

***Aspergillus* hypersensitivity in patients with chronic obstructive pulmonary disease: COPD as a risk factor for ABPA?**

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Allergic bronchopulmonary aspergillosis (ABPA) is a pulmonary disorder caused by hypersensitivity to *Aspergillus fumigatus* which primarily complicates the course of asthma and cystic fibrosis. There is a theoretical possibility that patients with chronic obstructive pulmonary disease (COPD) can also develop *Aspergillus* hypersensitivity (AH) and/or ABPA. The aim of this prospective case-control study conducted in the Chest Clinic was to evaluate the prevalence of AH/ABPA in patients with COPD. Two hundred subjects with COPD (17, 62, 74, 47; GOLD guidelines stages I–IV respectively) and 100 healthy volunteers were screened with an *Aspergillus* skin test. Patients were said to have AH if they demonstrated immediate cutaneous hyperreactivity to *A. fumigatus* antigen and those with positive responses were further investigated for ABPA. Of this patient population there were 179 (89.5%) males and 21 (10.5%) females with a mean age of 57.1 in the COPD arm and 88 males and 12 females with a mean age of 52.3 in the control arm. AH was found in 17 (8.5%) patients with COPD as compared to none in the control group. Two (1.0%) COPD patients fulfilled the serologic criteria for the diagnosis of ABPA. On univariate analysis, age of the patient, duration of COPD, smoking index and the COPD severity did not predict the occurrence of AH. On the basis of this study we concluded that AH/ABPA can occur in patients with COPD, and it is probable that COPD could be a predisposing factor for AH/ABPA. The clinical significance of AH and ABPA in COPD remains unclear.

Keywords chronic obstructive pulmonary disease (COPD), *Aspergillus fumigatus*, allergic bronchopulmonary aspergillosis, *Aspergillus* hypersensitivity

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality throughout the world, i.e., the fourth leading cause of death worldwide. In addition, a further increase in its prevalence and mortality is predicted in the coming decades [1]. Tobacco smoking is the most common risk factor for COPD, although in many

countries, air pollution resulting from the burning of wood and other biomass fuels has also been identified as a risk factor [2]. In India, the population prevalence of COPD is 4.1%, with a male to female ratio of 1.56 to one [3].

Members of the genus *Aspergillus* are ubiquitous soil-dwelling organisms and because some are thermophilic, they have a propensity to cause diverse pulmonary syndromes like invasive aspergillosis, aspergilloma, allergic rhinosinusitis and asthma, hypersensitivity pneumonitis, chronic necrotizing aspergillosis, and allergic bronchopulmonary aspergillosis (ABPA) [4]. The latter is a hypersensitivity disease of the lung almost always caused by *Aspergillus fumigatus*. Unlike invasive aspergillosis, lung damage from ABPA is the result of an allergic host response

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to the presence of *Aspergillus* species in the airways. ABPA is a well described clinical disorder, complicating the course of 8–19% of patients with bronchial asthma [5] and 2–15% of those with cystic fibrosis [4]. *Aspergillus* hypersensitivity (AH) is generally defined as the presence of an immediate cutaneous hypersensitivity to commercial extracts of *A. fumigatus* or to an *Aspergillus* mix. AH is generally regarded as the first step in the development of ABPA. The prevalence of AH in chronic asthma varies from 15–48% [5]. The prevalence of AH/ABPA is even higher in patients with severe acute asthma. In a recent study we found that the occurrence of AH and ABPA in patients with severe acute asthma was 51 and 39%, respectively [6]. The pathogenesis of ABPA involves the colonization of the airway mucus by *Aspergillus* spores with subsequent release of antigens by the growth of the fungus. This induces a shift to CD4⁺ Th2 clones and IL-5 (interleukin-5)-mediated eosinophilic airway inflammation. Fungal proteases also cause epithelial damage and IL-8 production with airway neutrophilia and ensuing bronchial wall damage [7].

We have previously reported a case of ABPA complicating the course of a patient with COPD [8]. There is also a biological plausibility of the relationship of COPD and *Aspergillus* species, and ABPA has been causally linked to the development of an acute exacerbation of COPD in animal studies [9]. However, these observations need to be investigated systematically to suggest any causal relationship in pathogenesis and in disease progression of COPD. Hence, the aim of the present study was to systematically evaluate the prevalence of AH and ABPA in patients with COPD.

Patients and methods

Design

This was a prospective case-control study conducted in the Chest Clinic of this institute between July 2007 and September 2008. The study was approved by the Institute's Ethics Committee and written informed consent was obtained from all patients and members of the control group. The cases involved 200 consecutive patients with COPD and the controls were 100 healthy volunteers who had no respiratory symptoms.

Patients

For inclusion in the study, the patients were diagnosed to have COPD as per the following criteria [2]: (i) age more than 40 years, (ii) progressive breathlessness and cough with minimal mucoid expectoration for at least three months in a year for two successive years, (iii) history of smoking defined by smoking index greater than 300 (where the smoking index is the number of cigarettes or bidis (0.2–0.5

grams of raw, dried and crushed tobacco flakes rolled by hand in tendu leaf [*Diospyrus melanoxylon*]) smoked per day multiplied by the number of years and/or significant history of exposure to biomass fuel for at least 8–10 years, and (iv) evidence of obstructive defect on spirometry defined as a ratio of forced expiratory volume in the first second (FEV1) to forced vital capacity (FVC) less than 70% with no bronchodilator reversibility (increment of FEV1 and/or FVC less than 12% and 200 ml after 400 µg of inhaled salbutamol). Patients were excluded from the study if they met any of the following criteria; (i) COPD patients receiving systemic steroid therapy (more than or equal to 10 mg per day for more than 7 days of prednisolone or the equivalent in the preceding 3 weeks), and (ii) patients on any other form of immunosuppressive therapy. The controls were healthy volunteers defined as asymptomatic individuals without any diagnosed illness and were found to be 'normal' on detailed physical examination. The controls were recruited from the healthy attendants accompanying the patients attending the outpatient departments of our institute. Hospital employees and direct family members of the study patients were not recruited as controls. All cases and controls meeting the inclusion criteria were subjected to a clinical history, physical examination followed by an *Aspergillus* skin test and spirometry.

Methods

Aspergillus skin test was performed by injecting 0.2 ml of 1:1000 *Aspergillus fumigatus* antigen (Department of Medical Mycology, PGIMER, Chandigarh) intradermally in the forearm. For control 0.2 ml of phosphate buffer saline was injected intradermally in the other forearm. The injection site was examined every 15 min for 1 h, and after 6 h for type III reactions. For delayed-type reactions, the patients were advised to return after 72 h for evaluation of skin injection sites. The reactions were classified as type I if a wheal and erythema developed within 1 min, reached a maximum after 10–20 min, and resolved within 1 h. The antigen arm skin reaction diameter must have been at least 8 mm or more than seen on the control arm. A type III reaction was observed after 6 h, and any amount of subcutaneous edema were considered to be a positive result. A type IV reaction was read after 72 h, and an induration of more than 5 mm was considered to be a positive result.

Aspergillus hypersensitivity (AH) was defined as the presence of immediate cutaneous hyperreactivity to the *Aspergillus* antigen. Patients who were found to be skin test positive were further evaluated for ABPA through studies of IgE levels (total and *A. fumigatus* specific), *A. fumigatus* precipitins and high-resolution computed tomography (HRCT) of the chest. The patients were considered to have ABPA if they met both of the following

criteria; elevated total IgE levels (more than 1,000 IU per ml) and elevated IgE levels against *A. fumigatus* (more than 0.35 kUA per liter). In addition, the patients must have two of the following criteria; presence of serum precipitating antibodies against *A. fumigatus*, radiographic pulmonary opacities (fixed/transient), absolute eosinophil count more than 1000 per microliter and/or central bronchiectasis on HRCT [10–12].

All subjects performed spirometry on a dry rolling seal spirometer (Spiro RS-232; P.K. Morgan Limited; Kent, UK). Spirometric indices such as forced expiratory volume in the first second (FEV1), forced vital capacity (FVC) and FEV1/FVC ratio were measured before and after administration of 400 µg of inhaled salbutamol using American Thoracic Society guidelines [13]. The highest measurements from among the three technically acceptable and reproducible results were expressed at body temperature and pressure saturated with water vapor. Age, gender, height, and spirometric data were recorded for all patients using software previously developed at our laboratory [14]. The spirometer was frequently calibrated to ensure performance. Predicted values for FVC, FEV1 and FEV1/FVC ratio were generated using previously defined prediction equations [15]. The patients were categorized as mild, moderate, severe and very severe as per the GOLD guidelines. The latter defines (i) stage I (mild) – FEV1/FVC ratio less than 70% with post-bronchodilator FEV1 > 80%; (ii) stage II (moderate) – FEV1/FVC ratio less than 70% with post-bronchodilator FEV1 between 50 and 80%; (iii) stage III (severe) – FEV1/FVC ratio less than 70% with post-bronchodilator FEV1 between 30 and 50%; and (iv) stage IV (very severe) – FEV1/FVC ratio less than 70% with post-bronchodilator FEV1 < 30% and/or presence of cor pulmonale or type 2 respiratory failure [2].

Statistical analysis

Data was analyzed using the statistical package SPSS (SPSS version 10, for Windows; Chicago, IL). Data are presented in a descriptive fashion as mean (95% confidence intervals [CI]) or number (percentage with 95% CI). The normalcy of distribution was evaluated using Kolmogorov-Smirnov test. The differences between continuous variables were analyzed using Mann-Whitney U if not normally distributed and student's *t*-test if normally distributed. The differences between categorical variables were analyzed using Fisher's exact test. Univariate analysis was performed to assess the risk factors for AH among COPD patients. *P* values less than 0.05 were considered as statistically significant.

Results

The 200 COPD patients consisted of 179 (89.5%) males and 21 females (10.5%) with a mean (95% CI) age of 57.1 (55.8–58.4) years. All patients indicated a history of cough with mucoid sputum and exertional dyspnea. A history of atopy was absent in all patients. The baseline characteristic of the 200 COPD patients and 100 controls is shown in Table 1. The majority of the COPD patients were smokers (94%) and the most common pattern was smoking bidi (86%). All 12 non-smokers with COPD (all females) gave a history of prolonged household exposure to biomass fuel. The majority of the patients of COPD (60.5%) belonged to the GOLD stages III and IV.

Aspergillus hypersensitivity was demonstrated in 17(8.5%) patients in the COPD group versus none in the control population. Among the 17 *Aspergillus* skin test positive patients, 14 were male and three were female and 15 were smokers and two had no history of smoking. Type

Table 1 Baseline characteristics of the study population.

Variables	Cases (<i>N</i> = 200)	Controls (<i>N</i> = 100)	<i>P</i> value
Age (years)	57.1 (55.8–58.5)	52.3 (50.5–54.1)	0.001
Male gender, No. (%; 95% CI)	179 (89.5, 84.5–93.0)	88 (88, 80.2–93.0)	0.85
Body mass index (kg/m ²)	21.9 (21.1–22.6)	22.4 (21.5–23.4)	0.26
Duration of COPD (years)	6.1 (5.6–6.7)	–	
History of smoking, No. (%; 95% CI)	188 (94, 89.8–93)	–	
Smoking index	606.4 (541.8–676.1)	–	
FEV1 (% predicted)	47.9 (44.4–51.4)	97.5 (93.7–101.2)	0.0001
FVC (% predicted)	66.4 (61.9–70.8)	97.9 (93.9–101.9)	0.0001
FEV1/FVC ratio	53.4 (51.7–54.9)	77.8 (75.9–79.6)	0.0001
COPD stage (GOLD), No. (%; 95% CI)			
Mild	17 (8.5, 5.4–13.2)	–	
Moderate	62 (31, 24.9–33.7)		
Severe	74 (37, 30.6–43.9)		
Very severe	47 (23.5, 18.2–29.8)		

All values are expressed as mean (95% confidence intervals [CI]) unless otherwise stated.

III skin reaction was observed in only one patient who was also found to have type I positivity. Type IV reaction was not seen in any patient. The 17 patients with AH were further investigated for ABPA. The total IgE levels were elevated above 1000 IU per milliliter in only two patients who also demonstrated elevated *A. fumigatus* specific IgE levels and positive for *Aspergillus* precipitins. High-resolution chest CT was done in these two patients which revealed extensive centrilobular emphysema but did not show any evidence of central bronchiectasis. However, the patients were not treated, and on follow-up at six weeks and three months demonstrated spontaneous improvement in IgE levels with no change in their clinical status.

Univariate analysis was performed to determine the risk factors predicting the occurrence of AH in patients with COPD. There was no relationship between *Aspergillus* hypersensitivity in COPD and age, gender, duration of COPD, smoking index and the severity of COPD as measured by percentage predicted of post-bronchodilator FEV1 (Table 2).

Discussion

This is the first proof-of-concept study to evaluate the presence of AH/ABPA in patients with COPD. The results of our investigation demonstrated the coexistence of COPD and ABPA, and suggest that COPD could also be a predisposing factor in AH/ABPA. The prevalence of AH and ABPA in COPD was 8.5% and 1.0%, respectively. We could not ascertain any specific risk factors associated with AH in patients with COPD. The profile of COPD patients was similar to that previously reported by us in literature, with the vast majority being bidi smokers with advanced lung disease [3].

The pathogenesis of ABPA has not been completely elucidated and it is not known why only a minority of patients with asthma go on to develop ABPA. The first step in the pathogenesis of ABPA is the development of *Aspergillus* hypersensitivity, which is followed by exaggerated host immune response with resultant eosinophilic pulmonary inflammation and lung damage [16]. It is believed that in a

genetically predisposed individual, *A. fumigatus* conidia are trapped in the mucus and narrowed airways of the patient with asthma/CF where they germinate to form hyphae [4]. They then release soluble and particulate antigens which cause marked airway inflammation. The epithelial damage results in diffusion of soluble antigen and mycelial fragments into interstitium with further release of inflammatory mediators, and influx of inflammatory cells, primarily eosinophils [17]. The antigens are also presented to Th2-cells (both Th1 and Th2, but primarily the Th2 CD4+ T-cells) with IL-4, IL-5 and IL-13 cytokine synthesis and secretion. The Th2 response leads to total and *A. fumigatus*-specific IgE synthesis, mast cell degranulation and promotion of a strong eosinophilic response. The inflammatory cells (contributed by proteases of *A. fumigatus*) lead to tissue injury and the characteristic pathology of ABPA [4].

Patients with COPD have mucus hypersecretion and impaired mucociliary clearance and there is a theoretical possibility that they could also develop ABPA during the course of their disease. There is also a biological plausibility of a link between ABPA and COPD as ABPA has been linked to COPD exacerbations in equines [9]. Thus it seems that AH/ABPA may have been under recognized in patients with COPD. However, the prevalence of AH/ABPA is less than half the prevalence of AH/ABPA in patients with bronchial asthma at our center [10–12]. There are several reasons for this lower prevalence [8]. First, ABPA is known to develop in only a small proportion of asthmatics and cystic fibrosis patients, implying that other host factors may be operative. Several host factors including MHC polymorphisms (HLA DR2), IL-10 polymorphisms, surfactant protein A2 and CFTR (cystic fibrosis transmembrane receptor) polymorphisms have been implicated [4]. Probably, such polymorphisms might be under-represented in COPD. Second, the airway inflammation in COPD is different from that in asthma and may not be conducive to the production of ABPA. Third, patients with COPD may have severe functional impairment which may protect them from activities that involve exposure to fungal spores.

We used an indigenous *Aspergillus* antigen for diagnosis of AH and one can question the validity and

Table 2 Univariate analysis of factors predicting occurrence of *Aspergillus* hypersensitivity in patients with COPD.

Variables	<i>Aspergillus</i> hypersensitivity (n = 17)	No <i>Aspergillus</i> hypersensitivity (n = 183)	Crude OR (95% CI)	p value
Age (years)	57.0 (51.9–62.1)	57.0 (55.8–58.5)	0.89 (0.94–1.05)	0.96
Female gender, No. (% , 95% CI)	2 (11.8, 3.3–34.3)	18 (10.9, 7.0–16.6)	1.22 (0.18–5.18)	0.76
Duration of COPD (years)	5.6 (3.9–7.3)	6.17 (5.6–6.8)	0.96 (0.84–1.1)	0.99
Smoking Index	587.4 (309.4–865.3)	608.2 (541.5–674.9)	1.0 (0.99–1.0)	0.86
Severity of COPD (post BD FEV1 % predicted)	52.9 (39.5–66.4)	48.9 (44.8–53.2)	0.59 (0.99–1.02)	0.86

All values are expressed as mean (95% confidence intervals [CI]) unless otherwise stated.

standardization of this product. But the fact that the skin tests were negative in all the 100 healthy controls suggests that the antigen was of good quality. The other important question is the validity of the diagnosis of COPD in this cohort of patients. To avoid confounding with asthmatics, we included only patients aged more than 40 years with a history of smoking and an obstructive defect on spirometry with no bronchodilator reversibility. To further strengthen our diagnosis, the only two patients with AH fulfilling the serologic criteria for ABPA showed centrilobular emphysema and bullae on HRCT. However, it may be possible that some patients with severe asthma who did not have 12% reversibility could, but not likely, be diagnosed as COPD given the criteria used in this paper.

ABPA is rarely reported in lung disorders other than bronchial asthma and cystic fibrosis [4]. By excluding patients with no response to bronchodilators, we have excluded asthmatic patients from this study. Occasionally, ABPA has been reported to complicate other lung diseases like idiopathic bronchiectasis [18], post-tubercular bronchiectasis [19], bronchiectasis secondary to Kartagener's syndrome [20], and chronic granulomatous disease and

hyper IgE syndrome [21]. This suggests that AH/ABPA do not occur exclusively in asthmatic patients. The Rosenberg-Patterson criteria are most often used for the diagnosis of ABPA [22,23], and the presence of six out of eight major criteria provides a relatively firm basis for the diagnosis. However, there is no agreement as to the number of criteria required to establish ABPA. Although widely adopted, the current criteria to define ABPA needs to be revised. For example, there is controversy as to the cutoff value of IgE level that should be used for the diagnosis of ABPA. Most studies have employed a cutoff value of 1000 international units (IU) per milliliter [10,11,23–28], but some authors have used a cutoff value of 500 IU per milliliter [29–32]. We believe that a cutoff of 500 IU per milliliter will contribute to the over diagnosis of ABPA as these levels are often seen in patients with AH without ABPA. Since the diagnosis of ABPA will lead to possible unwarranted steroid therapy, we recommend a cutoff value of 1000 IU per milliliter. An eosinophil count greater than 1,000 cells per microliter is also used as a major criterion for the diagnosis of ABPA. However, 53% of patients in our series [10] had an absolute eosinophil count less than

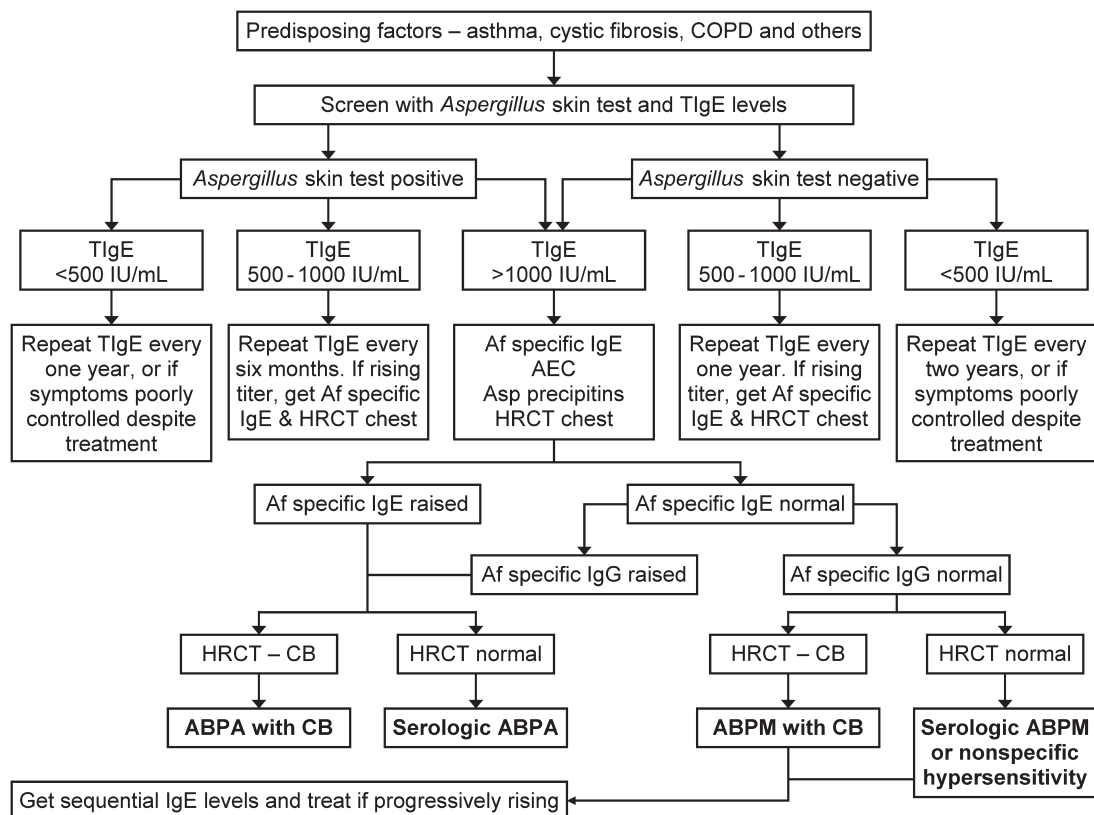


Fig. 1 Proposed screening criteria for allergic bronchopulmonary aspergillosis (ABPA) in patients with predisposing factors (asthma, cystic fibrosis, COPD and others) ABPM, allergic bronchopulmonary mycosis, AEC, absolute eosinophil count, Af, *Aspergillus fumigatus*, Asp, *Aspergillus*, CB, central bronchiectasis, HRCT, high resolution computed tomography, TlgE, total IgE levels.

Table 3 Proposed diagnostic criteria for allergic bronchopulmonary aspergillosis.*Predisposing conditions*

Bronchial asthma, cystic fibrosis, COPD, Others (hyper-IgE syndrome, chronic granulomatous disease, Kartagener's syndrome, occupational disorder in non-asthmatic patients working with soybean products or sugar cane mills)

Obligatory criteria

Elevated total IgE levels (more than 1000 IU per milliliter)

Elevated IgE or IgG levels against *Aspergillus fumigatus* (more than twice the pooled serum samples from patients with *Aspergillus*-hypersensitive asthma; will vary from center to center and should be established at each center)

Other criteria (at least three of five)

Aspergillus skin test – type I positive (immediate cutaneous hypersensitivity to *Aspergillus* antigen)

Presence of serum precipitating antibodies against *Aspergillus fumigatus*

Radiographic pulmonary opacities (fixed/transient)

Absolute eosinophil count more than 1000 cells per microliter

Central bronchiectasis on high resolution computed tomography

1,000 cells per microliter and thus a low eosinophil count does not exclude the diagnosis of ABPA. In view of these shortcomings, we propose new screening (Fig. 1) and diagnostic (Table 3) criteria for the diagnosis of ABPA.

Finally, our study is not without limitations. In general, controls must be selected so that their exposure to risk factors and confounders should be representative of that of the patients. As the controls were non-smoking healthy volunteers, they were not identical with respect to the same qualitative and quantitative risk factors of our patients. Future studies should also include tobacco smoking healthy volunteers so as to match this risk factor. In addition, as a majority of the tobacco smokers were bidi smokers, one might also conclude that the results observed in this study are not fully applicable to non-Indian COPD patients who smoke tobacco other than bidis. One can also postulate that the high frequency of *Aspergillus* allergy in the study was related to bidi being contaminated with *Aspergillus* spores. However, we are not aware of any investigation being conducted on fungal contamination of bidis. Further studies should also include a control group of bidi smokers with no COPD. Finally, we also did not follow these patients over prolonged periods to understand the clinical significance of AH/ABPA in this population. However, at the inception of this study we did not anticipate that such a large proportion of patients with COPD would show AH, and larger studies with longer follow-up duration is required to understand the exact clinical significance of AH/ABPA in pathogenesis of COPD.

Conclusions

The results of the study suggest the co-existence of COPD and AH/ABPA. The clinical significance of AH and ABPA in COPD is not clear. Larger studies with longer follow-up

periods are required to ascertain the exact clinical significance of AH/ABPA in COPD.

Author contributions

RA conceived the idea, recruited the cases and controls, and drafted and revised the manuscript; BH recruited the cases and controls and performed the spirometry; DG recruited the cases and controls, and drafted and revised the manuscript; ANA recruited the cases and controls and the statistical analysis; AC performed the *Aspergillus* skin test, drafted and revised the manuscript; SKJ drafted and revised the manuscript.

Declaration of interest: None.

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