



ELSEVIER

REVIEW

Allergic bronchopulmonary aspergillosis in paediatric cystic fibrosis patients

Marina Buarque de Almeida, Maria Helena F. Bussamra and Joaquim Carlos Rodrigues*

Paediatric Pulmonary Section, Child Institute, Clinical Hospital, University of São Paulo Medical School, São Paulo, Brazil

KEYWORDS

allergic
bronchopulmonary
aspergillosis;
Aspergillus fumigatus;
cystic fibrosis;
bronchiectasis;
recombinant allergens

Summary Allergic bronchopulmonary aspergillosis (ABPA) is a severe complication in children, adolescents and adults with cystic fibrosis (CF), the prevalence of which ranges from 6–25%. The disease is the result of the colonisation of the respiratory tract by fungi of the genus *Aspergillus*, commonly *Aspergillus fumigatus*, and subsequent host sensitisation to fungal antigens, accompanied by a Th2 CD4 type response mediated by the production of specific IgE. The consequent inflammatory and obstructive bronchopulmonary injury can progress to fibrosis. The diagnosis should be considered early in patients with CF who show wheezing, transient pulmonary infiltrates and reduced lung function. The objective diagnosis is not straightforward because of overlapping clinical and radiological signs, particularly the progression of bronchiectasis. Specific criteria are needed for the diagnosis of ABPA in patients with CF, such as those proposed by the Cystic Fibrosis Foundation. The study of specific IgE against recombinant antigens of *A. fumigatus* has contributed to the early diagnosis of ABPA with high sensitivity and specificity. The technique has also shown promise in the follow-up of patients after steroid therapy and the early detection of recurrences. Treatment consists of long-term systemic corticosteroid usage, the monitoring of their adverse effects, and of the measurement of total serum IgE levels. The concomitant use of oral itraconazole seems to promote a better control of the disease and to reduce the duration of systemic steroid therapy but its use continues to be controversial. Controlled studies involving larger numbers of patients are necessary if we are to better understand the management of ABPA.

© 2005 Elsevier Ltd. All rights reserved.

FUNGUS- AND PATHOGENESIS-RELATED ASPECTS

Aspergillus species are ubiquitous fungi in nature and are commonly found as saprophytes in soil, plants and

decomposing organic matter. Its spores measure 2–3.5 μm in diameter and are therefore able to reach the terminal airways and alveoli where they can grow at human body temperature.¹

Aspergillus species are typical opportunistic agents^{2–4} with 80% of clinical infections caused by the *Aspergillus fumigatus*. *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus terreus* are also pathogenic but are less frequently found in the environment and less frequently in infections.^{3,5}

* Corresponding author. Present address: Av Dr Enéas de Carvalho Aguiar 647, 05403 9000 São Paulo, Brazil.

Tel.: +55 11 30698566; fax: +55 11 30698566.

E-mail address: jocarod@uol.com.br (J.C. Rodrigues).

Pulmonary aspergillosis manifests as aspergilloma or as allergic or invasive disease. Aspergillomas occur in patients with pre-existing lung cavities. Invasive aspergillosis affects immunocompromised individuals, especially cancer or transplant patients. In atopic individuals, the development of allergies to *Aspergillus* will depend on the type and frequency of exposure to its antigens present in spores and hyphae. This sensitisation generally occurs associated with other aeroallergens, accompanied by the production of specific IgE.⁶ In contrast, allergic bronchopulmonary aspergillosis (ABPA) results from a hypersensitivity reaction to the fungus, and mainly affects asthmatic individuals and patients with cystic fibrosis (CF).⁷ ABPA is the most severe allergic complication among those caused by *Aspergillus* and is relatively uncommon during childhood.⁸

The allergic and inflammatory response of patients with ABPA is quantitatively greater than that of patients only sensitised to the fungus. The model proposed for the immunopathogenesis of ABPA suggests the inhalation of spores that are trapped in the mucus present in the bronchial tree, followed by their germination and formation of mycelia. These mycelia start to release allergens and mycotoxins that are presented to T lymphocytes by HLA-DR type antigen-presenting cells within the bronchoalveolar lymphoid tissue.⁹ The host reaction is characterised by the appearance of fungus-specific antibodies (IgG and IgE), as well as by an increase in total IgE and then by eosinophilic infiltration of the airways causing tissue damage.¹⁰ The presence of the fungus above and between epithelial cells probably plays an important role in the modulation of the immune response in favour of a T-helper 2 (H2) pattern.¹¹ *A. fumigatus* releases substances that have been shown *in vitro* to cause detachment of epithelial cells and to trigger the production of pro-inflammatory cytokines such as interleukin (IL)-8, IL-6 and MCP-1. In addition, some fungal toxins inhibit ciliary beating,¹² proteases are probably responsible for a decrease in phagocytic activity^{13,14} and elastases and collagenases secreted by the fungus are factors that increase and perpetuate airway damage.¹⁵

Some factors such as the prevalence of atopy, the increased production of bronchial secretion and a certain inability to remove micro-organisms from the respiratory tree facilitate sensitisation to the fungus in CF patients.^{16,17} Interestingly, patients with ABPA without CF show a higher frequency of mutations in the *CFTR* protein gene.¹⁸ The presence of HLA-DR2 or DR5 and possibly of DR4 or DR7, renders the individual more susceptible, whereas HLA-DQ2 plays a protective role.¹⁹ ABPA is more frequent in CF patients over 6 years of age²⁰ but early colonisation of the airways with *Pseudomonas aeruginosa* (PA) seems to be associated with the development of ABPA in younger patients.²¹ There is discussion whether aggressive treatment of PA may facilitate endobronchial colonisation with *Aspergillus* as the growth of the latter is normally inhibited in the presence of exoproducts of PA.²²

Conversely, PA by inhibiting the growth of *Aspergillus* hyphae may cause the release of fungal antigens, thus intensifying the immunological response.²³

The occurrence of ABPA in CF patients may further impair pulmonary function^{24,25} and the additional lung injury caused by ABPA in patients with already compromised pulmonary function may result in an unfavourable clinical picture. Therefore, early diagnosis of ABPA and rapid treatment are essential.²⁶

PREVALENCE OF ABPA IN PATIENTS WITH CF

ABPA is seen in 1–25% of asthmatic patients; in CF the prevalence ranges from 6–25%.^{2,17,27–31} North American statistics indicate its presence in 7–14% of corticosteroid-dependent asthmatics and in about 7% of patients with CF.³² Data obtained from European multicentre studies involving 12 447 CF patients show an ABPA prevalence of 7.8% (2.1–13.6%).²⁰ Geller et al.²³ reporting the results of the Epidemiological Study of Cystic Fibrosis (ESCF) in North American and Canadian centers, found a very low prevalence of ABPA of 2% in patients older than 5 years. The variations in ABPA prevalence are probably related to the lack of uniform criteria for the diagnosis of the disease, variations in laboratory techniques, as well as the lack of clinical recognition by medical teams.^{33–35} In addition, the prevalence of ABPA increases with patient age.²³

Growth of *Aspergillus* from respiratory secretions in CF patients is relatively frequent (9–57%). The wide variability in the prevalence of bronchial colonisation by the fungus is related to the degree of exposure to its spores. Patients living in rural areas show higher rates of colonisation as do those living in inadequately ventilated houses or houses containing moulds.^{23,36} However, the simple presence of the fungus is not associated with a worsening of lung function.³⁷

CLINICAL ASPECTS OF ABPA

The classical clinical and laboratory manifestations of ABPA include episodes of wheezing, transient pulmonary infiltrates, an immediate positive *A. fumigatus* skin test, elevated total serum IgE, elevated levels of *Aspergillus*-specific IgE and IgG, eosinophilia, positive precipitins and central bronchiectasis.^{38,39}

The system proposed by Patterson et al. in 1986 classifies ABPA into five stages.⁴⁰ This classification corresponds to the findings obtained in asthmatic patients but does not often apply to CF patients.¹³ In the acute phase (stage 1), the classical signs are present and corticosteroid therapy permits improvement of non-fibrotic pulmonary lesions, a decrease in serum IgE, control of symptoms and a decline in eosinophilia. The effect of steroid therapy on the levels of specific IgE and IgG is variable but nevertheless the

symptoms tend to remit. Remission (stage 2) occurs after treatment of the acute phase, accompanied by clearance of pulmonary lesions (improved chest X-ray) and a fall in serum IgE. After remission, the patient may again show symptoms as during the acute phase (stage 3) and may require regular bronchodilator treatment. This exacerbation phase can be clinically asymptomatic, showing only laboratory changes such as raised serum IgE and the presence of infiltrates on chest X-rays. A subgroup of patients is unable to discontinue corticotherapy without the occurrence of severe crises (stage 4). Long-term ABPA may result in severe and permanent changes consistent with pulmonary fibrosis (stage 5).

CLINICAL AND LABORATORY CRITERIA FOR THE DIAGNOSIS OF ABPA

The diagnosis of ABPA is time-consuming and requires the assessment of both clinical and laboratory data.²⁷ The clinical presentation of ABPA with its exacerbations and remissions, may cause diagnostic confusion.^{41,42} Paediatric patients with CF present in many different ways but do respond to treatment.^{8,43}

At least four criteria for the diagnosis of ABPA have been suggested, including those described by Rosenberg et al.³⁸ (Table 1) and modified by Patterson et al.³⁹ (Table 2). Many of these clinical and laboratory findings are observed in CF patients in the absence of ABPA.^{17,26} Due to the overlapping signs and symptoms of CF and ABPA, which confuse the clinical situation,¹⁷ the criteria have been adapted for patients with CF²³ (Table 3). It has been suggested that for a definitive diagnosis, clinical improvement and a 50% fall in serum IgE levels after systemic corticosteroid treatment are necessary.⁴⁴ In 2001, the Cystic Fibrosis Foundation agreed a consensus on the diagnosis of ABPA in CF patients¹³ and added a proposal of annual screening, emphasising the importance

Table 2 Modified criteria for the diagnosis of allergic bronchopulmonary aspergillosis.³⁹

MAJOR

- Obstructive pulmonary disease
- Eosinophilia $>1000/\text{mm}^3$
- Positive skin test to *Aspergillus fumigatus*
- Positive precipitins against *A. fumigatus*
- Elevated IgE
- Fixed or transient pulmonary infiltrates
- Central bronchiectasis in the superior lobes
- Elevated *A. fumigatus*-specific IgE

MINOR

- A. fumigatus*-positive sputum culture
- Brownish sputum
- Late-phase positive skin test
- Decrease in IgE levels after steroid therapy

Table 3 Adaptation of the diagnostic criteria of allergic bronchopulmonary aspergillosis to patients with cystic fibrosis.²³

Two of the following criteria

- Positive skin test for *Aspergillus*
- Positive precipitins against *A. fumigatus*
- Serum IgE higher than 1000 IU/ml

At least two of the following criteria

- Bronchoconstriction
- Eosinophilia higher than $1000/\text{mm}^3$
- History of pulmonary infiltrates
- A. fumigatus*-positive sputum culture
- Brownish sputum
- Response to steroid therapy

of early diagnosis and adequate treatment to prevent irreversible sequelae (Table 4).^{45,46}

As no gold standard for the diagnosis of ABPA in CF exists, the comparison of prevalence, diagnosis and response to treatment between different CF centres is

Table 1 Classical criteria for the diagnosis of allergic bronchopulmonary aspergillosis.³⁸

MAJOR

- Episodes of bronchial obstruction
- Eosinophilia
- Positive skin test to *Aspergillus fumigatus*
- Positive precipitins against *Aspergillus* antigens
- Elevated IgE
- Fixed or transient pulmonary infiltrates
- Central bronchiectasis

MINOR

- Presence of *A. fumigatus* in sputum (culture or bacterioscopy)
- Brownish sputum
- Late-phase positive skin test

Table 4 Consensus of the Cystic Fibrosis Foundation for the diagnosis of allergic bronchopulmonary aspergillosis.¹³

Classical criteria

- Clinical deterioration
- Immediate positive skin test or positive RAST
- IgE $>1000 \text{ kU/l}$ ($>2398 \text{ ng/ml}$)
- A. fumigatus*-positive precipitins or presence of anti-*A. fumigatus* IgG
- Altered chest X-ray

Suggestion for annual screening

- Persistence of a clinical suspicion:
- Serum IgE $>500 \text{ kU/l}$, perform immediate skin test or RAST
- Serum IgE $<500 \text{ kU/l}$, repeat if the clinical suspicion was important

difficult. Cunningham et al.⁴⁷ performed a survey of criteria used to diagnose ABPA in specialised CF clinics in the UK. Measurements of precipitins, specific IgE, *A. fumigatus* skin tests, eosinophil counts in peripheral blood, total IgE and chest X-rays were performed frequently and on average four of the six tests were carried out annually at the centres. However, a wide variation in the value attributed to these tests to confirm the diagnosis and initiate treatment was found.

The recent isolation of *A. fumigatus* antigens using molecular biology techniques has improved the serological diagnosis of ABPA.⁴⁸ The identification and reproducibility of *A. fumigatus* antigen fractions has permitted the detection of specific antibodies against each fraction. The recombinant allergens Asp f1, Asp f2, Asp f3, Asp f4 and Asp f6 have been evaluated in the diagnosis of ABPA in patients with asthma^{49–53} and CF.^{48,54–56} These studies have demonstrated that the presence of IgE antibodies against rAsp f1 and f3 characterised a serological profile of sensitised individuals with 100% specificity and 88% sensitivity.^{48,50} In contrast, specific IgE against the recombinant allergens Asp f4 and/or f6 was exclusively detected in the serum of individuals with ABPA,^{7,48,50,51} with 80% of the patients showing specific IgE against rAsp f4, 55% against rAsp f6, and 90% responding to at least one of these recombinant antigens.⁴⁸ These tests were simultaneously carried out in a blind fashion in two laboratories, one in the United States and the other in Switzerland. The response to rAsp f1 and f3 did not discriminate sensitised patients from those with ABPA, whereas the positive response to any of the recombinant antigens Asp f2, f4 and f6 showed 100% sensitivity and specificity in the diagnosis of ABPA.⁴⁹

The consensus of the Cystic Fibrosis Foundation confirms that the detection of antibodies against recombinant allergens can improve and substantially refine the diagnosis of allergic complications associated with *A. fumigatus*, including ABPA. The technique has the advantage that it employs standard extracts and the CAP system, permits quantitative and fully automated testing.¹³

Few studies using this technique in the clinical management of patients with CF and asthma are available so far. In Brazil, a systematic investigation of ABPA patients with CF has been conducted.⁵⁷ In a group of 52 paediatric patients with CF classified as being at high risk of ABPA, the technique permitted the identification of four patients with a serologic profile of ABPA. However, agreement with the diagnosis of ABPA based on the criteria of the Cystic Fibrosis Foundation was obtained in only two. In one of the patients where the diagnosis was in doubt, the patient showed low levels of IgE against rAsp f2 and f6 (classes II and I, respectively), and the clinical manifestations were weak. As no cut-off for a positive response has been clarified and the detection of antibodies might precede clinical manifestations, the patient was only observed and not treated. The other patient showing a serological profile compatible with ABPA which did not fulfil the clinical and

laboratory criteria of the Cystic Fibrosis Foundation was a patient previously treated for ABPA. The evolving behaviour of these serological profiles is still unknown. The only study dealing with this aspect has suggested that rAsp f3-specific IgE levels may discern the phase of the disease and remain elevated during remission.⁵⁸

TREATMENT

The treatment of choice for ABPA consists of the use of systemic corticosteroids to reduce the inflammatory response induced by the fungus. This treatment is able to control exacerbations and reduce pulmonary infiltrates but recurrences during the course of treatment have been reported.^{59,60} The use of systemic corticosteroids is problematic in patients with CF who are already predisposed to diabetes, osteopaenia and growth retardation. In addition, the pharmacology of many drugs has been shown to be altered in CF patients.¹³ Various dosage schemes have been proposed but the best option has yet to be defined. Initial daily doses of 1–2 mg/kg prednisolone have been shown to be adequate and should be followed by a slow and gradual reduction according to the remission of symptoms and of the pulmonary infiltrates, with a minimum duration of treatment of at least 3 months. Monitoring of serum total IgE levels is useful as a parameter of disease control. A reduction of 30–50% is expected with the commencement of therapy. In patients in whom treatment cannot be discontinued without recurrence, prophylaxis for *Pneumocystis carinii* and maintenance treatment for bone mineralisation should be considered, in addition to monitoring other adverse effects of prolonged steroid therapy.⁶¹ The follow-up of patients intermittently checking IgE levels is recommended as recurrences might be detectable early based on a rise in serum levels of this immunoglobulin.¹³

Itraconazole is a systemic antifungal agent that can be administered orally and has shown beneficial effects in the treatment of ABPA. There are reports showing a lower frequency and adequate control of exacerbations,^{25,62} and a possible reduction and even discontinuation of systemic steroid therapy.^{63,64} A randomised, double-blind, placebo-controlled study involving 55 patients with ABPA without associated CF has demonstrated beneficial effects of itraconazole in corticosteroid-dependent patients.³² However, based on available data, there is limited evidence (grade III) that itraconazole is useful in the control of ABPA exacerbations in CF patients or that it reduces the need for steroid therapy.¹³

FINAL CONSIDERATIONS

ABPA is a disease of variable prevalence, a fact due in part to the lack of a diagnostic gold standard. Future perspectives include the application of the detection of specific immunity against recombinant *Aspergillus* antigens as a useful and standard diagnostic tool. CF centres should

regularly check patients for the presence of ABPA because of the impact ABPA has on CF with its associated deterioration in lung function.

The diagnosis and treatment of ABPA still represents a challenge in clinical practice, particularly in patients with CF, as many controversies exist regarding diagnostic criteria. Data concerning the best treatment for ABPA and for patient follow-up remain scarce.

REFERENCES

- Greenberger PA. Allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 2002; **110**(5): 685–692.
- Kurup VP, Kumar A. Immunodiagnosis of aspergillosis. *Clin Microbiol Rev* 1991; **4**(4): 439–456.
- Bardana EJ Jr. The clinical spectrum of aspergillosis—part 1: epidemiology, pathogenicity, infection in animals and immunology of *Aspergillus*. *Crit Rev Clin Lab Sci* 1981; **13**(1): 21–83.
- Soubani AO, Chandrasekar PH. The clinical spectrum of pulmonary aspergillosis. *Chest* 2002; **121**(6): 1988–1999.
- Bardana EJ Jr. The clinical spectrum of aspergillosis—part 2: epidemiology, pathogenicity, infection in animals and immunology of *Aspergillus*. *Crit Rev Clin Lab Sci* 1981; **13**(1): 85–159.
- Knutsen AP, Bellone C, Kauffman H. Immunopathogenesis of allergic bronchopulmonary aspergillosis in cystic fibrosis. *J Cyst Fibros* 2002; **1**(2): 76–89.
- Nikolaizik WH, Weichel M, Blaser K, Cramer R. Intracutaneous tests with recombinant allergens in cystic fibrosis patients with allergic bronchopulmonary aspergillosis and *Aspergillus* allergy. *Am J Respir Crit Care Med* 2002; **165**(7): 916–921.
- Amin RS, Wilmott RW. Hypersensitivity pneumonitis and eosinophilic pulmonary diseases. In: Chernick VBT, ed: *Kendig's Disorders of the Respiratory Tract in Children*. Philadelphia: Saunders, 1998; pp. 731–753.
- Alfaham M, Goodchild MC. *Aspergillus* lung disease in cystic fibrosis. In: Dodge JA, Brock DJH, Widdicombe JH, eds: *Cystic fibrosis—Current topics*. Philadelphia: John Wiley & Sons LTDA, 1996; pp. 267–288.
- Slavin RG, Bedrossian CW, Hutcheson PS et al. A pathologic study of allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 1988; **81**(4): 718–725.
- Kurup VP, Choi H, Murali PS, Coffman RL. IgE and eosinophil regulation in a murine model of allergic aspergillosis. *J Leukoc Biol* 1994; **56**(5): 593–598.
- 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**(10): 1681–1687.
- Stevens DA, Moss RB, Kurup VP et al. Participants in the Cystic Fibrosis Foundation Consensus Conference. 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 Jan 1; **38**(1): 158.
- Kauffman HF, Tomee JF, van de Riet MA, Timmerman AJ, Borger P. Protease-dependent activation of epithelial cells by fungal allergens leads to morphologic changes and cytokine production. *J Allergy Clin Immunol* 2000; **105**(6 Pt 1): 1185–1193.
- Kurup VP, Seymour BW, Choi H, Coffman RL. Particulate *Aspergillus fumigatus* antigens elicit a TH2 response in BALB/c mice. *J Allergy Clin Immunol* 1994; **93**(6): 1013–1020.
- Robinson M, Bye PT. Mucociliary clearance in cystic fibrosis. *Pediatr Pulmonol* 2002; **33**(4): 293–306.
- Zeaske R, Bruns WT, Fink JN et al. Immune responses to *Aspergillus* in cystic fibrosis. *J Allergy Clin Immunol* 1988; **82**(1): 73–77.
- Miller PW, Hamosh A, Macek M Jr et al. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in allergic bronchopulmonary aspergillosis. *Am J Hum Genet* 1996; **59**(1): 45–51.
- Chauhan B, Santiago L, Hutcheson PS et al. Evidence for the involvement of two different MHC class II regions in susceptibility or protection in allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 2000; **106**(4): 723–729.
- Mastella G, Rainisio M, Harms HK et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis. A European epidemiological study. Epidemiologic Registry of Cystic Fibrosis. *Eur Respir J* 2000; **16**(3): 464–471.
- Mussaffi H, Greif J, Komreich L et al. Severe allergic bronchopulmonary aspergillosis in an infant with cystic fibrosis and her asthmatic father. *Pediatr Pulmonol* 2000; **29**(2): 155–159.
- Brown K, Rosenthal M, Bush A. Fatal invasive aspergillosis in an adolescent with cystic fibrosis. *Pediatr Pulmonol* 1999; **27**(2): 130–133.
- Geller DE, Kaplowitz H, Light MJ, Colin AA. Allergic bronchopulmonary aspergillosis in cystic fibrosis: reported prevalence, regional distribution, and patient characteristics. Scientific Advisory Group, Investigators, and Coordinators of the Epidemiologic Study of Cystic Fibrosis. *Chest* 1999; **116**(3): 639–646.
- Wojnarowski C, Eichler I, Gartner C et al. Sensitisation to *Aspergillus fumigatus* and lung function in children with cystic fibrosis. *Am J Respir Crit Care Med* 1997; **155**(6): 1902–1907.
- Nepomuceno IB, Esrig S, Moss RB. Allergic bronchopulmonary aspergillosis in cystic fibrosis: role of atopy and response to itraconazole. *Chest* 1999; **115**(2): 364–370.
- Slavin RG. ABPA in CF: a devastating combination. *Pediatr Pulmonol* 1996; **21**(1): 1–2.
- Laufer P, Fink JN, Bruns WT et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis. *J Allergy Clin Immunol* 1984; **73**(1 Pt 1): 44–48.
- Patterson R, Greenberger PA, Halwig JM, Liotta JL, Roberts M. Allergic bronchopulmonary aspergillosis. Natural history and classification of early disease by serologic and roentgenographic studies. *Arch Intern Med* 1986; **146**(5): 916–918.
- Hutcheson PS, Rejent AJ, Slavin RG. Variability in parameters of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *J Allergy Clin Immunol* 1991; **88**(3 Pt 1): 390–394.
- Marchant JL, Warner JO, Bush A. Rise in total IgE as an indicator of allergic bronchopulmonary aspergillosis in cystic fibrosis. *Thorax* 1994; **49**(10): 1002–1005.
- 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**(6 Pt 1): 1513–1518.
- Stevens DA, Schwartz HJ, Lee JY et al. A randomised trial of itraconazole in allergic bronchopulmonary aspergillosis. *N Engl J Med* 2000; **342**(11): 756–762.
- Brueton MJ, Ormerod LP, Shah KJ, Anderson CM. Allergic bronchopulmonary aspergillosis complicating cystic fibrosis in childhood. *Arch Dis Child* 1980; **55**(5): 348–353.
- Maguire S, Moriarty P, Tempary E, FitzGerald M. Unusual clustering of allergic bronchopulmonary aspergillosis in children with cystic fibrosis. *Pediatrics* 1988; **82**(6): 835–839.
- Becker JW, Burke W, McDonald G, Greenberger PA, Henderson WR, Aitken ML. Prevalence of allergic bronchopulmonary aspergillosis and atopy in adult patients with cystic fibrosis. *Chest* 1996; **109**(6): 1536–1540.
- Nelson LA, Callera ML, Schwartz RH. Aspergillosis and atopy in cystic fibrosis. *Am Rev Respir Dis* 1979; **120**(4): 863–873.
- Milla CE. Allergic bronchopulmonary aspergillosis and cystic fibrosis. *Pediatr Pulmonol* 1999; **27**(2): 71–73.
- Rosenberg M, Patterson R, 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.

39. Patterson R, Greenberger PA, Radin RC, Roberts M. Allergic bronchopulmonary aspergillosis: staging as an aid to management. *Ann Intern Med* 1982; **96**(3): 286–291.
40. Patterson R, Greenberger PA, Halwig JM, Liotta JL, Roberts M. Allergic bronchopulmonary aspergillosis. Natural history and classification of early disease by serologic and roentgenographic studies. *Arch Intern Med* 1986; **146**(5): 916–918.
41. Sharma OP, Chwogule R. Many faces of pulmonary aspergillosis. *Eur Respir J* 1998; **12**(3): 705–715.
42. 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 (ABPA). *Thorax* 1999; **54**(1): 44–50.
43. Maiz L, Cuevas M, Quirce S, Pacheco A, Escobar H. Allergic bronchopulmonary aspergillosis with low serum IgE levels in a child with cystic fibrosis. *J Allergy Clin Immunol* 1997; **100**(3): 431–432.
44. Hanley-Lopez J, Clement LT. Allergic bronchopulmonary aspergillosis in cystic fibrosis. *Curr Opin Pulm Med* 2000; **6**(6): 540–544.
45. Skov M, Koch C, Reimert CM, Poulsen LK. Diagnosis of allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis. *Allergy* 2000; **55**(1): 50–58.
46. Greenberger PA, Patterson R, Ghory A et al. Late sequelae of allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 1980; **66**(4): 327–335.
47. 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.
48. Cramer R. Recombinant *Aspergillus fumigatus* allergens: from the nucleotide sequences to clinical applications. *Int Arch Allergy Immunol* 1998; **115**(2): 99–114.
49. Kurup VP, Banerjee B, Hemmann S, Greenberger PA, Blaser K, Cramer R. Selected recombinant *Aspergillus fumigatus* allergens bind specifically to IgE in ABPA. *Clin Exp Allergy* 2000; **30**(7): 988–993.
50. Cramer R, Hemmann S, Ismail C, Menz G, Blaser K. Disease-specific recombinant allergens for the diagnosis of allergic bronchopulmonary aspergillosis. *Int Immunol* 1998; **10**(8): 1211–1216.
51. Hemmann S, Menz G, Ismail C, Blaser K, Cramer R. Skin test reactivity to 2 recombinant *Aspergillus fumigatus* allergens in *A. fumigatus*-sensitized asthmatic subjects allows diagnostic separation of allergic bronchopulmonary aspergillosis from fungal sensitisation. *Allergy Clin Immunol* 1999; **104**(3 Pt 1): 601–607.
52. Cramer R, Lidholm J, Gronlund H, Stuber D, Blaser K, Menz G. Automated specific IgE assay with recombinant allergens: evaluation of the recombinant *Aspergillus fumigatus* allergen I in the Pharmacia Cap System. *Clin Exp Allergy* 1996; **26**(12): 1411–1419.
53. Banerjee B, Kurup VP, Greenberger PA, Hoffman DR, Nair DS, Fink JN. Purification of a major allergen, Asp f 2 binding to IgE in allergic bronchopulmonary aspergillosis, from culture filtrate of *Aspergillus fumigatus*. *J Allergy Clin Immunol* 1997; **99**(6 Pt 1): 821–827.
54. Banerjee B, Greenberger PA, Fink JN, Kurup VP. Immunological characterisation of Asp f 2, a major allergen from *Aspergillus fumigatus* associated with allergic bronchopulmonary aspergillosis. *Infect Immun* 1998; **66**(11): 5175–5182.
55. Nikolaizik WH, Moser M, Cramer R et al. Identification of allergic bronchopulmonary aspergillosis in cystic fibrosis patients by recombinant *Aspergillus fumigatus* I/a-specific serology. *Am J Respir Crit Care Med* 1995; **152**(2): 634–639.
56. Hemmann S, Nikolaizik WH, Schoni MH, Blaser K, Cramer R. Differential IgE recognition of recombinant *Aspergillus fumigatus* allergens by cystic fibrosis patients with allergic bronchopulmonary aspergillosis or *Aspergillus* allergy. *Eur J Immunol* 1998; **28**(4): 1155–1160.
57. Almeida MB. Allergic bronchopulmonary aspergillosis diagnosis in cystic fibrosis patients through detection of recombinant *Aspergillus fumigatus* allergens. São Paulo, 2004. Dissertação (mestrado) – Faculdade de Medicina da Universidade de São Paulo.
58. Knutsen AP, Hutcheson PS, Slavin RG, Kurup VP. IgE antibody to *Aspergillus fumigatus* recombinant allergens in cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *Allergy* 2004; **59**(2): 198–203.
59. Wark P, Wilson AW, Gibson PG. Azoles for allergic bronchopulmonary aspergillosis associated with asthma. Cochrane Database Syst Rev. 2001; (4): CD001108. Review. Update in: Cochrane Database Syst Rev. 2003; (3): CD001108.
60. Wark PA, Gibson PG. Allergic bronchopulmonary aspergillosis: new concepts of pathogenesis and treatment. *Respirology* 2001; **6**(1): 1–7.
61. Vlahakis NE, Aksamit TR. Diagnosis and treatment of allergic bronchopulmonary aspergillosis. *Mayo Clin Proc* 2001; **76**(9): 930–938.
62. Denning DW, Van Wye JE, Lewiston NJ, Stevens DA. Adjunctive therapy of allergic bronchopulmonary aspergillosis with itraconazole. *Chest* 1991; **100**(3): 813–819.
63. Mannes GP, van der Heide S, van Aalderen WM, Genritsen J. Itraconazole and allergic bronchopulmonary aspergillosis in twin brothers with cystic fibrosis. *Lancet* 1993; **341**(8843): 492.
64. Lebeau B, Pelloux H, Pinel C et al. Itraconazole in the treatment of aspergillosis: a study of 16 cases. *Mycoses* 1994; **37**(5–6): 171–179.