RESPIRATORY MEDICINE (1999) **93**, 835–838

Prophylactic antibiotic therapy is associated with an increased prevalence of *Aspergillus* colonization in adult cystic fibrosis patients

J. BARGON*, N. DAULETBAEV*, B. KÖHLER*, M. WOLF*, H.-G. POSSELT† AND T. O. F. WAGNER*

**Division of Pulmonary Medicine, Department of Internal Medicine II and †Department of Pediatric Medicine, Frankfurt University Hospital, Frankfurt/Main, Germany*

Aspergillus colonization is a common phenomenon in adult cystic fibrosis (CF) patients. The clinical significance of *Aspergillus* for the pathogenesis of CF lung disease remains unclear and factors predisposing to such colonization are still completely unknown.

We investigated the prevalence of *Aspergillus* colonization in 104 adult CF patients who attended our outpatient clinic in 1997. With respect to demographic and clinical data, and antibiotic therapy received, we further examined which factors were associated with Aspergillus colonization in these patients.

Repeated investigations of CF sputum samples revealed Aspergillus species in 43/104 (41·3%; 95% confidence interval 30·2–52·5%) of the patients. We found no significant relationship between *Aspergillus* colonization and age ($P > 0{\cdot}4$), gender ($P = 0{\cdot}4$), colonization with *pseudomonas* species ($P > 0{\cdot}6$), lower lung function values ($P > 0{\cdot}9$), or worse chest radiography ($P > 0{\cdot}1$). Surprisingly, the prevalence of *Aspergillus* colonization was higher in CF patients receiving prophylactic antibiotic therapy (oral antibiotics: $P = 0{\cdot}05$; inhalative antibiotics: $P = 0{\cdot}035$; both antibiotics: $P = 0{\cdot}048$).

Prophylactic antibiotics are widely used to eradicate or decrease chronic bronchopulmonary infection in CF. Our results indicate that long-term antibiotic therapy may predispose CF patients to *Aspergillus* colonization.

RESPIR. MED. (1999) **93**, 835–838

Introduction

Chronic bronchopulmonary infection leads to progressive lung destruction in patients with cystic fibrosis (CF). In adult patients, the most common pathogen is *Pseudomonas aeruginosa*. *Aspergillus* species are isolated from respiratory tract secretions of many CF patients; the prevalence reported varies from 25–60% (1-3). *Aspergillus* infection is rarely invasive; however, in approximately 10% of the CF population *Aspergillus* recovery is associated with allergic bronchopulmonary aspergillosis (ABPA) (4,5), which seriously complicates the course of CF lung disease and is difficult to diagnose, since the symptoms overlap those of chronic bronchopulmonary infection (4,6).

Factors predisposing to *Aspergillus* colonization remain unclear. Some studies suggest an association between increasing age, and living in a rural environment, and the prevalence of *Aspergillus* colonization (7,8). The clinical significance of such colonization remains controversial. Several studies report that sensitization to *Aspergillus* in the presence of increased total IgE and increased antibody titre alone is associated with decreased lung function in CF patients (9,10). Colonization alone is not suggested as a risk factor for more advanced lung disease in one study (9), whereas other publications find a correlation between *Aspergillus* colonization and severity of disease (5,11).

In this study, we investigated which demographic or clinical factors may be associated with *Aspergillus* colonization in the adult CF patients of our outpatient clinic. We analysed (1) the prevalence of *Aspergillus* colonization in the CF population and (2) the relationship between *Aspergillus* colonization and (a) age, (b) gender, (c) colonization with *Pseudomonas aeruginosa* and *Burkholderia cepacia*, and duration of this colonization, and (d) antibiotic therapy regimen and duration of the therapy. We also examined whether *Aspergillus* colonization is associated with more advanced lung disease in these patients.

Paper received 19 January 1999 and accepted 8 July 1999.
Correspondence to: Dr. Joachim Bargon, Division Pulmonary Medicine, Department of Internal Medicine II, Frankfurt University Hospital, Theodor Stern Kai 7, 60590 Frankfurt/Main, Germany. Fax: +49 69 6301 7391; e-mail: bargon@t-online.de

Methods

STUDY GROUP AND DATA ANALYSED

All adult CF patients who attended our outpatient clinic in 1997 were included in this study. The diagnosis of CF was

0954-6111/99/110835+04 $12·00/0

confirmed by positive sweat chloride tests and/or DNA analysis. Sputum samples were cultured as described elsewhere (12). *Aspergillus* colonization was assumed when *Aspergillus* species were found in at least two of four sputum samples during the 1 yr observation period. Patients colonized with *Staphylococcus aureus* received prophylactic oral antibiotics with anti-staphylococcal properties continuously. In the case of *Pseudomonas* colonization, we usually recommended inhalation therapy beginning with tobramycin (Gernebcin®, Lilly, Stolberg, Germany) or, when the strain was resistant to tobramycin, with colistin (Colistin®, Gruenenthal, Bad Homburg, Germany), also continuously. The severity of CF lung disease was estimated by lung function investigation and evaluation of the Crispin–Norman score (CNS). For the analysis we used average values of at least the three best lung functions and CNS values.

The data analysed included age, gender, lung function parameters, CNS values, colonization with *Pseudomonas aeruginosa* and antibiotic therapy regimen.

STATISTICAL ANALYSIS

All continuous variables are given as median (25–75 percentiles) or median (range), if appropriate. Categorical variables are expressed as number of cases (%; 95% confidence interval). To compare the data, the Mann–Whitney test for independent non-parametric samples was used. Categorical variables were compared using the Pearson's χ^2 procedure. Significance was defined as a P value of $\leq 0{\cdot}05$.

Results

One hundred and four CF patients (46 women) of median age 25 years (Table 1) were evaluated. Eleven patients had a history of ABPA with no recent manifestations for at least 2 yr. The majority of patients (80·8%; Table 1) was colonized with *Pseudomonas aeruginosa* or *Burkholderia cepacia*, or with both strains. Although lung function parameters were normal in 17 of 104 patients (16·3%) [median (range) forced expiratory volume in 1 sec (FEV_1) 101 (81–129)% pred.], in most patients we observed airway obstruction of various degree, with median FEV_1 for the whole group of 48·5% pred. (Table 1). When compared to *Pseudomonas* negative patients, *Pseudomonas* colonized patients showed worse lung function [FEV_1 47·6 (32·7–68·9) *vs.* 56·3 (38–104)% pred. in non-colonized] and chest radiography [CNS 12 (9–16) *vs.* 9 (5–12) in non-colonized; $P<0{\cdot}05$, both comparisons). Colonization with *Pseudomonas* in these patients was not age- or gender-associated ($P>0{\cdot}3$; data not shown).

Repeated investigations of CF sputum samples revealed *Aspergillus* species in 43 of 104 (41·3%) patients (Tables 1 and 2). There was no significant difference in age ($P>0{\cdot}4$), gender ratio ($P = 0{\cdot}4$), lung function ($P>0{\cdot}9$) and CNS ($P>0{\cdot}1$) values between *Aspergillus* positive and negative patients (Table 2). With regard to colonization with *Pseudomonas* strains, we found no significant relationship with *Aspergillus* colonization. Thus, neither the prevalence of colonization with *Pseudomonas aeruginosa* or *Burkholderia cepacia*, nor the duration of this colonization was associated with a higher prevalence of *Aspergillus* colonization ($P>0{\cdot}5$, both comparisons; Table 2). Regarding the sensitization to *Aspergillus* we correlated total serum IgE and results of skin prick test with *Aspergillus* antigen with *Aspergillus* colonization and non-colonization and could not find any difference between both groups ($P>0{\cdot}3$). The next question we analysed concerned the relationship between antibiotic therapy regimen and the prevalence of *Aspergillus* colonization. The finding that the percentage of patients who received prophylactic therapy with oral antibiotics was significantly higher in the *Aspergillus* positive group was interesting (74·4 *vs.* 55·7% in *Aspergillus* negative group, $P = 0{\cdot}05$; Table 2). The association between antibiotic therapy and *Aspergillus* colonization was even stronger when the subgroup treated with inhaled

TABLE 1. Demographic and clinical data of CF patients evaluated in the study

Age, (years)	25 (20–30)
Women	46 [44·2%; 23·9–55·4%]
VC, l	3·1 (2·4–4·2)
VC, % pred.	74·5 (55·3–87·3)
FEV_1, l	1·8 (1·3–2·9)
FEV_1, % pred.	48·5 (33·2–73·7)
Crispin–Norman Score	11 (9–15)
Colonization with *Pseudomonas aeruginosa*, cases	84 [80·8%; 0·1–88·4%]
Colonization with *Aspergillus* species, cases	43 [41·3%; 0·2–52·5%]
Colonization with both species, cases	36 [34·6%]
Prophylactic antibiotic therapy:	
Oral antibiotics, cases	66 [63·5%; 51·7–73·6%]
Inhaled antibiotics, cases	55 [52·9%; 41·2–63·8%]
Both, oral and inhaled, cases	46 [44·2%; 32·2–56%]

Data are expressed as median (25–75 percentiles) or cases number [%; 95% confidence interval]. VC: vital capacity.

TABLE 2. Data of *Aspergillus* positive *vs. Aspergillus* negative patients

	Aspergillus positive (n = 43)	*Aspergillus* negative (n = 61)
Age (years)	26 (21–30)	24 (20–30)
Women	24 [55·8%; 37–72·2%]	22 [36·1%; 48·2–77]
FEV_1, % pred.	49·2 (33·3–71·8)	47·2 (32–77·6)
Normal values, cases	4 [9·3%; 1·2–25·1%]	13 [21·3%; 9·4–36·5%]
Mild obstruction, cases	12 [27·9%; 12–47%]	7 [11·5%; 2–24·8%]
Moderate obstruction, cases	13 [30·2%; 13·6–49·4%]	20 [32·8%; 18·1–48·8%]
Severe obstruction, cases	14 [32·6%; 15·4–51·2%]	21 [34·4%; 19·1–50·5%]
Crispin–Norman Score	12 (9–16)	11 (9–15)
Colonization with Pseudomonas species, cases	36 [83·7%; 65·3–93·5%]	49 [80·3%; 65·6–90%]
Colonization with Pseudomonas species, yrs	9·5 (6–13)	8 (5–13)
Continuous prophylactic therapy with:		
Oral antibiotics, cases	32 [74·4%; 55·6–87·3%]*	34 [55·7%;40–69·6%]
Oral antibiotics, yrs	8 (5–14)	10 (5·5–13·5)
Inhaled antibiotics, cases	28 [65·1%; 46–80%]§	27 [44·3%; 29·4–58·9%]
Inhaled antibiotics, yrs	5 (2·5–7·5)	5 (2–7·3)
No antibiotics, cases	8 [18·6%; 6·3–36%]	21 [34·4%; 20–50%]
Both antibiotics	25 [58·1%; 37·9–75%]¶	21 [34·4%; 20–50%]

Data are expressed as (25–75 percentiles) or cases number [%; 95% confidence interval]. *P= 0·05; §P= 0·036; ¶P = 0·048. The duration of prophylactic therapy with antibiotics was defined as the start of continuous medication with either oral or inhaled antibiotics from the beginning to the study period in years and as % of all patients, who received this continuous therapy. Pulmonary function was defined as follows: normal values: FEV_1 > 80% pred; mild obstruction FEV_1; 60–80% pred; moderate obstruction: FEV_1 40–60% pred; and severe obstruction: FEV_1 < 40% pred.

antibiotics was analysed (65·1 *vs.* 44·3% in *Aspergillus* negative group, P = 0·035; Table 2). Additionally, *Aspergillus* positive patients received significantly more often antibiotics in both applications, than did *Aspergillus* negative patients (58·1 *vs.* 34·4%, respectively, P = 0·048; Table 2).

Discussion

The role of *Aspergillus* species in the pathogenesis of CF lung disease is still not clear. The data on the prevalence of *Aspergillus* colonization in CF, and the course of lung disease after being colonized, are rare and vary from centre to centre. In this study, we analysed data of 104 adult CF patients of our outpatient clinic in order to evaluate the prevalence of *Aspergillus* colonization, and to reveal factors which may be associated with this colonization. Age of the patients, gender, colonization with *Pseudomonas aeruginosa* or *Burkholderia cepacia*, severity of CF lung disease and therapy regimen were analysed with regard to *Aspergillus* colonization.

Our data about the prevalence of *Aspergillus* colonization in adult CF patients (41·3%) are in a good agreement with data reported in literature (1-3,11,13,14). In contrast to previous investigations (7), we did not find any relationship between age of the patients and the prevalence of *Aspergillus* recovery. This fact may be explained through differing study groups: we evaluated data of adult CF patients only and did not compare them with data of CF children. *Aspergillus* colonization in this study was not gender-associated. In contrast to colonization with Pseudomonas species, which was found in our and other studies to be associated with worse lung disease, *Aspergillus* recovery did not relate to more advanced lung disease.

The most striking finding of this study was the significant relationship between prophylactic antibiotic therapy and *Aspergillus* colonization. This relationship was true for oral (P = 0·05) and inhaled antibiotics (P = 0·036) and their combination (P = 0·048). The use of prophylactic antibiotics is an established therapy to improve lung function and has been shown to reduce the frequency of exacerbations, to slow the progress of CF lung disease and to improve lung function (15,16,17). Until now, antibiotic therapy is believed to rarely induce side effects, and a majority of CF patients uses inhaled or oral antibiotics for many years. Our data may lead to concerns about the side effects of such therapy unknown thus far. To explain these findings, the following can be suggested: firstly, antibiotics can change the natural milieu of mucosal surfaces and therefore predispose to fungal infection. The increased prevalence of *Aspergillus* colonization as a side effect of a long-term antibiotic therapy of CF patients would not then be surprising, although not yet reported. Another possible explanation arises from the observation, that *Pseudomonas* and *Aspergillus* species function as antagonists, and *Pseudomonas* species have been reported to suppress fungal growth in culture (18). It is therefore tempting to postulate,

that complete or incomplete eradication of *Pseudomonas* in CF airways due to the inhalation of antibiotics could alter the counterbalance between *Pseudomonas* and *Aspergillus* species, and thus facilitate the fungal infection. Since the data about the association between *Aspergillus* colonization and more rapid lung disease are controversial (5,9, and our data) the clinical relevance of this observation remains unclear. Some *in vitro* data, however, suggest that *Aspergillus* causes damage to human respiratory epithelium (19). Nonetheless, the benefit of prophylactic therapy should not be questioned at this point. Further prospective studies have to confirm our results, since our retrospective analysis precluded randomization and 'confounding by indication' cannot be completely excluded.

In conclusion, our data indicate that prophylactic antibiotic therapy with inhaled and, possibly, oral antibiotics may predispose CF patients to *Aspergillus* colonization. Further observations are necessary to evaluate the clinical significance of this colonization in CF patients.

Acknowledgement

This study was supported by Mukoviszidose e. V. Bonn. We thank Dr Ackerman, Dept. of Biomathematics, for his help with the statistical analysis.

References

1. Jacquot J, Puchelle E, Hinnrasky J, *et al.* Localization of the cystic fibrosis transmembrane conductance regulator in airway secretory glands. *Eur Respir J* 1993; **6:** 169–176.
2. Haase G, Skopnik H, Groten T, Kusenbach G, Posselt HG. Long-term cultures from sputum of patients with cystic fibrosis. *Mycosis* 1991; **34:** 373–376.
3. Burns JL, Emerson J, Stapp JR, *et al.* Microbiology of sputum from patients at cystic fibrosis centers in the United States. *Clin Infect Dis* 1998; **27:** 158–163.
4. Zeaske R, Bruns WT, Fink JN *et al.* Immune response to *Aspergillus* in cystic fibrosis. *J Allergy Clin Immunol* 1988; **82:** 73–77.
5. Mroueh S, Spock A. Allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *Chest* 1994; **105:** 32–36.
6. Tobin MJ, Maguire O, Reen D, Tempany E, Fitzgerald MX. Atopy and bronchial reactivity in older patients with cystic fibrosis. *Thorax* 1980; **35:** 807–813.
7. Milla CE, Wielinksi CL, Regelmann WE. Clinical significance of the recovery of *Aspergillus* species from the respiratory secretions of cystic fibrosis patients. *Pediatr Pulmonol* 1996; **21:** 6–10.
8. Simmonds EJ, Littlewood JM, Hopewood V, Evans EG. *Aspergillus* fumigatus colonisation and population density of place of residence in cystic fibrosis. *Arch Dis Child* 1994; **70:** 139–140.
9. Wojnarowski C, Eichler I, Gartner C *et al.* Sensitization to *Aspergillus* fumigatus and lung function in children with cystic fibrosis. *Am J Respir Crit Care Med* 1997; **155:** 1902–1907.
10. Nicolai T, Arleth S, Spaeth A, Bertele-Harms R-M, Harms HK. Correlation of IgE antibody titer to *Aspergillus* fumigatus with decreased lung function in cystic fibrosis. *Pediatr Pulmonol* 1990; **8:** 12–15.
11. Schonheyder H, Jensen T, Hoiby N, Andersen P, Koch C. Frequency of *Aspergillus* fumigatus isolates and antibodies to *Aspergillus* antigens in cystic fibrosis. *Acta Pathol Microbiol Immunol Scand* 1985; **93:** 105–112.
12. Kommission für Verfahrensrichtlinien. Die mikrobiologische Diagnose von Infektionen der tieferen Atemwege und der Lunge. *Zbl Bak Hyg I Ab Orig* 1980; **248:** 162–176.
13. Burns JL, Emerson J, Stapp JR *et al.* Microbiology of sputum from patients at cystic fibrosis centers in the United States. *Clin Infect Dis* 1998; **27:** 158–163.
14. El Dahr JM, Fink R, Selden R, Arruda LK, Platts-Mills TA, Heymann PW. Development of immune responses to *Aspergillus* at an early age in children with cystic fibrosis. *Am J Respir Crit Care Med* 1994; **150:** 513–1518.
15. Ramsey BW, Dorkin HL, Eisenberg JD *et al.* Efficacy of aerosolized tobramycin in patients with cystic fibrosis. *N Engl J Med* 1993; **328:** 1740–1746.
16. Touw DJ, Brimicombe RW, Hodson ME, Heijerman HG, Bakker W. Inhalation of antibiotics in cystic fibrosis. *Eur Respir J* 1995; **8:** 1594–1604.
17. Ramsey BW, Pepe MS, Quan JM *et al.* Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. *N Engl J Med* 1999; **340:** 23–30.
18. Kerr J. Inhibition of fungal growth by Pseudomonas aeruginosa and Pseudomonas cepacia isolated from patients with cystic fibrosis. *J Infect* 1994; **28:** 305–310.
19. Amitani R, Murayama T, Nawada R., *et al. Aspergillus* culture filtrates and sputum sols from patients with pulmonary aspergillosis cause damage to human respiratory ciliated epithelium in vitro. *Eur Respir J* 1995; **8:** 1681–1687.