Official Journal of the Asian Pacific Society of Respirology
Respirology

ORIGINAL ARTICLE

The effects of antifungal therapy on severe asthma with fungal sensitization and allergic bronchopulmonary aspergillosis

ALESSANDRO C. PASQUALOTTO,[1,2,3] GEORGINA POWELL,[3] ROBERT NIVEN[2] AND DAVID W. DENNING[1,3]

[1]*The University of Manchester, Manchester, UK,* [2]*Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, and* [3]*Wythenshawe Hospital, Manchester, UK*

ABSTRACT

Background and objective: **Very little is known about the response rates to or appropriateness of treatment for patients with allergic fungal diseases of the lung. This study assessed the effect of antifungal therapy in patients with severe asthma with fungal sensitization (SAFS) and allergic bronchopulmonary aspergillosis (ABPA).**

Methods: **A retrospective cohort study of 33 adult patients who fulfilled the criteria for either SAFS ($n = 22$) or ABPA ($n = 11$) was conducted. All patients had received antifungal therapy for at least 6 months. The primary study end point was the effect of antifungal therapy on patients' lung function.**

Results: **Overall, total IgE values and radioallergosorbent test (RAST) for *A. fumigatus* markedly decreased after 6 months of therapy in both SAFS and ABPA patients ($P = 0.004$ and $P = 0.005$, respectively). Reduction was seen in the eosinophil count ($P = 0.037$), dose of oral steroids ($P = 0.043$) and courses of systemic steroids required ($P = 0.041$). Lung function also improved ($P = 0.016$). Four of 10 patients discontinued oral steroids after 6 months of therapy. Reduction in IgE levels ($P = 0.015$) and RAST for *A. fumigatus* was also observed ($P = 0.006$) for those patients treated for at least 1 year with antifungal drugs.**

Conclusions: **Both ABPA and SAFS patients benefited from oral antifungal therapy. The antifungal therapy may act by reducing the antigenic load, interacting with corticosteroids or by a direct immunological effect.**

SUMMARY AT A GLANCE

This retrospective cohort study evaluated the clinical effect of antifungal therapy in patients with allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitization (SAFS). Both ABPA and SAFS patients benefited from antifungal therapy.

Key words: allergens, allergic bronchopulmonary aspergillosis, aspergillosis, asthma, itraconazole, voriconazole.

INTRODUCTION

Until recently oral corticosteroids were regarded as the only effective therapy for patients with allergic bronchopulmonary aspergillosis (ABPA). Since its introduction, oral itraconazole has shown therapeutic benefit in ABPA patients, in part by reducing the need for systemic steroids.[1–6] The benefit of itraconazole has also been demonstrated in two double-blind, placebo-controlled trials in patients with ABPA.[7,8] Individuals with steroid-dependent ABPA taking a 16-week course of itraconazole benefited from a reduction in oral steroid dose, lower total IgE levels, and improved exercise tolerance or resolution of pulmonary infiltrates.[7] In another double-blind placebo-controlled trial involving stable patients with ABPA,[8] itraconazole for 16 weeks was associated with improvement in immunologic parameters such as eosinophilia, total IgE, and *Aspergillus*-specific IgG suggestive of a potential anti-inflammatory and immunological effect.

Correspondence: Alessandro C Pasqualotto, Av Independência 155, Santa Casa-Complexo Hospitalar, Serviço de Controle de Infecção Hospitalar (SCIH), Hospital Dom Vicente Scherer, 90035-075, Porto Alegre, Brazil. Email: acpasqualotto@hotmail.com

Conflict of interest statement: In the past 5 years, Dr Pasqualotto has received grant support from Pfizer, Merck, CAPES, CNPq, the Fungal Research Trust, Sigma-Tau and Myconostica. He has been a speaker to Pfizer, Schering-Plough, United Medical, Merck and Biometrix, and has received support to attend conferences from Pfizer, Schering-Plough, United Medical, Bago and Merck.

Dr Denning has received grant support from Astellas, Merck, Pfizer, F2G, OrthoBiotech, Indevus, Basilea, AM Pharma, the Fungal Research Trust, the Welcome Trust, the Moulton Trust, The Medical Research Council, the National Institute of Allergy and Infectious Diseases and the European Union. He has been an advisor/consultant to Basilea, Vicuron (now Pfizer), Schering Plough, Indevus, F2G, Nektar, Daiichi, Sigma Tau, Astellas, Gilead and York Pharma. He has been paid for talks on behalf of Astellas, Merck, GSK, Chiron, AstraZenca and Pfizer. He holds founder shares in F2G Ltd and Myconostica Ltd, both university spin-out companies.

All other authors report no potential conflict of interest.

Received 7 January 2009; invited to revise 20 February 2009; revised 17 March 2009; accepted 14 April 2009 (Associate Editor: Jerry Brown).

There are well-accepted diagnostic criteria for ABPA.[9] However, many patients with asthma have evidence of sensitization to many different fungi but do not fulfil these criteria. Recently, the term SAFS (severe asthma with fungal sensitization) was proposed for these patients.[10] As the pathophysiological mechanisms underlying these conditions appear to be similar, a response to antifungal therapy might also be expected for patients with SAFS. The purpose of this retrospective study was to evaluate the effect of antifungal therapy in a cohort of patients with SAFS and ABPA.

METHODS

Study design

A retrospective cohort study was undertaken to assess the response to treatment of 33 adult patients with *Aspergillus*-related diseases treated with antifungal medication.

Study subjects

All consecutive patients attending a hospital chest clinic who fulfilled the clinical criteria for SAFS or ABPA and who had received at least 6 months of antifungal therapy were eligible for inclusion in the study.

Definitions

Diagnostic criteria for ABPA were:[9] (i) asthma; (ii) immediate cutaneous reaction to *A. fumigatus*; (iii) total serum IgE $\geq$1000 IU/mL; (iv) elevated *A. fumigatus*-specific serum IgE levels (RAST); (v) precipitating antibodies to *A. fumigatus* in the serum; and (vi) central bronchiectasis. Minor diagnostic criteria were: (i) peripheral blood eosinophilia (often absent in patients receiving steroids); (ii) repeated detection of *Aspergillus* in sputum; (iii) a history of expectoration of brown plugs or flecks; and (iv) a history of recurrent pulmonary infiltrates (transient or fixed). Some patients with long-standing ABPA do not maintain detectable precipitins in their serum. Patients with ABPA were not specifically tested for immediate skin reactivity to *Aspergillus*; a raised serum specific IgE was regarded as equivalent.

Asthma severity was defined according to the British Thoracic Society (BTS) severity grade for asthma.

Criteria for the diagnosis of SAFS were:[10] (i) severe (poorly controlled) asthma, BTS level four or five; (ii) total IgE <1000 IU/mL; and (iii) either a positive skin test or raised specific IgE to any fungus. In this study, we included only patients with SAFS related to *Aspergillus* species, with or without detectable serum precipitins to *A. fumigatus*.

The study protocol was approved by an independent local ethics committee and patient's confidentiality was maintained at all times.

Study end points

The null hypothesis was that 6 months of treatment with antifungal drugs would have no impact on the lung function and laboratory parameters of patients with SAFS or ABPA. The primary study end point was the effect of antifungal therapy on patients' lung function. Secondary end points were: number of hospitalizations or emergency room visits for respiratory diseases, total IgE value, RAST value for *A. fumigatus*, number of eosinophils in the peripheral blood, dosage of oral steroids, dosage of inhaled steroids and courses of systemic steroids.

Study methods

Clinical data from 6 months prior to the commencement of antifungal therapy (baseline) were obtained retrospectively, and were again collected after 6 and after 12 months of antifungal therapy. Patients who had received 6–12 months of antifungal therapy at the time of this study were evaluated for the 6-month end point only. The number of hospitalizations or emergency room visits and dose of steroids were routinely asked at clinic visits, and subject recall for these variables is thought to be quite reliable. Lung function was estimated by FEV_1 performed either in the laboratory or by the attending physician at the time of a clinic visit using a dry bellows wedge spirometer calibrated regularly. Prednisolone was used as the reference when comparisons of oral therapy with steroids were required. Similarly, beclomethasone equivalent dose was used as reference for inhaled therapy with steroids.

Analysis

Descriptive statistics were used to summarize the data. Pearson's chi-square test was used to evaluate categorical variables (Fisher's exact test where appropriate). To compare continuous variables before and after antifungal therapy, non-parametric Wilcoxon signed ranks T test for related samples was used. For all comparisons, P values ≤ 0.05 were considered statistically significant. All analyses were performed with the software SPSS 11.5.0 for Windows.

RESULTS

Patients' characteristics

There were 33 patients included in the study, 22 of whom had a diagnosis of SAFS and 11 a diagnosis of ABPA (Table 1). All patients had asthma and were on treatment with inhaled steroids. The median duration of asthma was 42 years (range 5–68 years), which was similar for SAFS and ABPA patients. Most patients had severe asthma (BTS severity grade 4 in 71.9% and grade 5 in 25.0%). Women were significantly over-represented in the SAFS group ($P = 0.003$). As

Table 1 Baseline characteristics of patients included in the study

	SAFS ($n = 22$)	ABPA ($n = 11$)	*P* value	All patients ($n = 33$)
Women (%)	16 (72.7%) (16/22)	2 (18.2%) (2/11)	0.003	18 (54.5%) (18/33)
Age, years (range)	51.5 (34–75) (22/22)	52.0 (42–67) (11/11)	0.925	52.0 (34–75) (33/33)
Total IgE, IU/mL (range)	337 (76–990) (22/22)	3 317 (1 000–39 000) (11/11)	<0.0001	600.0 (76–39 000) (33/33)
RAST for *A. fumigatus,* IU/mL (range)	3.6 (0.5–34.9) (21/22)	51.2 (0.8–350.0) (11/11)	0.001	8.75 (0.5–350.0) (33/33)
% with positive precipitins to *A. fumigatus*	28.6% (4/14)	71.4% (5/7)	0.159	27.3% (9/21)
Duration of asthma, years (range)	35.0 (7–68) (21/22)	44.0 (5–64) (11/11)	0.180	42.0 (5–68) (32/33)
FEV_1, L, (range)	1.60 (1.30–3.00) (17/22)	1.75 (1.40–3.60) (10/11)	0.223	1.69 (1.30–3.60) (27/33)
Inhaled steroid dose, μg daily (range)	1000 (400–2000) (22/22)	1 000 (800–2 000) (11/11)	0.985	1 000 (400–2 000) (33/33)
Eosinophils $\times 10^9$/L, (range)	0.395 (0.10–1.81) (20/22)	0.330 (0.06–0.76) (9/11)	0.908	0.37 (0.01–1.81) (29/33)
% continuously on oral steroids	22.7% (5/22)	45.5% (5/11)	0.240	31.3% (10/33)
% exacerbations requiring steroids†	50.0% (11/22)	36.4% (4/11)	0.458	45.5% (15/33)
% hospitalized for asthma†	13.6% (3/22)	18.2% (2/11)	1.000	15.2% (5/33)

† In 6 months before starting antifungal therapy.

Continuous variables are presented as median values and range. Fractions represent the number of patients tested for the particular variable.

expected by the diagnostic criteria, median values of total IgE and RAST for *A. fumigatus* were higher in ABPA patients than in patients with SAFS. Elevated titres of IgE against mixed moulds occurred for 18.2% of ABPA patients ($n = 2$) and 27.3% of SAFS patients ($n = 6$). One additional patient with ABPA had a strongly positive IgE against *Candida* (55.6 IU/mL).

Antifungal therapy

All patients were initially treated with itraconazole, in dosages ranging from 100 to 450 mg daily (median 300 mg daily). Mean itraconazole levels were therapeutic (>5 mg/L) for 75% of patients. Side effects occurred in 19.3% of patients, and included tendonitis (6.4%), oedema (3.2%), diarrhoea and rash (3.2%), abnormal liver function (3.2%) and abdominal pain (3.2%). Antifungal therapy was modified to voriconazole in three patients due to adverse effects, persistently low itraconazole levels or clinical deterioration. All patients received at least 6 months of therapy (range 6–60 months). No patient with overt Cushing's syndrome was noted during the study.

Evaluation after 6 months of antifungal therapy

Patients' response to 6 months of antifungal treatment is summarized in Figure 1. Total IgE values decreased significantly by 24.5% in SAFS patients ($P = 0.004$) and 36.8% in ABPA patients ($P = 0.005$). RAST for *A. fumigatus* decreased significantly by 14.8% in the overall population studied ($P = 0.005$), and by 34.1% in SAFS patients ($P = 0.019$). Although RAST values actually increased by 21.6% in ABPA patients ($P = 0.139$), a decrease of 30.5% ($P = 0.017$) was seen when the only ABPA patient for whom RAST values markedly increased (from 85.2 to 459.2 IU/mL) was excluded. Similarly, a significant decrease occurred in the number of eosinophils in the peripheral blood. Excluding one outlier ABPA patient whose eosinophil counts increased from 0.13 (at baseline) to 0.9×10^9/L (at 6-month evaluation), eosinophil counts decreased by 33.5%, 36.9%, 26.7% for all patients ($P = 0.011$), SAFS ($P = 0.016$) and ABPA patients ($P = 0.400$), respectively. Lung function improved overall (median FEV_1 increased by 190 ml; $P = 0.016$), with a non-significant improvements in both subgroups (SAFS, 170 mL increment, $P = 0.075$; ABPA, 230 mL increment, $P = 0.080$).

Of the 10 patients treated with continuous oral steroids at the time of recruitment, four (40%) discontinued oral steroids during the 6 months of antifungal therapy. Of these four patients, three were diagnosed with SAFS (60%) and one with ABPA (20%). Only one patient who was not on steroids at baseline required oral steroids continuously after 6 months of antifungal therapy. Although the average oral dosage of steroids was reduced by 51.5% when all patients were analysed ($P = 0.043$), the dose reduction within each diagnostic subgroup was not statistically significant (SAFS patients had 60% reduction; $P = 0.144$; ABPA patients had 43% reduction; $P = 0.109$). Overall, antifungal therapy reduced the number of courses of systemic steroids required by 57.4% ($P = 0.041$). A non-significant reduction in steroid courses was seen for SAFS patients (average reduction 53.7%; $P = 0.062$) and ABPA patients (average reduction 65.0%; $P = 0.416$). Of the 15 patients who at baseline had required courses of systemic steroids, 46.7% did not

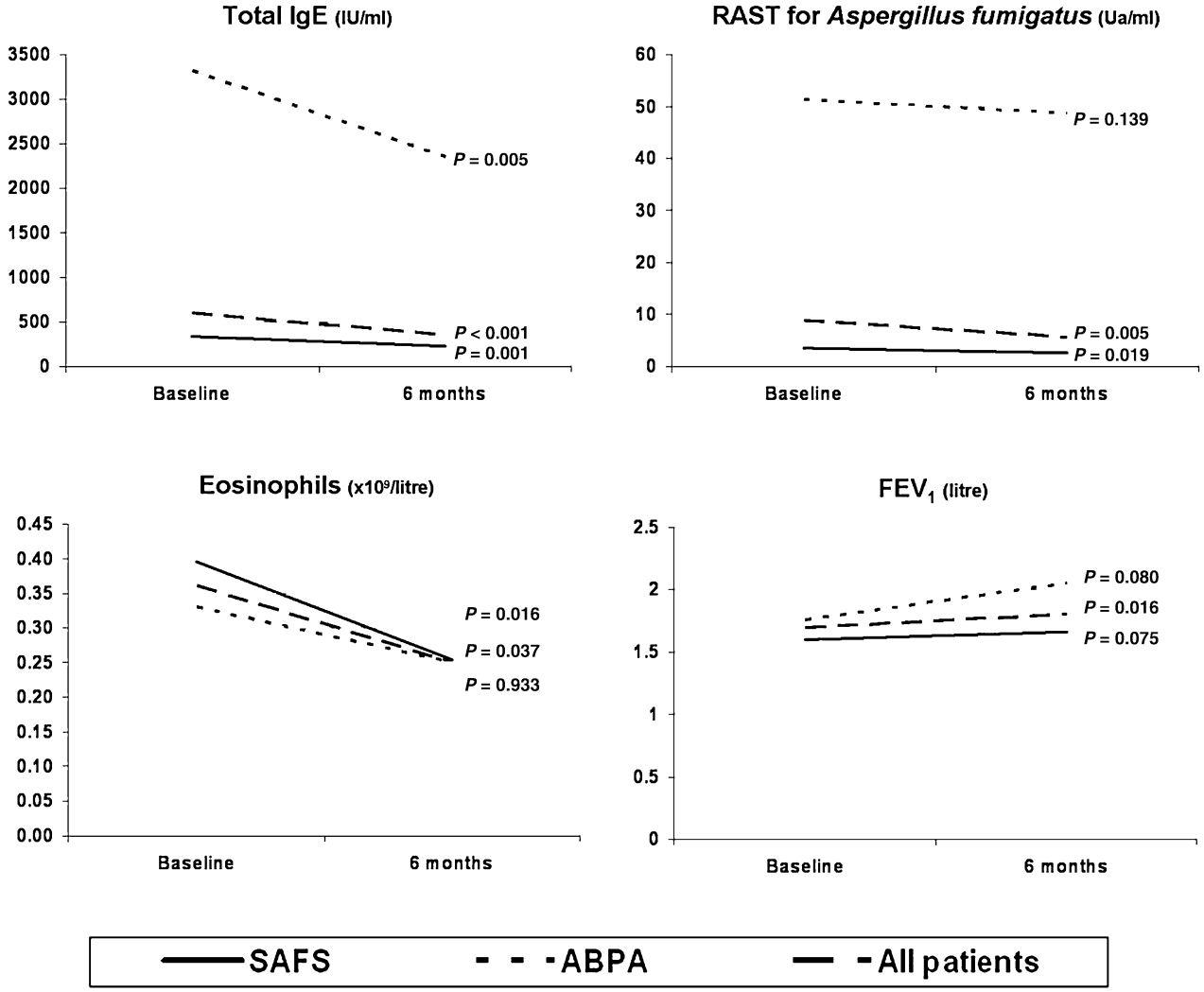

Figure 1 Response to 6-month antifungal therapy, as determined by total IgE value, RAST for *Aspergillus fumigatus*, eosinophils count and FEV_1. —, SAFS; ---, ABPA; — -, All patients.

receive any additional steroids in the first 6 months of antifungal therapy. The dosage of inhaled steroids was not modified after antifungal therapy ($P = 0.577$ for all patients). Similarly, there was no significant reduction in the number of hospitalizations or emergency room visits due to respiratory diseases during the antifungal treatment ($P = 0.589$ for all patients); however, only five patients had been admitted to the hospital in the 6 months preceding the start of antifungal therapy.

Evaluation after 12 months of antifungal therapy

From the 33 patients initially included in the study, data from 17 patients who were on treatment with antifungal drugs for more than 1 year were obtained (13 SAFS patients and 4 ABPA patients). In these patients overall, total IgE values decreased by 22.6% ($P = 0.015$); however, there was no significant reduction in the respective subgroups (SAFS, reduction by 10.2%; $P = 0.155$; ABPA, reduction by 41.1%; $P = 0.068$). For the group overall, RAST values significantly decreased (average reduction 32.7%; $P = 0.006$); however, there was no significant reduction in the respective subgroups (SAFS, 27.4% reduction, $P = 0.053$; ABPA, 40.9% reduction, $P = 0.068$). A non-significant decrease was observed in the dosage of inhaled steroids (in all patients an 8.6% reduction, $P = 0.059$; in SAFS patients a 6.3% reduction, $P = 0.180$; in ABPA patients a 13.3% reduction, $P = 0.157$). Overall, antifungal therapy at 12 months resulted in no statistically significant changes in the key variables of the number of eosinophils (2.7% reduction, $P = 0.779$), dosage of oral steroids (59.0% reduction, $P = 0.109$), courses of systemic steroids (54.1% reduction, $P = 0.589$), FEV_1 values (reduction by 290 mL, $P = 0.726$) and the number of hospitalizations (11.1% reduction; $P = 0.257$), compared with baseline.

DISCUSSION

Asthma is common, affecting at least one in every seven children and one in 25 adults in the United Kingdom.[11] Although most asthma patients have mild symptoms, a minority of patients suffer from severe symptoms requiring multiple hospital admissions. Severe asthma seems to be strongly associated with atopy, especially to mould allergens. In addition, a strong temporal relationship exists between high environmental spore counts and asthmatic attacks.[12] Asthma deaths and admissions to hospital in the United Kingdom of patients under the age of 35 years have been shown to coincide with the summer-autumn increase in ambient mould spores.[13] Among patients with persistent asthma requiring specialist referral, 20–25% will have skin test reactivity to *Aspergillus* or other fungi. *A. fumigatus* plays an important role as an allergen in these patients.[10]

Although about 1% of adult asthmatic patients are thought to have ABPA,[14] fungal sensitization occurs in 20–25% of patients with 'persistent' asthma. SAFS is a recently proposed acronym for the group of patients with severe asthma and fungal sensitization who do not meet the criteria for ABPA.[10] The similarities and differences between SAFS and ABPA are not completely clear, and they most likely represent a spectrum of fungal allergic disease, although discrete toll-like receptor profiles have now been detected.[15] Total IgE has been used to distinguish the two syndromes. Accordingly, all patients in this study with IgE levels $\geq$1000 IU/mL were considered to have ABPA. Eosinophil counts were similar between these groups, as previously demonstrated.[9] Of patients with SAFS included in this study, 72.7% were women.

Bowyer *et al*.[9] showed that patients with ABPA and SAFS seem to react to different *Aspergillus* allergens. Both ABPA and SAFS patients reacted with Asp f1 and Asp f2, but no single allergen caused a reaction in 100% of patients. Although SAFS patients manifested raised RAST responses to total *A. fumigatus* protein mixture, extremely low reactivity was observed among these patients to the *Aspergillus* allergens Asp f1, Asp f2 and Asp f6. No SAFS patient showed reactivity with Asp f4.

A characteristic feature of ABPA is the production of mucous plugs full of *A. fumigatus* hyphae. Indeed almost all ABPA patients are colonized by *A. fumigatus*; occasional patient are colonized with other fungi. In contrast, it is exceptional for SAFS patients to produce mucous plugs and fungal colonization is rare. Further, sensitization occurs to a wide range of fungi, of which *A. fumigatus* is the most common. Some SAFS patients are apparently only sensitized to one fungus (the testing panel is not very large considering that >1 000 000 fungi are thought to be present on earth).

Growing evidence suggests that patients' hypersensitivity to *Aspergillus* allergens might be genetically predetermined. Knutsen suggested that the exacerbated Th2 CD4 T cell response seen in ABPA could be associated with certain HLA-DR2 and DR5 genotypes, single nucleotide polymorphisms (SNPs) of the IL-4 receptor alpha chain, and IL-13 and -10 polymorphisms.[16] Vaid *et al*.[17] also showed particular SNPs in surfactant protein A genes to be associated with ABPA. An association between ABPA and particular cytokine profiles has also been proposed.[18]

In the present study, we demonstrated that both SAFS and ABPA patients benefited from antifungal therapy. Analyses of the overall results after 6-month treatment revealed improved lung function, decreased levels of IgE and *A. fumigatus*-specific IgE, and reduced eosinophils. In addition, a diminution in the total oral dosage of steroids and courses of systemic steroids was observed. No modification occurred in the dosage of inhaled steroids or number of hospitalizations. Analysis of the results for the patients who completed 12 months of treatment also revealed lower levels of total IgE and specific IgE against *A. fumigatus*, in addition to a non-significant reduction in the dosage of inhaled steroids. Although no impact was observed after 1 year of therapy on variables such as eosinophils, dosage and courses of oral steroids, lung function and hospitalizations, only 17 patients were evaluated for these end points. The difference in the findings at 6 and 12 months may be explained by smaller numbers for analysis, longer/continued therapy in patients who were less well or slower to respond or a loss of effect of continued therapy.

The limited number of patients reduced the power of the study, which was evident when the subgroups of patients with SAFS or ABPA were evaluated separately. After 6 months of antifungal therapy, patients with SAFS showed lower IgE levels, *A. fumigatus*-specific IgE levels and eosinophils. A non-significant improvement occurred for lung function ($P = 0.075$) and need of systemic steroids ($P = 0.062$). Analyses of results after 1 year of therapy for SAFS patients were limited by the small number of patients. Results for patients with ABPA were similar, except that eosinophil counts and courses of systemic steroids did not improve after 6 months of antifungal therapy.

The results of this retrospective study support the findings from the FAST study, a double-blind placebo-controlled randomized trial of itraconazole in patients with SAFS.[19] In this multicentre study, SAFS patients treated with itraconazole for 32 weeks had a significant improvement in quality of life, which was evaluated by the Asthma Quality of Life Questionnaire (AQLQ) score. In addition, the benefit of antifungal treatment was demonstrated by total IgE levels, respiratory function and rhinitis score. Follow-up 4 months after discontinuation of the assigned therapy showed the AQLQ score had reverted to nearly pre-study levels. Similar findings were observed for the rhinitis score, suggesting that continuing antifungal therapy beyond 8 months is important for maintaining quality of life in these patients. Based on the results from the current cohort and the FAST study, a minimum of 6–8 months of antifungal therapy seems to be required by these patients, although larger studies would be required to evaluate more accurately the ideal duration of antifungal therapy. As with the FAST study,[19] results from this cohort showed that therapy with itraconazole was associated with considerable toxicity. However, few

patients needed the antifungal therapy to be modified due to side effects, and the clinical benefit of antifungal therapy seemed to outweigh any associated risk. In order to minimize the risk of antifungal resistance and toxicity, therapy is best guided by the monitoring of itraconazole serum levels.

Three possible mechanisms of action are postulated to explain the benefits of antifungal therapy for allergic fungal disease. Reduction of the local fungal airway load (e.g. colonization of the lung) may reduce the overall allergic response. Systemic antifungal therapy may also reduce fungal exposure in the sinuses, gut and/or skin. Understanding whether the impact is local to the lung or systemic is important when considering local inhaled antifungal therapy versus systemic therapy.

Alternatively itraconazole may increase inhaled steroid exposure resulting in a more anti-inflammatory effect, although we have no direct evidence for this. Itraconazole (the antifungal drug most frequently used in this study) is known to be an inhibitor of CYP3A4. Patients treated with itraconazole show higher exposure to budesonide[20] and probably fluticasone;[21] beclomethasone has not been tested. Itraconazole has no effect on prednisolone levels (although voriconazole increases prednisolone exposure by approximately 30%). There are also reports of drug interactions using inhaled budesonide and fluticasone in combination with itraconazole resulting in adrenal suppression in patients with ABPA.[21,22]

Immunomodulatory effects could contribute to the response to itraconazole. Itraconazole has been shown to suppress in a dose-dependent manner the secretion of type 2 cytokines (IL-4 and IL-5) in both T cells obtained from patients with atopic dermatitis and healthy controls (58% inhibition by 1 mmol/L of itraconazole).[23] In addition, itraconazole has been shown to suppress the elicitation phase of the allergic contact hypersensitivity reaction in mice challenged with haptens, possibly via inhibition of interferon (IFN)-γ production from hapten-immunized effector T cells.[24] There is no effect of itraconazole on polymorphonuclear leukocyte oxidative metabolism and phagocytosis,[25] whereas suppression of neutrophil random motions, chemotaxis and metabolism (at 10 μg/mL) are demonstrable.[26] Itraconazole also inhibits the generation of allospecific cytolytic activity in human mixed lymphocyte culture (an effect as suppressive as cyclosporine A).[27] In one investigation, a large number of genes encoding chemokines and inflammation-related cytokines were strongly up-regulated in the presence of voriconazole.[28] Hohl *et al*.[29] also showed increased TNF-α production by mouse alveolar macrophages after incubation with *A. fumigatus* in the presence of 0.5 μg/mL voriconazole. These three possible mechanisms are not mutually exclusive.

In summary, results from this retrospective study suggest that both ABPA and SAFS patients benefit from a 6-month course of antifungal therapy, which is in accordance with clinical experience and evidence from a randomized clinical trial. The primary mechanism of azole action in this context as well as the benefit of longer therapy with antifungal drugs requires considerably more research.

ACKNOWLEDGEMENTS

Dr Pasqualotto was supported by The Fungal Research Trust and Georgina Powell was funded by The Moulton Trust.

REFERENCES

1 Denning DW, Van Wye JE, Lewiston NJ, Stevens DA. Adjunctive therapy of allergic bronchopulmonary aspergillosis with itraconazole. *Chest* 1991; **100**: 813–19.
2 Mannes GP, van der Heide S, van Aalderen WM, Gerritsen J. Itraconazole and allergic bronchopulmonary aspergillosis in twin brothers with cystic fibrosis. *Lancet* 1993; **341**: 492.
3 Pacheco A, Martin JA, Cuevas M. Serologic response to itraconazole in allergic bronchopulmonary aspergillosis. *Chest* 1993; **103**: 980–1.
4 Germaud P, Tuchais E. Allergic bronchopulmonary aspergillosis treated with itraconazole. *Chest* 1995; **107**: 883.
5 Salez F, Brichet A, Desurmont S, Grosbois JM, Wallaert B *et al.* Effects of itraconazole therapy in allergic bronchopulmonary aspergillosis. *Chest* 1999; **116**: 1665–8.
6 De Beule K, De Doncker P, Cauwenbergh G, Koster M, Legendie R *et al.* The treatment of aspergillosis and aspergilloma with itraconazole, clinical results of an open international study (1982–1987). *Mycoses* 1988; **31**: 476–85.
7 Stevens DA, Schwartz HJ, Lee JY, MosKovitz BL, Jerome DC *et al.* A randomized trial of itraconazole in allergic bronchopulmonary aspergillosis. *N. Engl. J. Med.* 2000; **342**: 756–62.
8 Wark PA, Hensley MJ, Saltos N, Boyle MJ, Tonegvzzi RC *et al.* Anti-inflammatory effect of itraconazole in stable allergic bronchopulmonary aspergillosis: a randomized controlled trial. *J. Allergy Clin. Immunol.* 2003; **111**: 952–7.
9 Bowyer P, Blightman O, Denning DW. Relative reactivity of aspergillus allergens used in serological tests. *Med. Mycol.* 2006; **44** (Suppl.): S23–8.
10 Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven R. The link between fungi and asthma—a summary of the evidence. *Eur. Respir. J.* 2006; **27**: 615–26.
11 *Bridging the Gap*. A report from the respiratory alliance. Direct Publishing Solutions, Maidenhead, December 2002.
12 Targonski PV, Persky VW, Ramekrishnan V. Effect of environmental molds on risk of death from asthma during the pollen season. *J. Allergy Clin. Immunol.* 1995; **95** (5 Pt 1): 955–61.
13 Khot A, Burn R. Seasonal variation and time trends of deaths from asthma in England and Wales 1960–1982. *BMJ (Clin. Res. Ed.)* 1984; **289**: 233–4.
14 Donnelly SC, McLaughlin H, Bredin CP. Period prevalence of allergic bronchopulmonary mycosis in a regional hospital outpatient population in Ireland 1985–1988. *Ir. J. Med. Sci.* 1991; **160**: 288–90.
15 Carvalho A, Pasqualotto AC, Pitzurra L, Romani L, Denning DW *et al.* Polymorphisms in Toll-like receptor genes and susceptibility to pulmonary aspergillosis. *J. Infect. Dis.* 2008; **197**: 618–21.
16 Knutsen AP. Genetic and respiratory tract risk factors for aspergillosis: ABPA and asthma with fungal sensitization. *Med. Mycol.* 2006; **44** (Suppl. 1): 61–70.
17 Vaid M, Kaur S, Sambatakou H, Madan T, Denning DW *et al.* Distinct alleles of mannose-binding lectin (MBL) and surfactant proteins A (SP-A) in patients with chronic cavitary pulmonary aspergillosis and allergic bronchopulmonary aspergillosis. *Clin. Chem. Lab. Med.* 2007; **45**: 183–6.
18 Sambatakou H, Pravica V, Hutchinson IV, Denning DW. Cytokine profiling of pulmonary aspergillosis. *Int. J. Immunogenet.* 2006; **33**: 297–302.

19 Denning DW, O'Driscoll BR, Powell G, Chew F, Atherton GT *et al.* Randomized controlled trial of oral antifungal treatment for severe asthma with fungal sensitisation (SAFS), the FAST study. *Am. J. Respir. Crit. Care Med.* 2009; **179**: 11–18.
20 Raaska K, Niemi M, Neuvonen M, Neuvonen PJ, Kivistö KT. Plasma concentrations of inhaled budesonide and its effects on plasma cortisol are increased by the cytochrome P4503A4 inhibitor itraconazole. *Clin. Pharmacol. Ther.* 2002; **72**: 362–9.
21 Parmar JS, Howell T, Kelly J, Bilton D. Profound adrenal suppression secondary to treatment with low dose inhaled steroids and itraconazole in allergic bronchopulmonary aspergillosis in cystic fibrosis. *Thorax* 2002; **57**: 749–50.
22 Skov M, Main KM, Sillesen IB, Muller J, Koch C *et al.* Iatrogenic adrenal insufficiency as a side-effect of combined treatment of itraconazole and budesonide. *Eur. Respir. J.* 2002; **20**: 127–33.
23 Kanda N, Enomoto U, Watanabe S. Anti-mycotics suppress interleukin-4 and interleukin-5 production in anti-CD3 plus anti-CD28-stimulated T cells from patients with atopic dermatitis. *J. Invest. Dermatol.* 2001; **117**: 1635–46.
24 Ausaneya U, Kawada A, Aragane Y. Itraconazole suppresses an elicitation phase of a contact hypersensitivity reaction. *J. Invest. Dermatol.* 2006; **126**: 1028–35.
25 Johnson EM, Warnock DW, Richardson MD, Douglas CJ. In-vitro effect of itraconazole, ketoconazole and amphotericin B on the phagocytic and candidacidal function of human neutrophils. *J. Antimicrob. Chemother.* 1986; **18**: 83–91.
26 Vuddhakul V, Mai GT, McCormack JG, Seow WK, Thong YH. Suppression of neutrophil and lymphoproliferative responses in vitro by itraconazole but not fluconazole. *Int. J. Immunopharmacol.* 1990; **12**: 639–45.
27 Pawelec G, Jaschonek K, Ehninger G. The anti-fungal agent itraconazole exerts immunosuppressive effects on alloreactivity but not on natural immunity in vitro. *Int. J. Immunopharmacol.* 1991; **13**: 875–9.
28 Simitsopoulou M, Roilides E, Likartsis C, Ioannidis J, Orfanev A *et al.* Expression of immunomodulatory genes in human monocytes induced by voriconazole in the presence of Aspergillus fumigatus. *Antimicrob. Agents Chemother.* 2007; **51**: 1048–54.
29 Hohl TM, Van Epps HL, Rivera A, Morgan LA, Chen PL *et al.* *Aspergillus fumigatus* triggers inflammatory responses by stage-specific beta-glucan display. *PLoS Pathog.* 2005; **1**: e30.