

Sensitization to *Aspergillus* and Allergic Bronchopulmonary Aspergillosis in a Cystic Fibrosis Population

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ABSTRACT

The prevalence of sensitization to *Aspergillus* and of allergic bronchopulmonary aspergillosis (ABPA) was studied in 222 patients with cystic fibrosis (CF) older than 6 years of age. Sensitization to *Aspergillus* was evaluated by both skin prick and intradermal testing. Signs of ABPA were considered: positive prick or intradermal test or both, total serum IgE (PRIST) levels above 2 SD of the mean for age, specific IgE (RAST) ≥ 0.7 PRU/ml, serum precipitating antibodies, peripheral eosinophilia ($>0.35 \times 10^9$ cells/L), and asthma. ABPA was considered *probable* if five criteria were achieved and *possible* if only four were present (always including a positive *Aspergillus* skin test). Of 222 patients, 124 (56%) were sensitized to *Aspergillus*, and they showed a worse clinical score and pulmonary function than did nonsensitized subjects. Fifty-two (23%) had ABPA without any substantial clinical difference (age, Chrispin-Norman chest x-ray score, clinical score, pulmonary function) between those with probable ($n = 27$) and those with possible ($n = 25$) ABPA. Subjects with ABPA had worse clinical score, radiologic score, and pulmonary function than those who were not sensitized to *Aspergillus*. These data point out the high prevalence of sensitization to *Aspergillus* and of ABPA in CF and their relationship with a worse clinical condition.

INTRODUCTION

ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA) is a hypersensitivity response to *Aspergillus fumigatus* (Af), a ubiquitous mold that can colonize the bronchial tree. ABPA has been observed frequently in patients with bronchial asthma and in patients with cystic fibrosis (CF).⁽¹⁻⁷⁾ Diagnosis of ABPA in CF mainly relies on immunologic evidence, in particular on the immediate cutaneous reactivity to Af and on the in vitro demonstration of specific serum IgE, IgG, and precipitating antibodies. Asthma and peripheral eosinophilia are important adjunctive criteria.⁽²⁻⁶⁾ Pulmonary infiltrates and proximal bronchiectasis on chest x-ray are commonly encountered in CF patients as a result of chronic bacterial infection and should be viewed with caution as selected criteria for diagnosing ABPA.^(4,6) Positive sputum cultures for Af and expectoration

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of brown mucous plugs are commonly considered secondary markers of the disease.⁽²⁻⁴⁾ It has been reported that as many as 10%–23% of CF patients have criteria suggestive of ABPA.⁽²⁻⁶⁾

In the present study, we evaluated the prevalence of sensitization to *Aspergillus* and of ABPA in a large CF population. To assess early stages of the disease, criteria of increasing probability of ABPA were adopted.⁽³⁾

PATIENTS AND METHODS

Two hundred twenty-two CF patients (111 male and 111 female) aged between 6 and 36 years (mean $\pm$ SD 16.1 $\pm$ 5.8 years) were prospectively examined in a cross-sectional study. They represented approximately one third of the total population regularly attending the CF Center of Verona. All inpatients and outpatients older than 6 years of age during an 8-month period were enrolled in the study. None of them had been treated with corticosteroids during the 6 months preceding the study. Diagnosis of CF was based on characteristic clinical findings⁽⁸⁾ and on at least two abnormal sweat tests (chloride concentration > 60 mEq/L by the Gibson-Cooke method).

Clinical Studies

All patients had physical examinations. To assess the clinical severity of CF, a modification of the Shwachman-Kulczycki (S-K) score,⁽⁹⁾ including the chest x-ray score, was used in each patient, a maximum of 100 points being allocated for the best clinical condition. Chest radiographs were evaluated according to the Chrispin-Norman (C-N) score.⁽¹⁰⁾ Standard pulmonary function studies, including forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV₁), and forced expiratory flow between 25% and 75% of FVC (FEF₂₅₋₇₅), were performed using a Bell spirometer (Biomedin, Padova, Italy). Reference values were from Polgar and Promadhat.⁽¹¹⁾ Patients were defined to have asthma if they reported episodes of wheezing in the past, if improvement of airway obstruction after inhalation of 0.2 mg salbutamol (increase of FEV₁ $> 15\%$ with respect to initial value) was demonstrated, or if both conditions existed.

Skin Tests

Skin prick tests were performed with standard technique in all patients, using 12 common allergen extracts (Lofarma, Milano, Italy), including 4 molds (*Aspergillus* mix, *Alternaria*, *Penicillium*, and *Candida*), mites (*Dermatophagoides pteronyssinus* and *D. farinae*), grass mix, parietaria, cat, dog, milk and egg, plus a saline control and histamine. Skin tests were evaluated 15 min after challenge. A positive test was considered to be a wheal greater than 2 $\times$ 2 mm over the saline control.⁽¹²⁾ Patients were considered atopic if they had three or more positive prick test reactions. Additionally, an intradermal skin test was performed in all patients with the standardized *Aspergillus* mix (*Aspergillus fumigatus*, *A. nidulans*, *A. niger*) and a saline control (Lofarma, Milano, Italy) by introducing 0.02 ml into the flexor surface of the forearm. The test was considered positive if there was a wheal greater than 5 $\times$ 5 mm.⁽¹³⁾

Immunologic Studies

Blood was obtained for peripheral eosinophil count and immunologic studies only in CF patients who had a positive *Aspergillus* skin test (either prick or intradermal).

Total eosinophil count was done by standard hemocytometer technique. A value $> 0.35 \times 10^9$ cells/L was regarded as abnormal.

Total serum IgE was evaluated by the Phadebas-PRIST technique (Pharmacia, Uppsala, Sweden). The upper IgE limit ($\bar{x} + 2$ SD) for age groups of 6–8 years, 8–10 years, and over 10 years were 160, 180, and 200 IU/ml, respectively.

The radioallergosorbant test (RAST) for Af-specific IgE was performed using the Phadebas RAST system from Pharmacia. Results were expressed in PRU/ml and scored as follows: + = 0.35–0.70 PRU/ml,

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++ = 0.70–3.50 PRU/ml, +++ = 3.50–17.5 PRU/ml, and ++++ = >17.5 PRU/ml. A result ≥ 0.7 PRU/ml was considered positive.

Precipitins to Af antigens (Lofarma) were detected by testing patient's serum in agar double diffusion (Ouchterlony technique). The presence of precipitins was considered abnormal.

Probable and Possible ABPA

Criteria for diagnosis of ABPA were similar to those of Feanny et al.⁽³⁾ They included positive *Aspergillus* skin tests (either prick or intradermal), total IgE level above 2 SD of the normal mean for age, Af RAST $\geq ++$, positive precipitins, peripheral eosinophilia ($>0.35 \times 10^9$ cells/l), and evidence of asthma. The positive skin test was necessary for the diagnosis of ABPA. If five criteria were present, the diagnosis of ABPA was considered probable, and if only four criteria were present, the diagnosis was considered possible.

Statistical Analysis

Chi-square contingency table analysis and Student's *t*-test were used. A probability level of 0.05 or less was considered statistically significant.

RESULTS

Aspergillus skin tests (either prick or intradermal) were positive in 124 (56%) of 222 patients (their clinical features are shown in Table 1), 81 had a positive prick test and 121 a positive intradermal test. Positive prick tests for other molds were found in 44 (20%) of 222 subjects (*Candida* in 9 patients, *Alternaria* in 20, and *Penicillium* in 22), without any statistical relationship with positive *Aspergillus* skin tests. Patients with positive *Aspergillus* skin tests were comparable for sex, mean age, and C-N score with negative subjects, although they had significantly worse S-K scores and FEV₁ and a higher prevalence of atopy (Table 1). Of the 124 patients sensitized to *Aspergillus*, 41 (33%) had asthma, 66 (53%) had high serum IgE, 74 (60%) had a positive RAST to Af, 41 (33%) had precipitins to Af, and 42 (34%) had peripheral eosinophilia.

According to previously accepted criteria,^(3,4) 27 (12.2%) patients had probable ABPA (five criteria including positive skin test) and 25 (11.2%) had possible ABPA (four criteria including positive skin test). Table 2 shows the clinical features and the results of immunologic studies of patients with probable and possible ABPA. The main clinical features (age, S-K score, C-N score, and FEV₁) were statistically

TABLE 1. CLINICAL FEATURES OF 124 PATIENTS POSITIVE AND 98 NEGATIVE TO ASPERGILLUS SKIN TESTS^a

	Positive	Negative	
Gender (M:F)	61:63	50:48	NS ^b
Age (years) ($\bar{x}$) ($\pm$ SD)	15.9 (6.2)	16.3 (5.5)	NS
S-K score ^c ($\bar{x}$) ($\pm$ SD)	68.8 (12.5)	72.7 (12.1)	*
C-N score ^d ($\bar{x}$) ($\pm$ SD)	13.5 (4.8)	12.3 (4.8)	NS
FEV ₁ ^e (% pred.) ($\bar{x}$) ($\pm$ SD)	63.2 (22.1)	71.6 (28.6)	*
Atopy (No. of patients)	27	10	*

^aPrick test, intradermal test, or both.

^bNS, not significant.

^cS-K score, Shwachman-Kulczycki score.

^dC-N score, Chrispin-Norman x-ray score.

^eFEV₁, forced expiratory volume in 1 sec.

**p* < 0.05.

TABLE 2. CLINICAL FEATURES OF 27 PATIENTS WITH PROBABLE AND 25 WITH POSSIBLE ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA)^a

	<i>Probable ABPA</i>	<i>Possible ABPA</i>	
Gender (M:F)	12:15	15:10	NS ^b
Age (years) ($\bar{x}$) ($\pm$ SD)	14.8 (5.3)	18.1 (6.7)	NS
S-K score ^c ($\bar{x}$) ($\pm$ SD)	68.9 (10.7)	65.8 (11.9)	NS
C-N score ^d ($\bar{x}$) ($\pm$ SD)	12.9 (3.5)	15.0 (4.2)	NS
FEV ₁ ^e (% pred.) ($\bar{x}$) ($\pm$ SD)	61.7 (21)	58.0 (20.3)	NS
IgE (IU/ml) ($\bar{x}$) ($\pm$ SD)	1129 (953)	692 (571)	NS
Positive Af ^f RAST (No. of patients)	27	20	*
Positive Af precipitins (No. of patients)	20	9	**
Peripheral eosinophils ($\times 10^9$ cells/l) ($\bar{x}$) ($\pm$ SD)	0.55 (0.23)	0.32 (0.19)	***
Asthma (No. of patients)	19	11	NS

^a Criteria for ABPA: all patients had a positive *Aspergillus* skin test. Probable ABPA had five positive criteria, and possible ABPA had four positive criteria, including positive *Aspergillus* skin test, total IgE above 2 SD of mean for age, positive *Aspergillus* RAST, positive *Aspergillus* precipitins, peripheral eosinophilia, and asthma.

^b NS, not significant.

^c S-K score, Shwachman-Kulczycki score.

^d C-N score, Chrispin-Norman x-ray score.

^e FEV₁, forced expiratory volume in 1 sec.

^f Af, *Aspergillus fumigatus*.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

TABLE 3. CLINICAL FEATURES (MEAN $\pm$ SD) OF 52 PATIENTS WITH ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA), 72 SENSITIZED TO ASPERGILLOS AND 98 NEGATIVE TO ASPERGILLOS SKIN TESTS^a

	<i>ABPA^{b,c}</i>	<i>Sensitized</i>	<i>Negative</i>
Age (years)	16.4 (6.3)	16.1 (5)	16.3 (5.5)
S-K score	67.4 (11.4)	69.8 (13.3)	72.7 (12.1)
C-N score	13.9 (4.0)	13.2 (5.4)	12.3 (4.8)
FEV ₁ (% pred.)	59.9 (20.7)	65.6 (22.8)	71.6 (28.6)

^a See footnote^a of Table 2.

^b ABPA vs. Negative: Age, p = not significant; S-K score, p = 0.01; C-N score, p = 0.04; FEV₁, p = 0.01.

^c ABPA vs Sensitized and Sensitized vs. Negative: p = not significant for each variable considered.

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comparable in the two groups. Therefore, the 52 patients with ABPA (probable and possible ABPA), considered as a whole, were compared with the remaining 72 sensitized and the 98 nonsensitized subjects (Table 3). Patients with ABPA had significantly worse S-K score, C-N score, and FEV₁ than nonsensitized subjects. No difference was noted between patients with ABPA and those with intermediate degrees of sensitization and between the latter and nonsensitized patients.

DISCUSSION

The need for early diagnosis and treatment of ABPA in CF to prevent irreversible lung disease due to immunologic reaction to *Aspergillus* has been stressed previously by several authors.^(3,4,6,14-16) In the present study, we identified—using widely accepted diagnostic criteria⁽²⁻⁴⁾—a group of CF patients with the highest probability to have or to develop ABPA. Particular attention was paid to immunologic evidence of host response to *Aspergillus* and, among clinical markers, to the presence of asthma. Minor diagnostic criteria (such as positive sputum culture for *Aspergillus*, expectoration of brown plugs or flacks, and late skin reactivity to *Aspergillus*) were ignored. The radiologic evidence of lung infiltrates or central bronchiectasis was considered to be of limited value in early ABPA diagnosis in CF, especially in a cross-sectional study.^(4,6)

Fifty-six percent of our patients were sensitized to *Aspergillus* antigens. This prevalence was higher than that obtained using similar criteria by Feanny et al. (31%),⁽³⁾ although it is in good agreement with data of Laufer et al. (53%)⁽⁴⁾ and Zeaske et al. (58.6%).⁽⁵⁾ In our experience a positive *Aspergillus* skin test in patients over 6 years was unrelated to age, and it was associated with a worse clinical and respiratory condition and with an increased prevalence of atopy. Patients with ABPA (probable and possible) represented 23% of the population studied. Again, this prevalence was higher than the 10% previously reported by Feanny et al.⁽³⁾ but in agreement with the 23% observed by Zeaske et al.⁽⁵⁾

Some considerations could be given to these sometimes contrasting data on ABPA in CF. First, ABPA is a polymorphic disease, and a variable pattern of clinical, radiologic, and immunologic criteria has been considered in different studies to achieve the diagnosis. Moreover, the specificity of immunologic assays to detect *Aspergillus*-specific antibodies has been questioned.⁽¹⁷⁾ Available extracts of Af are complex mixtures of cross-reacting antigens, and identification of specific major antigenic components is still a matter of research.⁽¹⁸⁾ Second, an intermediate group of patients with different degrees of sensitization to *Aspergillus* has been considered.^(3,4) Poor homogeneity of this group in various studies may have influenced the results. Other possibly influencing factors, such as differences in populations studied (clinical status, microbial colonization in the lungs, severity of pulmonary damage), in therapeutic strategies adopted (systemic or nebulized antibiotics, antifungal agents, corticosteroids, prolonged ultrasonic nebulization), or in environmental conditions (climate) should be taken into account.⁽¹⁹⁻²¹⁾

We did not observe any significant clinical difference between subjects with probable and possible ABPA. When considered as a whole, however, these subjects showed a significantly worse clinical condition, radiologic score, and pulmonary function than those who had negative skin tests to *Aspergillus*. The cause-effect relationship between immune response to *Aspergillus* and lung damage cannot be demonstrated by this study. Nevertheless, it was suggested that besides full-fledged forms,^(22,23) milder degrees of hypersensitivity to *Aspergillus* may affect pulmonary function in CF.⁽¹⁴⁾ The matter of debate, in our opinion, is if early treatment with corticosteroids, before the appearance of overt clinical and radiologic signs of acute ABPA, can prevent further lung damage. Hutcheson et al.⁽²⁴⁾ showed that serologic markers of sensitization to *Aspergillus* in CF may spontaneously vary at any time, and they stressed that systemic corticosteroids should not be prescribed solely on the basis of immunologic evidence of disease. Moreover, side effects due to long-term systemic corticosteroids should suggest a careful cost-benefit evaluation.⁽²⁵⁾

In conclusion, this study indicated that in a large CF population older than 6 years, more than half the patients were sensitized to *Aspergillus* and that about one fourth were at high risk of ABPA. These subjects showed a worse clinical status than did nonsensitized patients. A screening and follow-up program of both intermediate and advanced degrees of hypersensitivity to *Aspergillus*—before the appearance of acute ABPA—should lead to early diagnosis and gain further insight into the natural history of the disease.

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