick testing (SPT) along with *A. fumigatus* precipitins as the most valid predictors of ABPA in CF (13). These investigations tend to be more universally available in CF units.

The incidence of allergic bronchopulmonary mycosis (ABPM) and fungal atopy in CF involving common fungi other than the *Aspergillus* species is unknown. Other fungi, most commonly *Candida albicans*, have been reported to cause this syndrome in chronic asthmatics (14,15). In India, over 8% of patients with chronic respiratory disease have been found to suffer from ABPM (16). In light of these findings, we carried out a case-control study to determine the levels of fungal atopy in an adult CF cohort as determined by total serum IgE levels, specific IgE to common fungi and specific skin prick testing to the same fungi.

Patients and methods

We reviewed 21 adult CF patients (22.1 ± 0.9 , 18–35 years, 11 males) and 20 age and sex matched controls (24.2 ± 0.4 , 21–27 years, eight males). The CF patients attended the adult CF outpatient clinic at the regional adult CF unit ($n=40$ adult CF patients) at Cork University Hospital, Ireland. Patients had no evidence of a recent acute exacerbation of respiratory disease requiring supplementary antibiotics or corticosteroids. Only one patient from this group had suffered from proven ABPA using standard criteria (6). The control population who were healthy volunteers, had no history of atopic disease, including asthma, allergic rhinitis or eczema. The study was approved by the local clinical research ethics committee.

Further investigations carried out included the following: (a) serum samples were analysed for total IgE, using the UniCAP automated immunoassay system 16 (Pharmacia, Dublin, Ireland) according to the method of Paganelli *et al.*, (17). Diagnostic levels consistent with fungal atopy were set at $\geq 100 \text{ KU l}^{-1}$. Diagnostic levels consistent ABPA or ABPM or with high risk of developing either, were set at $\geq 200 \text{ KU l}^{-1}$. (b) Specific serum IgE measurements, using solid phase radioimmunoassay, to nine common fungi were measured only if total serum IgE was $> 100 \text{ KU l}^{-1}$. Serum specific antibody measurements were carried out using the UniCAP system described (17). The specific IgE antibodies were assigned to (i) *Aspergillus fumigatus*, (ii) *Candida albicans*, (iii) *Cladosporidium herbarum*, (iv) *Penicillium*

notatum, (v) *Helminthosporium halodes*, (vi) *Curvularia lunata*, (vii) *Rhizopus nigricans*, (viii) *Alternaria alternata* and (ix) *Mucor mucado*. Responses were graded 0–4 as described by the manufacturer as follows: immunoassay class 0, $\leq 0.34 \text{ IU ml}^{-1}$, class 1, $0.35\text{--}0.69 \text{ IU ml}^{-1}$, class 2, $0.7\text{--}3.49 \text{ IU ml}^{-1}$, class 3, $3.5\text{--}17.49 \text{ IU ml}^{-1}$, class 4, $\geq 17.5 \text{ IU ml}^{-1}$. Diagnostic levels consistent with fungal atopy were set at specific IgE $\geq$ class 1. Diagnostic levels consistent with ABPA or ABPM or with high risk of developing either, were set at specific IgE $\geq$ class 2. (c) Type I immediate hypersensitivity skin prick testing (SPT) to the fungi outlined above was carried out using Allergo-Pharma antigens (Allergo-Pharma Biodiagnostics, Worcestershire, U.K.) with positive histamine and negative saline controls measured on the opposite arm. Reactions were measured after 15 min. Wheals measuring $\geq 3 \text{ mm}$ in diameter after 15 min were considered to be consistent with a diagnosis of fungal atopy and wheals measuring $\geq 6 \text{ mm}$ in diameter after 15 min were considered to be consistent with a diagnosis of ABPA or ABPM or with high risk of developing either. We adapted the anergy band Multitest skin test method of measuring total skin induration induced by all the fungi to give a total skin test score in mm for each subject and control (18).

STATISTICS

Results are expressed as mean values $\pm \text{SEM}$ and ranges where appropriate. Statistical analysis was performed using the Mann-Whitney *U* non-parametric analysis to compare variables between groups. A χ^2 -squared test was used to determine independence. Sensitivity and specificity values were determined in accordance with previously published guidelines (23). The Instat II software package, (San Diego, CA, U.S.A.) was used for statistical calculation. A two-tailed *P*-value < 0.05 was considered significant.

Results

Twenty-one CF patients, mean age 22.1 ± 0.9 years took part in this study. Results were compared to those of 20

TABLE 1. Comparison between adult CF patients and age and sex matched controls, outlining differences between group mean (SEM) total skin prick testing score (mm) and total serum IgE (KU l^{-1}). * $P < 0.05$ considered significant

	CF group ($n=21$)	Control group ($n=20$)	
Age (years)	22.1 (0.9)	24.2 (0.4)	
Range	18–35	21–27	
M:F	11:10	8:12	
Skin prick test	6.76	0.7	* $P=0.005$
Induration (mm)	(2.0)	(0.4)	
Serum IgE (KU l^{-1})	439.0 (166.0)	88.7 (26.1)	$P=0.05$

matched control subjects, mean age 24.2 ± 0.4 years. The CF group had a significant higher mean total skin prick test (SPT) score (CF: 6.76 ± 2.0 mm; control: 0.7 ± 0.4 mm; $P=0.005$) (Table 1). Likewise, the CF group had a significantly higher mean serum IgE than the control group (CF: 439.0 ± 166.0 KU l^{-1} ; control: 88.7 ± 26.1 ; $P<0.05$). These results demonstrate a substantial overall atopic tendency to fungi in the CF group. The mean IgE and SPT scores for the CF group are in the range described for ABPM as outlined above (13). We quantified fungal atopy and 'high risk' for ABPM in both the CF and control population. The diagnoses were made on the basis of the criteria outlined in Table 2, i.e. subjects with fungal atopy satisfy all three criteria as follows: total IgE >100 KU l^{-1} at least one specific IgE measurement $>$ class 1 and SPT

≥ 3 mm to the same fungus. ABPM 'high risk' subjects satisfy all three criteria as follows: total IgE >200 KU l^{-1} , at least one specific IgE measurement $>$ class 2 and SPT ≥ 6 mm to the same fungus. Nine out of 21 (43%) adult CF patients fulfilled the criteria for fungal atopy to at least one fungus on the basis of these three criteria previously shown to have the strongest predictive value (11) (Table 2). Our figures are higher than those previously quoted for fungal atopy in CF populations (1) and may reflect the effect of age in the adult only population studied, the evaluation of atopy to fungi other than *A. fumigatus* and the increasing prevalence of aspergillus colonization in the lower respiratory tracts of CF patients (11). In the presence of a total IgE >100 KU l^{-1} , we have demonstrated that a SPT ≥ 3 mm has a specificity for fungal atopy of

TABLE 2. Outlines differences within the adult CF group, between fungal atopy and allergic bronchopulmonary mycosis (ABPM) 'high risk' subgroups

Criteria	CF (n=21)	Control (n=20)
Fungal atopy		
IgE >100 (KU l^{-1})	10	5
Specific IgE $>$ class 1	9	0
SPT ≥ 3 mm	12	1
All three criteria	9	0
<i>A. fumigatus</i>	n=9	
<i>P. notatum</i>	n=4	
<i>C. herbarum</i>	n=3	
<i>C. albicans</i>	n=2	
<i>A. alternaria</i>	n=2	
Serum IgE		
' <i>A. fumigatus</i> only' group (n=4)	458.0 (82.3)	
Serum IgE 'Overlap atopy' group (n=5) (KU l^{-1})	1007.9 (112.2)	
Skin prick test	5	
' <i>A. fumigatus</i> only' group (n=4)	(1.2)	
Skin prick test 'Overlap atopy' group (n=5) (Induration mm)	17.16 (7.6)	
ABPM 'high risk'		
Skin prick test 'Overlap' group (n=0) (Induration mm)	12	
IgE >200 (KU l^{-1})	10	3
Specific IgE $>$ class 2	9	0
SPT ≥ 6 mm	4	0
All three criteria	4	0
<i>A. fumigatus</i>	n=3	
<i>A. alternaria</i>	n=1	
Serum IgE ' <i>A. fumigatus</i> only' group (n=3)	1787.5 (667.2)	
Serum IgE 'Overlap' group (n=0) (KU l^{-1})		
Skin prick test	10.5	
' <i>A. fumigatus</i> only' group (n=3)	(1.5)	
Skin prick test 'Overlap' (n=0)	12	
(Induration mm)		

Diagnostic criteria for fungal atopy include all of the following: (1) Total serum IgE >100 KU l^{-1} , (2) RAST $>$ class 1, 3. Skin prick test (SPT) ≥ 3 mm for the same fungus. Diagnostic criteria for ABPM include all of the following: (1) Serum IgE >200 KU l^{-1} , (2) RAST $>$ class 2, (3) SPT ≥ 6 mm for the same fungus. Mean (SEM) serum IgE and SPT values in the subgroups fulfilling the criteria for fungal atopy and ABPM 'high risk' to *A. fumigatus* only and to an 'overlap' group to more than a single fungus are included.

TABLE 3. Outlines the presence of fungal atopy or ABPM 'high risk' within the case and control populations. The individual fungi studied are shown in *italics*. Similar diagnostic criteria for fungal atopy and ABPM are used as in Table 2

	Fungal atopy IgE $\geq 100 \text{ KU l}^{-1}$ Specific IgE $\geq$ class 1 SPT $\geq 3 \text{ mm}$		ABPM 'high risk' IgE $\geq 200 \text{ KU l}^{-1}$ Specific IgE $\geq$ class 2 SPT $\geq 6 \text{ mm}$	
	Case (n=9)	Control (n=0)	Case (n=4)	Control (n=0)
<i>A. fumigatus</i>	9	0	3	0
<i>P. notatum</i>	4	0	0	0
<i>C. herbarum</i>	3	0	0	0
<i>C. albicans</i>	2	0	0	0
<i>A. alternaria</i>	2	0	1	0
<i>M. mucado</i>	0	0	0	0
<i>R. nigricans</i>	0	0	0	0
<i>H. halodes</i>	0	0	0	0
<i>C. lunata</i>	0	0	0	0

90%, whereas specific IgE $>$ class 1 has a specificity of only 80%. It is worth noting that none of the control subjects fulfilled the diagnostic criteria for fungal atopy on the basis of these three diagnostic criteria alone.

Four CF patients (19%) had all three diagnostic criteria for ABPM 'high risk'. Three of these patients fulfilled our criteria for allergic bronchopulmonary aspergillosis (ABPA) and the fourth fulfilled the criteria for allergic bronchopulmonary alternariosis. SPT $\geq 6 \text{ mm}$ for an individual fungus was 100% specific in this group, however specific IgE $>$ class 2 was only 66% specific for the diagnosis of ABPM in this small group of CF patients. Once again none of the control group fulfilled the ABPM criteria.

As previously outlined, fungal atopy itself can be a predictor of poor respiratory function in CF. It is important to highlight that of the nine fungi tested, the CF group demonstrated atopy to five fungi: in order of frequency these included *A. fumigatus* (n=9), *P. notatum* (n=4), *C. herbarum* (n=3), *C. albicans* (n=2) and *A. alternata* (n=2) (Table 3). There are overlap of fungal atopy to more than one fungus in five (55%) adult CF patients. These 'overlap atopy' syndromes occurred to the following combination of fungi: patient 3, *A. fumigatus* + *P. notatum* + *A. alternata*; patient 4, *A. fumigatus* + *P. notatum* + *A. alternata* + *C. herbarum* + *C. albicans*; patient 6, *A. fumigatus* + *P. notatum* + *C. herbarum*; patient 7, *A. fumigatus* + *C. albicans*; patient 9, *A. fumigatus* + *P. notatum* + *C. herbarum*. All four CF patients with ABPM 'increased risk' were allergic to a single fungus only.

Discussion

The experience of allergic bronchopulmonary aspergillosis in patients with CF suggests that there should be an increased awareness of the condition and its capacity to

cause significant pulmonary morbidity. The concomitant physical and radiological signs of ABPA and CF have led to confusion and delay in making this very important diagnosis in CF. Greenberger has defined separate isotypes of ABPA with and without central bronchiectasis in asthmatics (6). The importance of concentrating on serological and immediate hypersensitivity skin testing in order to make the diagnosis of ABPA and indeed, ABPM in cystic fibrosis, has been highlighted in several recent papers (11,19–21). This study confirms the high prevalence of fungal atopy in CF. We have not attempted to relate these findings to pulmonary function. Previous studies which have attempted to do this have shown conflicting results (3,7,8), however none of these studies have tried to correlate a fall in pulmonary function over time with the presence or absence of the paraclinical findings outlined in this paper. This study uses recently suggested diagnostic criteria (13,19,20) for ABPA and fungal atopy to *A. fumigatus* in CF and extends the criteria to include other common fungi, which may cause fungal atopy and ABPM in CF.

While others have highlighted the importance of a rise in total serum IgE (13), specific IgG subclasses (21), *A. fumigatus* precipitins (11,13,19,20) and SPT-*A. fumigatus* (11,13,19) as serological predictors of ABPA in CF, this study suggests that in the presence of a serum IgE $> 100 \text{ KU l}^{-1}$, a SPT result $\geq 3 \text{ mm}$ for a specific fungus is sufficient to make the diagnosis of fungal atopy. Using the paraclinical parameters of a serum IgE $> 200 \text{ KU l}^{-1}$ and a SPT result $\geq 6 \text{ mm}$ for an individual fungus we identified a group of CF patients who are at least at 'high risk' of developing ABPA and ABPM. The addition of specific serum IgE measurement adds little to the diagnostic panel, however in the absence of skin testing facilities, specific IgE $\geq$ class 1 for fungal atopy or $\geq$ class 2 for ABPM are also suggestive markers.

We have demonstrated a marked atopic tendency to a variety of common fungi in our adult CF patients. We also suggest that on the basis of the criteria used that four out of twenty-one (21%) CF patients suffered from ABPM or were at *high risk* of developing ABPM with *A. fumigatus* being the offending fungus in most but not all cases. While it is suggested that prednisolone and itraconazole have a definite role to play in the treatment of ABPA in asthma (4,22), it is not clear whether treating these CF patients with fungal atopy or ABPM would alter their disease status.

Our results demonstrate that greater awareness needs to be shown, amongst clinicians treating CF patients, of the possibility of either fungal atopy or ABPM within the CF population. A narrower focus of diagnostic tests concentrating on serum IgE, SPT and, to a lesser extent, specific IgE immunoassay testing, is recommended to diagnose fungal atopy and to highlight those who in the correct clinical context should be considered for treatment for ABPM. On the other hand a wider range of fungi other than *A. fumigatus* need to be considered as potential pathogens within an adult CF population. Our results would suggest that it may be worthwhile concentrating on a panel of common fungi including *A. fumigatus*, *P. notatum*, *C. albicans*, *A. alternata* and *C. herbarum*. Further studies examining the effect of fungal atopy and ABPM on pulmonary phenotype over time in CF are recommended.

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