Aspergillus/Allergic Bronchopulmonary Aspergillosis in an Irish Cystic Fibrosis Population: A Diagnostically Challenging Entity

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BACKGROUND: Patients with cystic fibrosis (CF) can become colonized by aspergillus, which can act as an allergen and cause allergic bronchopulmonary aspergillosis (ABPA). OBJECTIVE: To determine the rate of aspergillus colonization and ABPA in a population of Irish patients with CF. METHODS: In 50 consecutive patients with CF who presented with exacerbations, we looked for the presence of aspergillus in their sputum and signs and symptoms of ABPA. RESULTS: Fifteen patients (30%) grew aspergillus species in their sputum cultures. Six patients (12%) had ABPA. Matched for age, sex, genotype, and microbiology, there was no significant difference in forced expiratory volume in the first second (percent predicted, FEV_1%) in subjects with aspergillus-positive sputum compared to those not colonized with aspergillus. Subjects with ABPA experienced sharp short-term deterioration in lung function (mean 6.7% predicted FEV_1), which returned to baseline following at least 4 weeks of treatment. CONCLUSIONS: The prevalence of ABPA was 12%. Aspergillus-positive sputum of itself was not a poor prognostic sign in terms of lung function over the 5-year study course. ABPA produces short-term reversible declines in lung function and responds to treatment. The frequency of aspergillus isolates did not correlate with the occurrence of ABPA. A low threshold for the diagnosis of ABPA should be maintained in any patient with CF who does not improve with antibiotics. *Key words: cystic fibrosis*, *aspergillus*, *aspergillosis*, *lung function*, *ABPA*, *CF*. [Respir Care 2008;53(8):1035–1041. © 2008 Daedalus Enterprises]

Introduction

Patients with cystic fibrosis (CF) can become chronically colonized by a multitude of organisms, of which the genus *Aspergillus* can be particularly troublesome. Aspergillus is thermo-tolerant, resilient, and ubiquitous; its pulmonary manifestations in CF are complex and poorly understood; it is a diagnostic challenge; and it causes a spectrum of disease, from saphrophytic infestation to invasive (life-threatening) and allergic disease (including hypersensitivity pneumonitis). When it acts as an allergen, aspergillus can induce a hypersensitivity reaction in the CF lung, leading to allergic bronchopulmonary aspergillosis (ABPA). Although *A. fumigatus* has been implicated in the majority of cases, allergic bronchopulmonary fungosis may also occur in association with other fungi (*Stemphylium lanuginosum*, *Helminthosporium* species, *Candida* species, *Curvularia* species, *Schizophyllum* commune, *Dreschslera hawaiiensis*, *Fusarium vasinfectum*) and other species of aspergillus (*A. niger*, *A. flavus*, *A. nidulans*, *A. orizae*, or *A. glaucis*).[1-3] Ireland has the highest prevalence of asthma in Europe, and the highest incidence of CF in the world, which makes the Irish population an interesting one in which to study ABPA. CF remains the most common life-threatening genetically inherited disease; it affects 1 in 1,400 live births. This is the first study to evaluate the rate of aspergillus colonization and

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ABPA during CF exacerbations in an Irish population at a major tertiary referral center.

Methods

Between January 2001 and December 2005 we consecutively analyzed 50 adults with CF exacerbation in our tertiary referral center, for the presence and frequency of aspergillus in their sputum cultures. A pulmonary exacerbation was defined as the presence of at least 4 of the following: change in sputum production, new or increased hemoptysis, increased coughing, increased dyspnea, increased fatigue or lethargy, fever $>$ 38° C (measured orally), increased chest discomfort, anorexia or weight loss ($>$ 5% of ideal body weight in 2 weeks), $\geq$ 10% reduction in forced expiratory volume in the first second (FEV_1) or forced vital capacity, new findings on chest radiograph, or new findings on chest auscultation.[4] All individuals with CF who presented with exacerbation in the period January 2001 through December 2005 had their sputum evaluated for aspergillus species at presentation or in the following 12 months. Sputum samples were sent at 3-month intervals (4 samples annually) from each patient. Aspergillus species were isolated in the laboratory. Sputum loops were streaked over a plate of Sabouraud's dextrose agar, which was incubated at 37° C. All patients were evaluated for ABPA at the time of sputum positivity and at 6-month intervals (twice annually) thereafter. ABPA was defined by criteria set out by the CF consensus conference on ABPA in CF.[5] The diagnostic criteria were:

- Acute or subacute clinical deterioration (cough, wheeze, exercise intolerance, exercise-induced asthma, decline in pulmonary function, increased sputum) not attributable to another etiology
- Total serum immunoglobulin E (IgE) $>$ 1,200 ng/mL (if total IgE was 480–1,200 ng/mL, we repeated the testing in 1–3 months)
- Immediate skin-test reactivity to aspergillus or in vitro presence of anti-aspergillus IgE antibodies
- And one of: serum precipitins or IgG antibody to *A. fumigatus*, or new or recent abnormalities on chest radiograph (infiltrates or mucus plugging), or chest computed tomography [CT] (bronchiectasis) that did not clear with antibiotics and physiotherapy

The exclusion criteria were steroid use within the year prior to this presentation, and any prior aspergillus colonization. Routine screening for ABPA is performed twice annually on all patients, via measurement of serum IgE (irrespective of sputum culture results), but only sputum-positive patients were selected and included in this study. The patients with aspergillus-positive sputum were matched for age, sex, genotype, microbiology, and disease severity to a non-aspergillus group; lung function (FEV_1 % predicted) was measured via spirometry, and the 2 groups were compared. A Wilcoxon test was applied to the data, with statistics software (Prism 4.0, GraphPad Software, San Diego, California).

Results

Fifteen patients (30%) grew aspergillus species in their sputum culture on at least one occasion. Ten (66.7%) of those 15 grew aspergillus on more than one occasion, and 2 (13.3%) of those had positive sputum cultures on $\geq$ 10 occasions (Table 1). All the patients grew *A. fumigatus* on at least one occasion, and *A. fumigatus* was the most common aspergillus species grown. Other aspergillus species (*A. flavus*, *A. terreus*, *A. versicolor*, and *A. niger*) occurred in 4 patients, either alone or in combination with *A. fumigatus*.

Six (12%) of the 50 patients screened had ABPA. Six (40%) of the 15 patients with aspergillus-positive sputum had ABPA per consensus conference criteria. Of the 6 with ABPA, 3 had aspergillus-positive sputum at diagnosis. The remaining 3 did not have aspergillus in the sputum at initial diagnosis but subsequently were found to have aspergillus-positive sputum cultures. Thus, all the patients diagnosed with ABPA in the study grew aspergillus in the sputum at some point in the study period. Only sputum-positive patients were considered in this study; however, in our CF population there were instances of "questionable ABPA" that were deemed sputum-negative (and did not meet consensus conference criteria) and were thus excluded. Such cases included exacerbations (with clinical and radiological deterioration) that despite negative sputum cultures did not respond to antibiotics but did respond to a course of corticosteroids, suggested by clinical, radiological, and pulmonary function variables. In such cases, their IgE remained between 300 ng/mL and 1,000 ng/mL, which is far below the accepted range in the consensus conference criteria. Such individuals were diagnosed as having ABPA but did not meet current diagnostic guidelines.

In 4 of the 6 patients diagnosed with ABPA, the serum IgE rose above baseline (range 511–4337 ng/mL), but in all cases considered for this study, consensus conference diagnostic guidelines were met. Furthermore, 4 patients who were initially treated with antibiotics for infective exacerbations had no improvement until administration of corticosteroids. In the remaining 2 patients (notably 1 patient who had 5 ABPA episodes), diagnostic suspicion at presentation was high, so corticosteroids were commenced as initial treatment.

Chest radiograph, although performed in all patients in this study, is rarely useful in the diagnosis of CF ABPA, so those findings were excluded from this study (it is men-

Table 1. Sputum Culture Results

Patient Number	Number of Times Aspergillus Was Isolated in Sputum	*Aspergillus* Species Isolated	Number of Episodes of Allergic Bronchopulmonary Aspergillosis
1	3	*A. fumigatus*	0
2	4	*A. fumigatus*	0
3	1	*A. fumigatus*	1
4	1	*A. fumigatus*	0
5	3	*A. fumigatus*	1
6	5	*A. fumigatus* *A. flavus*	0
7	4	*A. fumigatus* *A. terreus* *A. versicolor*	0
8	1	*A. fumigatus*	1
9	2	*A. niger* *A. fumigatus*	0
10	1	*A. fumigatus*	0
11	2	*A. fumigatus*	0
12	10	*A. fumigatus*	5
13	30	*A. fumigatus*	0
14	1	*A. fumigatus*	1
15	4	*A. fumigatus* *A. niger*	1

tioned as a sub-point within the consensus conference criteria). There is substantial inter-observer variation in the detection of new infiltrates against a background of chronic lung destruction, damage, and fibrosis, so although new infiltrates were detected in 2 of our 6 cases of ABPA, we consider chest radiograph findings of limited value in making a diagnosis of CF ABPA, unless in the experienced hands of a radiologist who subspecializes in CF ABPA. This is an important point for practicing clinicians and a weakness of the available diagnostic criteria.

Patients with ABPA were treated with a 40-mg tapering dose of oral prednisolone. One individual was also given the anti-fungal agent itraconazole (400 mg once a day for 6 weeks). The patient treated with itraconazole experienced only one episode of ABPA, and grew aspergillus in sputum culture on that occasion. None of the patients with recurrent ABPA were treated with itraconazole. The duration of corticosteroid treatment was 4–8 weeks, depending on clinical response (improvement in pulmonary symptoms or pulmonary function test results), biological response (IgE returned to baseline), and radiological response (clearing of new infiltrates on CT). Inhaled bronchodilator was used adjunctively in one patient, who reported symptom improvement. All 6 patients with ABPA improved following corticosteroid treatment; they returned to their baseline pulmonary function 4–12 weeks after their ABPA episodes.

The aspergillus-positive-sputum group (mean age 23.7 y) was matched to an aspergillus-negative-sputum group (mean age 23.9 y). Each group included 10 males and 5 females, 12 patients with ΔF508 mutations, and 3 with R117H mutations. These groups had identical numbers (12) of *Pseudomonas aeruginosa* colonizers, but 3 in the aspergillus-positive group were also colonized with *Stenotrophomonas maltophilia*, whereas only 2 in the non-aspergillus group were dual-colonized. No staphylococcus colonization was detected in either group. The 2 groups were comparable in disease course and severity; their average number of exacerbations in the year preceding the study was almost equal: 2.54 versus 2.32, respectively.

There was no significant difference in percent of predicted FEV_1 between the aspergillus-positive group and the similar aspergillus-negative group. However, there was a trend toward lower lung-function abnormalities in those with aspergillus-positive sputum. The analysis between the 2 groups was performed on first appearance of aspergillus in the sputum. The mean percent predicted FEV_1 in the aspergillus group was 50.2% (standard error 6.95) versus 67.5% (standard error 6.76) in a matched non-aspergillus group ($P = .09$). Subjects with ABPA experienced sharp short-term deterioration in lung function (mean FEV_1 6.7% predicted), which returned to baseline following at least 4 weeks of treatment with systemic corticosteroids. Each of the 6 patients who experienced ABPA demonstrated reversibility (to differing extents) in their lung function following treatment (Fig. 1).

Discussion

This descriptive study of an Irish CF population analyzed sputum cultures at initial presentation for exacerbation and then for a 1-year period thereafter for aspergillus and ABPA. Identification of aspergillus in sputum from an individual with ABPA does not necessarily mean that the fungus is implicated in disease. In fact, aspergillus is detected in only approximately 50% of ABPA patients.[6] It therefore remains mainly a clinical diagnosis, an under-recognized entity, and must always be considered even if sputum culture yields no growth. Furthermore, *P. aeruginosa* may inhibit the growth of aspergillus and therefore explain the high percentage of negative aspergillus cultures in CF ABPA.

Nearly one third of our patients had aspergillus in their sputum on at least one occasion. There was a trend toward declining lung function in those with aspergillus-positive sputum. The incidence of ABPA in the young adult CF population was reported by Nepomuceno et al[7] as approximately 9%, which is similar to the 12% at our center, but the literature has reported a wide range in the incidence of CF ABPA,[8-10] which reflects the different populations studied. ABPA produces short-term reversible de-

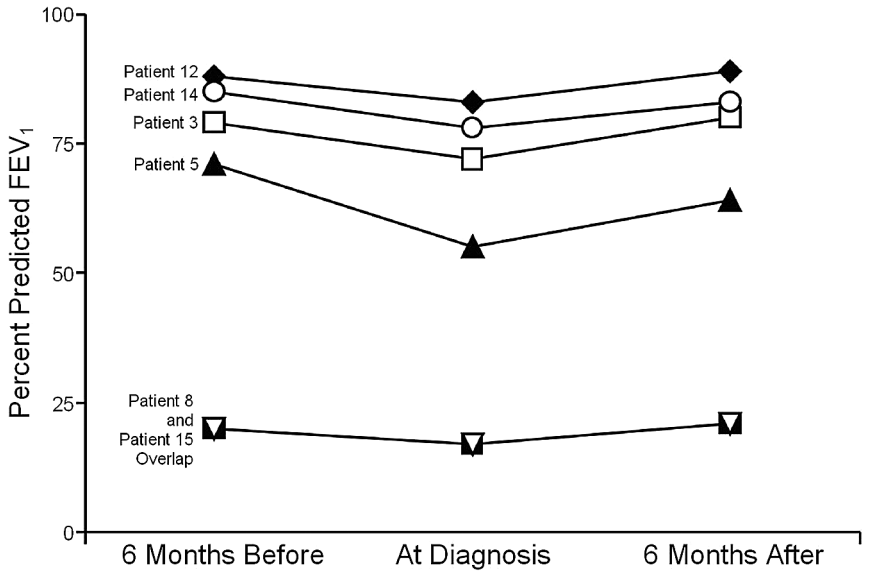

Fig. 1. Response in forced expiratory volume in the first second to treatment of allergic bronchopulmonary aspergillosis.

cline in lung function. The frequency of aspergillus isolates did not correlate with the occurrence of ABPA.

Although our study number was small, it illustrates the diagnostic difficulty of CF ABPA. Patients who had aspergillus-positive sputum within 1 year of presenting with an ABPA exacerbation were considered in this study, but we encountered instances of sputum-negative "questionable ABPA" (did not meet consensus conference criteria) during the study and excluded those from the study.

The factors that underlie the development of ABPA remain unclear, which makes the identification of at-risk individuals difficult, and most often retrospective, by which time lung destruction has already occurred. One of the risk factors for ABPA is genetic[11,12] (pre-activation of airway epithelial cells, such as in asthma or CF). The extent to which this activation facilitates development of aspergillus spores into hyphae, bronchial penetration of aspergillus, the immune response, and bronchial inflammation and destruction remains poorly understood. Another independent risk factor is mucus quality;[13] thick, viscid mucus associated with impaired clearance in the CF airways allows easier aspergillus colonization. Patients with ABPA but without CF have higher frequencies of the cystic fibrosis transmembrane regulator mutation,[14] which make that mutation itself an independent risk factor.

The high incidence and severity of bacterial infection in CF leads to more use of antibiotics, which has been suggested to "pave the way" for fungal infection.[15] The severity of an ABPA episode depends on the quantity and virulence of the inhaled organism,[5] which explains why some individuals may develop more ABPA episodes with fewer positive sputum cultures, as we saw in our population. The effects of long-term exposure to aspergillus spores in the environment are still unclear. Recent studies of specific IgE against recombinant antigens of *A. fumigatus* have contributed to earlier diagnosis of ABPA, with high sensitivity and specificity, and described a new group of patients: aspergillus-sensitized without ABPA. Nevertheless, before that method can be used for diagnosis, a longitudinal study with a larger number of patients is required.[16]

Generally, it is stated that IgE to recombinant *A. fumigatus* allergens rAspf1 and rAspf3, are markers for sensitization, and that rAspf4 and rAspf6 indicate serologic ABPA. Used in conjunction with the consensus criteria, a larger number of ABPA sufferers may be detected. Ritz et al[17] assessed risk factors associated with ABPA and aspergillus sensitization and found that patients with CF and ABPA had more frequent *S. maltophilia* in sputum than did controls. Furthermore, longer duration of *P. aeruginosa* colonization and higher cumulative dose of inhaled corticosteroids were found to be risk factors for *A. fumigatus* sensitization.

ABPA classically affects patients with persistent asthma and most work involving diagnostic criteria has been done in such cases. Table 2 shows the classic criteria for the diagnosis of ABPA.[18-20]

Based on the criteria in Table 2, the diagnosis of CF ABPA is difficult and often delayed because several of the diagnostic criteria overlap with common manifestations of CF (bronchial obstruction, pulmonary infiltrates, and bronchiectasis occur routinely in CF, because of recurrent and chronic bacterial infection). Patients with CF also have immune responses to aspergillus (IgE, IgA, and IgG antibody production and elevated total serum IgE) in the ab-

Table 2. Classic Criteria for the Diagnosis of Allergic Bronchopulmonary Aspergillosis in Patients With Persistent Asthma

Asthma
Chest infiltrates on radiograph or CT
Immediate cutaneous reactivity to *Aspergillus* species
Elevated total serum IgE (> 1,000 ng/mL)
Serum precipitating antibodies to *A. fumigatus*
Central bronchiectasis on chest CT
Peripheral blood eosinophilia
Elevated serum IgE and/or IgG to *A. fumigatus*

CT = computed tomogram
IgE = immunoglobulin E

sence of ABPA, which makes it difficult to define the separating boundary from ABPA. It is noteworthy that a staging system has been used for patients with asthma and ABPA,[21] although this is not often applied to patients with ABPA and CF.

Clinical suspicion should always supersede any laboratory or radiologic test, and the workup in any suspected case of ABPA in CF should include skin, serologic, radiologic, and sputum tests.

Cutaneous Skin Test for Aspergillus

The cutaneous reaction (wheal > 3 mm) to *A. fumigatus* antigen is a good tool to support a diagnosis of ABPA.[22,23] The antigen is subcutaneously injected into the patient's forearm, and any reactions (namely, immediate [type I] or late [type III]) at 48–72 hours are noted. A person who is allergic to aspergillus will experience swelling, itching, and reddening of the injection site, usually within 20 min, but the skin reaction may take as long as 8 hours to develop in some people. For the wheal of immediate skin sensitivity some authorities have suggested that a diameter of > 4 mm should be considered a positive result, whereas others have stated that it should be > 3 mm with surrounding erythema, Reactions may be found in up to a third of CF patients without ABPA,[24] which again confirms that the results have to be interpreted in the context of the clinical setting and concomitant symptoms and together with other diagnostic tests for ABPA.

Total Serum IgE and Serum *A. fumigatus* Antibody (IgE/IgG)

Total IgE is an easily accessible test to most clinicians and is valuable for diagnosing ABPA in CF;[22] however, the value varies with age. Various diagnostic cut-offs have been suggested, including total IgE > 500 IU/mL,[25] and even > 1,000 IU/mL.[10] Like other diagnostic tools in CF ABPA, IgE and IgG have to be interpreted along with the constellation of other clinical and laboratory variables to reach a diagnosis.

Specific Antibodies

The presence of specific antibodies (via serum measurement) is a sensitive indicator of ABPA in CF.[22] Such antibodies are generally of the IgG isotype (in particular the IgG1, IgG2, and IgG4 subclasses). There have been reports of specific IgA and IgM antibodies, but these are not routinely used in CF. In CF, IgE and IgG are the subtypes most markedly elevated. In addition, the IgG isotype pattern described above is very suggestive of a diagnosis of ABPA in CF[26] and may be of great value to the clinician. The availability of these tests may be restricted at some centers.

Serum *A. fumigatus* Antibody Precipitins

Precipitating antibodies are antibodies specific to aspergillus that bind with the spores and form tiny solids (precipitates) that can be detected. These are a sensitive marker in CF ABPA. However, although they are a key diagnostic feature of CF ABPA in the consensus criteria, they have been found in CF patients without ABPA. Furthermore, of importance to the clinician, the levels of these antibodies fluctuate over time, which complicates their use in diagnosis.[22]

Chest Radiograph

Although use of chest radiography has been advocated in the diagnosis of ABPA, its use is limited in CF, as compared to patients with asthma who (between exacerbations) would be expected to have relatively clear radiographs. This is in stark contrast to the status quo CF chest radiograph, which shows substantial lung damage, especially in the later stages, which makes it difficult to detect a new infiltrate and thus fulfill the stated diagnostic criteria. We believe that in advanced CF chest radiography has a very limited—if any—role in the diagnosis of ABPA.

High-Resolution CT

High-resolution CT is much more useful than radiography, but inter-observer differences in interpretation must be considered. Though a radiology opinion from an expert in aspergillus lung disease is useful, such expertise is not readily available in some centers. Transient findings may include pulmonary infiltrates, presence of fluid/inflammation in bronchi ("ring sign"), and lobar/segmental collapse linked to mucus plugs ("glove finger appearance"). Permanent patterns include bronchiectasis (most frequently

in the upper lobes, but also more centrally, which suggests ABPA, evidenced by "parallel lines" or "tram tracking"). But, again, even in the baseline CF chest CT, such findings may occur because of recurrent bacterial infections, and only an experienced eye can differentiate this from ABPA.

Pulmonary Function Testing

Pulmonary function tests are helpful in identifying reversibility and response to treatment, so they are most useful once a diagnosis has been reached. Deterioration in lung function differs among ABPA patients; in some, lung function remains stable, whereas others have progressive deterioration in FEV_1, forced vital capacity, functional residual capacity, and occasionally diffusion capacity. It is suggested that co-existent pseudomonas infection contributes to a more marked decline in lung function during an ABPA episode,[27] so it is useful to document most current colonizers prior to interpreting lung function test results.

Sputum Test

Sputum testing is not essential, because ABPA may be present despite negative sputum. However, sputum specimens are an important screening tool for the presence of aspergillus and thus the consideration of the diagnosis in a patient who is not improving with antibiotics. Sputum specimens are also useful to identifying the concurrent microbiology in the airways, which may aid in the interpretation of the CF pulmonary function testing. Sputum cultures are inexpensive and the most readily available method in an out-patient setting, and their value should not be underestimated, despite the possibility of sputum-negative ABPA.

The diagnosis of CF ABPA remains a clinical one, because of the recognized weaknesses in the available consensus criteria. Table 3 shows our advocated criteria for the diagnosis of CF ABPA.

One of the major difficulties with ABPA in the context of CF is illustrated by a survey of 58 CF centers, by Cunningham et al,[28] which found marked non-conformity with diagnostic and treatment approaches in 45 of the centers. That finding highlights the continuing difficulty of diagnosing and treating CF ABPA, and it also probably explains the differences in the literature about the incidence of CF ABPA. We believe the diagnosis should remain clinical, with consensus criteria and diagnostic test results used to back up the diagnosis in individual cases. Furthermore, clinicians should maintain a high suspicion for ABPA in patients $>$ 6 years of age, in whom other allergic conditions are less commonly observed. Biannual total serum IgE concentration is another useful screening tool. If the concentration is $>$ 500 IU/mL, clinicians should proceed to skin and serology testing. However, if the IgE concentration is 200–500 IU/mL, the consensus criteria recommend repeating the test immediately if there is a high clinical index of suspicion (disease exacerbation), or at 3–6 months if the patient remains asymptomatic.

Table 3. Suggested Criteria for the Diagnosis of Allergic Bronchopulmonary Aspergillosis in Patients With Cystic Fibrosis

Two of the following 3 criteria:
Immediate cutaneous reactivity
Presence of precipitating antibodies
Total serum IgE $>$ 1,000 IU/mL
Plus at least 2 of the following 6 criteria:
Bronchoconstriction
Peripheral blood eosinophilia
History of new pulmonary infiltrates
Raised serum anti-aspergillus IgE/IgG
Positive sputum culture
Response to steroids

IgE = immunoglobulin E

The long-term prognosis of ABPA is usually good. Most patients maintain adequate respiratory status. Detecting exacerbations secondary to aspergillus is crucial in preventing further airway destruction in an already fragile CF lung. Treatment requires corticosteroids to treat inflammatory response and improve symptoms, and occasionally anti-fungal agents should be used to suppress the proliferation of *A. fumigatus* and limit bronchial inflammation.[29] All the patients diagnosed with ABPA in this study were treated. Corticosteroid treatment (0.5 mg/kg/d) for the first 2 weeks, followed by a decreasing dose over the following 6–8 weeks is recommended, but the exact duration of therapy is still debated. Several anti-fungal agents (amphotericin B, ketoconazole, clitromazole, nystatin, and natamycin) have been proposed as treatments for ABPA, but no significant benefit was observed in tests, and in many cases these agents had adverse effects.[30-32] In contrast, orally administered itraconazole (200–400 mg/d $\times$ 16 wk) is an effective adjunctive therapy for ABPA.[33] Itraconazole decreases the corticosteroid requirement and exacerbations and halts disease progression without toxic effects. It has also been suggested that it has anti-inflammatory properties similar to azithromycin, but the mechanisms of that are poorly understood.[29] Adrenal suppression and resistance due to long-term use are the only potential concerns. Pulse intravenous methylprednisolone has been used with some success in cases of resistant CF ABPA.[34]

Conclusions

The diagnosis and treatment of ABPA in CF is a substantial challenge in clinical practice, despite consensus guidelines and recent developments. Many controversies

persist regarding diagnostic criteria, and data concerning the best treatment and patient follow-up remain scarce. Case reports of life-threatening ABPA in CF[35] remind us of the need to consider a diagnosis of aspergillus/ABPA, even in the most atypical of presentations in the setting of CF.

REFERENCES

1. Lake FR, Tribe AE, Mcaleer R, Froudist J, Thompson PJ. Mixed allergic bronchopulmonary fungal disease due to *Pseudallescheria boydii* and *Aspergillus.* Thorax 1990;45(6):489-491.
2. Crompton G. Fungal Disease. Bronchopulmonary aspergillosis. In: Brewis RAL, Gibson GJ, Geddes DM, editors. Respiratory medicine. London: Bailliere Tindal; 1990:1035-1050.
3. Greenberger PA. Diagnosis and management of allergic bronchopulmonary aspergillosis. Allergy Proc 1994;15(6):335-339.
4. Wilmott RW, Amin RS, Colin AA, DeVault A, Dozor AJ, Eigen H, et al. Aerosolized recombinant human DNase in hospitalized cystic fibrosis patients with acute pulmonary exacerbations. Am J Respir Crit Care Med 1996;153(6 Pt 1):1914-1917.
5. Stevens DA, Moss RB, Kurup VP, Knutsen AP, Greenberger P, Judson MA, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis–state of the art: Cystic Fibrosis Foundation Consensus Conference. Clin Infect Dis 2003;37(Suppl 3):S225-S264. Erratum in: Clin Infect Dis 2004;38(1):158.
6. McCarthy DS, Pepys J. Allergic bronchopulmonary aspergillosis. Clinical Immunology I. Clinical features. Clin Allergy 1971;1:261-286.
7. Nepomuceno IB, Esrig S, Moss RB. Allergic bronchopulmonary aspergillosis in cystic fibrosis. Chest 1999;115(2):364-370.
8. Skov M, McKay K, Koch C, Cooper PJ. Prevalence of allergic bronchopulmonary aspergillosis in cystic fibrosis in an area with a high frequency of atopy. Respir Med 2005;99(7):887-893.
9. Moss RB. Allergic bronchopulmonary aspergillosis. Clin Rev Allergy Immunol 2002;23(1):87-104.
10. Mastella G, Rainisio M, Harms HK, Hodson ME, Koch C, Navarro J, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis. A European epidemiological study. Eur Respir J 2000;16(3):464-471.
11. Chauhan B, Knutsen AP, Hutcheson PS, Slavin RG, Bellone CJ. T cell subsets, epitope mapping, and HLA-restriction in patients with allergic bronchopulmonary aspergillosis. J Clin Invest 1996;97(10):2324-2331.
12. Chauhan B, Santiago L, Kirschmann DA, Hauptfeld V, Knutsen AP, Hutcheson PS, et al. The association of HLA-DR alleles and T-cell activation with allergic bronchopulmonary aspergillosis. J Immunol 1997;159(8):4072-4076.
13. Knutsen A, Bellone CJ, Kauffman HF. Immunopathogenesis of allergic bronchopulmonary aspergillosis in cystic fibrosis. J Cystic Fibrosis 2002;1(2):76-89.
14. Miller PW, Hamosh A, Macej M Jr, Greenberger PA, MacLean J, Walden SM, et al. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in allergic bronchopulmonary aspergillosis. Am J Hum Genet 1996;59(1):45-51.
15. Burns JL, Van Dalfsen JM, Shawar RM. Effect of chronic intermittent administration of inhaled tobramycin on respiratory microbial flora in patients with cystic fibrosis. J Infect Dis 1999;179(5):1190-1196.
16. Almeida MB, Bussamra MH, Rodrigues JC. ABPA diagnosis in cystic fibrosis patients: the clinical utility of IgE specific to recombinant Aspergillus fumigatus allergens. J Pediatr (Rio J) 2006;82(3):215-220.
17. Ritz N, Ammann RA, Casaulta Aebischer C, Schoeni-Affolter F, Schoeni MH. Risk factors for allergic bronchopulmonary aspergillosis and sensitisation to *Aspergillus fumigatus* in patients with cystic fibrosis. Eur J Pediatr 2005;164(9):577-582.
18. Greenberger PA. Immunological aspects of lung diseases and cystic fibrosis. JAMA 1997;278(22):1924-1930.
19. Rosenberg M, Mintzer R, Cooper BJ, Roberts M, Harris KE. Clinical and immunologic criteria for the diagnosis of allergic bronchopulmonary aspergillosis. Ann Intern Med 1977;86(4):405-414.
20. Greenberger PA, Patterson R. Diagnosis and management of allergic bronchopulmonary aspergillosis. Ann Allergy 1986;56(6):444-452.
21. Patterson R, Greenberger PA, Radin RD, Roberts M. Allergic bronchopulmonary aspergillosis: staging as an aid to management. Ann Intern Med 1982;96(3):286-291.
22. Hutcheson PS, Knutsen AP, Rejent AJ, Slavin RG. A 12 year longitudinal study of Aspergillus sensitivity in cystic fibrosis patients. Chest 1996;110(2):363-366.
23. Cockrill BA, Hales CA. Allergic bronchopulmonary aspergillosis. Annu Rev Med 1999;50:303-316.
24. Hemmann S, Nikolaizik WH, Schöni MH, Blaser K, Crameri R. Differential IgE recognition of recombinant *Aspergillus fumigatus* allergens by cystic fibrosis patients with allergic bronchopulmonary aspergillosis. Eur J Immunol 1998;28(4):1155-60.
25. Skov M, Koch C, Reimert CM, Poulsen LK. Diagnosis of allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis. Allergy 2000;55(1):50-58.
26. Skov M, Pressler T, Jensen HE, Hoiby N, Koch C. Specific IgG subclass antibody pattern to *Aspergillus fumigatus* in patients with cystic fibrosis with allergic bronchopulmonary aspergillosis. Thorax 1999;54(1):44-50.
27. Wojnarowski C, Eichler I, Gartner C, Götz M, Renner S, Koller DY, Frischer T. Sensitization to Aspergillus fumigatus and lung function in children with cystic fibrosis. Am J Respir Crit Care Med 1997;155(6):1902-1907.
28. Cunningham S, Madge SL, Dinwiddie R. Survey of criteria used to diagnose allergic bronchopulmonary aspergillosis in cystic fibrosis. Arch Dis Child 2001;84(1):89.
29. Wark PA, Hensley MJ, Saltos N, Boyle MJ, Toneguzzi RC, Epid GD, et al. Anti-inflammatory effect of itraconazole in stable allergic bronchopulmonary aspergillosis: a randomized control trial. J Allergy Clin Immunol 2003;111(5):952-957.
30. Fournier EC. Trial of ketoconazole in allergic bronchopulmonary aspergillosis. Thorax 1987;42(10):831.
31. Wark PA, Gibson PG, Wilson AJ. Azoles for allergic bronchopulmonary aspergillosis associated with asthma. Cochrane Database Syst Rev 2003;(3):CD001108.
32. Leon EE, Craig TJ. Antifungals in the treatment of allergic bronchopulmonary aspergillosis. Ann Allergy Asthma Immunol 1999;8(6)2:511-517.
33. Leblonde I, Tonnel AB. Allergic bronchopulmonary aspergillosis. Allergy 2005;60:1004-1013.
34. Thomson JM, Wesley A, Byrnes CA, Nixon GM. Pulse intravenous methylprednisolone for resistant allergic bronchopulmonary aspergillosis in cystic fibrosis. Pediatr Pulmonol 2006;41(2):164-170.
35. Skowronski E, Fitzgerald DA. Life-threatening allergic bronchopulmonary aspergillosis in a well child with cystic fibrosis. Med J Aust 2005;182(9):482-483.
36. Kraemer R, Delosea N, Ballinari P, Gallati S, Crameri R. Effect of allergic bronchopulmonary aspergillosis on lung function in children with cystic fibrosis. Am J Respir Crit Care Med 2006;174(11):1211-1220.