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Original Article

Clinical and immunological characteristics of severe asthma with fungal sensitization and allergic bronchopulmonary aspergillosis: potential utility for differential diagnosis

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Abstract: Fungal-sensitized asthmatic condition SAFS and the Aspergillus-related asthmatic condition ABPA are the focus of intense research. To investigate the clinical and immunological characteristics of severe asthma with fungal sensitization (SAFS) and allergic bronchopulmonary aspergillosis (ABPA) and provide for the early differential diagnosis and treatment of ABPA. The following parameters were measured in the serum of all recruited patients: total IgE (tIgE) levels, specific IgE (sIgE) and IgG (sIgG) antibodies against *Aspergillus fumigatus*, and sIgE antibodies against mixed fungal cultures (sIgE-M.x). Pulmonary function, fractional exhaled nitric oxide, and induced sputa were examined; routine blood tests and computerized tomography of the lungs were performed. For SAFS patients, IgE antibody levels against eight types of fungi were measured. The percentage level of bronchiectasis, ratio of eosinophils in the induced sputa, and total number of blood eosinophils were significantly higher in ABPA than in SAFS. There were no significant differences in lung indicators, (percentage of predicted forced vital capacity [FVC% pred], percentage of predicted forced expiratory volume in 1 s [FEV_1% pred], FEV1%, and FVC). SIgE-M.x, sIgE, and sIgG antibodies against *A. fumigatus*, and tIgE antibodies were significantly higher in ABPA than in SAFS. The clinical reference values for total blood eosinophils, sIgE-M.x antibodies, and sIgE as well as sIgG antibodies against *A. fumigatus* for differential diagnosis of SAFS and ABPA were 0.41 × 10^9/L, 3.22 kU/L, 3.86 kU/L, and 28.75 mgA/L, respectively. This panel should be considered for the differential diagnosis of ABPA and SAFS.

Keywords: Bronchial asthma, allergic bronchopulmonary aspergillosis, fungal allergen, specific IgE

Introduction

Fungi are common allergens, known to exacerbate the severity of asthma in asthmatic patients. Moreover, *Aspergillus fumigatus*, *Penicillium chrysogenum*, *Candida albicans*, *Alternaria alternata*, and *Cladosporium herbarum* are commonly implicated in exacerbating asthma [1-3], while *Setomelanomma rostrata*, *Mucor racemosus*, and *Botrytis cinerea* are less commonly reported. Fungal allergens are widely found both indoors and outdoors [4]. Long-term exposure to an environment containing fungi is correlated with poor management of asthma, an increased incidence of acute asthmatic attacks, higher hospitalization rates, and increased mortality [3, 5-8]. Simon-Nobbe *et al.* [9] additionally reported that over half the patients with asthma were sensitized by fungi. Denning [10] showed that fungal sensitization was prevalent in asthmatic patients who required hospitalization and that the severity of asthma was correlated with the degree of fungal sensitization [11]. However, studies of patients with severe asthma with fungal sensitization (SAFS) suggest that prolonged antifungal therapy has beneficial effects in the context of severe persistent asthma. Furthermore, anti-

fungal treatment has been reported to improve clinical symptoms in patients with allergic bronchopulmonary aspergillosis (ABPA) [12]. In 1952, Hinson [13] described ABPA for the first time as a potentially fatal lung disease triggered by allergic responses to *Aspergillus* antigens in the human bronchi, with inappropriate treatment leading to irreversible pathological damage. SAFS did not reach the diagnostic standard of ABPA, so it is necessary to differentiate from ABPA. At present, bronchiectasis mustn't be recognized in the diagnosis of ABPA, That may be the early stage of ABPA, called serological ABPA (ABPA-S). SAFS and ABPA-S may have a certain degree of overlap. The diagnosis of SAFS patients with mild ABPA inflammation is very similar. Thus, it is believed that SAFS and ABPA represent different stages of the same disease [14, 15]; however, it is difficult to distinguish between them by clinical doctors. Because of the overlapping features of ABPA with asthma, cystic fibrosis, and other diseases, this condition often remains underdiagnosed and there may be a long delay (of up to 10 years) between the first occurrence of symptoms and subsequent diagnosis [16]. Therefore, further understanding of clinical and immunological characteristics of SAFS and ABPA is necessary. The present findings should enable clinical standardization and early diagnosis of SAFS and support early treatment of SAFS to prevent its progression to ABPA.

Materials and methods

Research participants

Sixty-seven patients admitted to the First Affiliated Hospital of Guangzhou Medical University from 2014 to 2016 were recruited in this study. Of these, 31 patients presented with SAFS, and 36 patients presented with ABPA. Diagnosis of SAFS was carried out as recommended by the 2015 Global Initiative for Asthma [17], wherein a positive reaction in the specific IgE (sIgE) antibody test against certain fungi (one or more positive results against eight types of fungi) is indicative of SAFS, and in accordance with the diagnostic criteria proposed by Agarwal *et al.* [14]. ABPA diagnosis was carried out in accordance with the consensus set of diagnostic criteria proposed by Agarwal *et al.* in 2013 [18]. Patients with the following conditions were excluded: active, acute, or chronic pulmonary diseases, severe immune diseases, blood diseases, malignant tumors, coronary heart disease, and hypertension. In addition, pregnant and breastfeeding women and individuals prescribed a daily oral medication were excluded. The following information was recorded for each patient: gender, age, history of asthma and sinusitis, and bronchiectasis status as indicated by lung computerized tomography scanning. Tests were carried out to analyze the levels of sIgE antibodies against mixed fungi (sIgE-M.x), sIgE antibodies against *A. fumigatus* (sIgE-A.f), specific IgG antibodies against *A. fumigatus* (sIgG-A.f), serum total IgE (tIgE), blood eosinophil counts, erythrocyte sedimentation rate (ESR), fractional exhaled nitric oxide (FeNO), induced sputum, and pulmonary function.

Assessment of total IgE and allergen-specific IgE and IgG antibodies

Serum samples for biomarker analysis were stored at -80°C until analysis. sIgE-M.x, sIgE antibodies against eight types of fungi (*A. fumigatus* [sIgE-A.f], *C. albicans* [sIgE-C.a], *P. chrysogenum* [sIgE-P.c], *C. herbarum* [sIgE-C.h], *A. alternata* [sIgE-A.a], *M. racemosus* [sIgE-M.r], *B. cinerea* [sIgE-B.c], and *S. rostrata* [sIgE-S.r]), as well as sIgG-A.f antibodies and serum tIgE, were determined by using a fluoro-immunoassay technique using an UniCAP 1000 instrument (Thermo Fisher Scientific, Uppsala, Sweden) according to the manufacturer's instructions. IgE and IgG measurements were reported in kU/L and mg/L, respectively [19, 20]. A positive sIgE result was set at a value of ≥0.35 kU/L. The detection range was 2-200 mg/L for sIgG. For sIgG levels higher than 200 mg/L, blood samples were diluted 10-fold before testing.

FeNO measurement

For all patients, standard measurements of FeNO (NioxMino, Aerocrine, Sweden) were carried out at a flow rate of 50 mL/s [21] using signals fed back for the control. This test was performed prior to body plethysmography and bronchial provocation to prevent the distortion of FeNO data that may result from the breathing maneuvers involved.

Induced sputum

Sputum induction was carried out according to previous studies [22]. Briefly, salbutamol (200

Table 1. General characteristics of patients

	SAFS (n = 31)	ABPA (n = 36)	*P*-value
Female (number of patients [%])	10 (32.7%)	16 (44.4%)	0.059
Age (years)	46 ± 13	47 ± 15	0.167
Weight (kg)	60.0 (52.0-67.0)	52.5 (48.0-61.2)	0.006
Height (cm)	163 (159-170)	160 (152-168)	0.304
BMI (kg/m^2)	22.2 (19.0-24.5)	21.2 (19.1-23.1)	0.510
Smoking history [number of patients (%)]	4 (12.9%)	9 (25%)	0.423
Obesity history [number of patients (%)]	2 (4.7%)	0 (0%)	0.059
Sinusitis [number of patients (%)]	25 (80.6%)	23 (63.9%)	0.491
Bronchiectasis [number of patients (%)]	11 (25.6%)	32 (88.9%)	0.001
Disease duration (years)	10 (4-30)	10 (3-20)	0.871

ABPA, allergic bronchopulmonary aspergillosis; SAFS, severe asthma with fungal sensitization. BMI, body mass index [weight (kg)/height^2 (m^2)]; Obesity, BMI values greater than 30 kg/m^2; Smoking history, smoking more frequently than once a week, with the cumulative number of smoked cigarette packs >10 per year.

Table 2. Clinical characteristics of the two groups

	SAFS (n = 31)	ABPA (n = 36)	*P*-value
FeNO (ppb)	29 (9-65)	68 (14-92)	0.177
Total leukocytes (10^9/L)	8.62 (5.80-10.46)	8.09 (6.21-10.65)	0.921
Neutrophils (10^9/L)	6.00 (3.30-8.50)	4.70 (3.10-7.50)	0.498
Eosinophils (10^9/L)	0.15 (0.00-0.30)	0.85 (0.30-1.41)	0.001
Induced sputum EOS (%)	11.50 (6.50-45.50)	13.00 (1.50-23.50)	0.047
FVC% pred	84.70 (74.40-99.50)	78.30 (66.00-89.00)	0.408
FEV_1% pred	62.80 ± 22.82	55.005 ± 24.41	0.628
FEV_1%/FVC	60.84 (48.70-69.00)	53.36 (41.53-74.20)	0.514
FEF_{25-75}% pred	22.50 (15.10-51.65)	14.85 (7.45-41.76)	0.062
Erythrocyte sedimentation rate (mm/h)	9 (4-17)	22 (15-40)	0.005
C-reactive protein (mg/L)	0.75 (0.1-4.3)	0.1 (0.1-5.2)	0.457
Glucocorticoids (mg/day)[a]	10 (0-25)	15 (10-20)	0.373
Inhaled corticosteroids (μg/day)[b]	500 (320-640)	320 (320-640)	0.154
1-3-β-D-glucan (pg/mL)	10 (10-17)	10 (10-49)	0.320
Use of glucocorticoids [number of patients (%)]	23 (74.2%)	29 (80.6%)	0.815
Use of antibiotics [number of patients (%)]	12 (38.7%)	21 (58.7%)	0.235

EOS, eosinophil; FeNO, fractional exhaled nitric oxide; FVC% pred, percentage of predicted forced vita capacity; FEV_1% pred, percentage of predicted forced expiratory volume in 1 s. [a]Glucocorticoid use was assessed via the measurement of prednisone acetate. [b]Inhaled corticosteroid use was assessed via the measurement of beclomethasone dipropionate.

μg) was inhaled; 15 min later, two concentrations of saline (0.9% and 3%, for 7 min each) were nebulized and inhaled. Sputum was then induced, and differential counts of inflammatory cells were analyzed in the sputum.

Lung function measurements

Lung function measurements were carried out in asthmatic patients to evaluate the pulmonary ventilation function and the lung function index, including the percentage of predicted forced vital capacity (FVC% pred), percentage of predicted forced expiratory volume in 1 s (FEV_1% pred), and the ratio FEV_1%/FVC, as well as the percentage of predicted peak expiratory flow rate (PEF% pred) and forced expiratory flow (FEF_{25-75}% pred).

Statistical analyses

All analyses were carried out using the IBM SPSS 19.0 software. Results were expressed as the number of patients (%), the mean ± standard deviation for normally distributed variables and the median and interquartile range

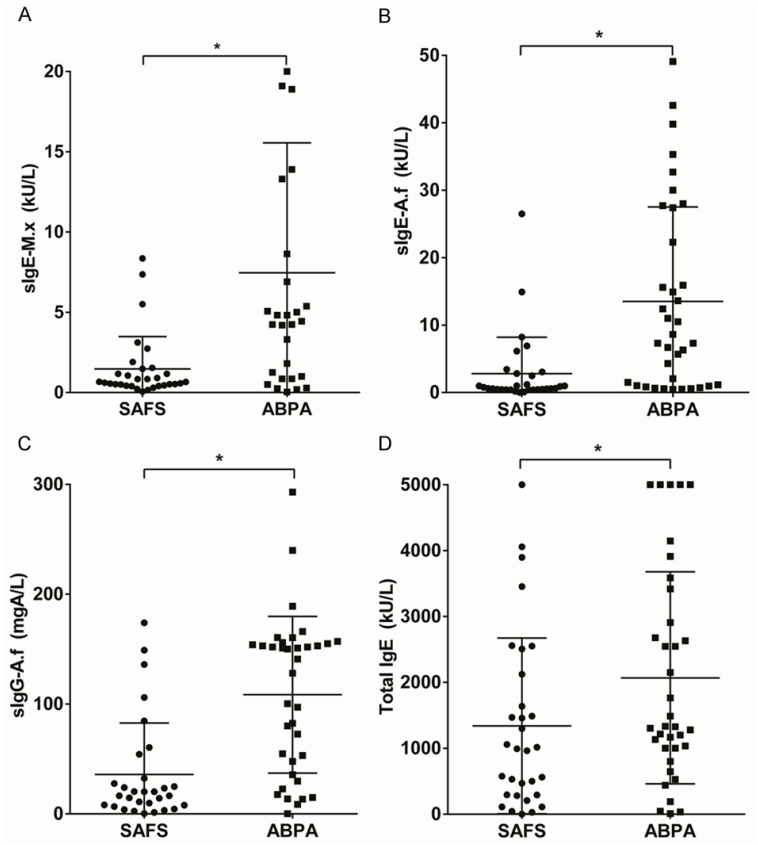

Figure 1. Analysis of sIgE-M.x, sIgE-A.f, sIgG-A.f, and total IgE levels in patients with SAFS and ABPA. ABPA, allergic bronchopulmonary aspergillosis; SAFS, severe asthma with fungal sensitization; sIgE-A.f, specific IgE against *Aspergillus fumigatus*; sIgE-M.x, specific IgE against mixed mycosis agents; sIgG-A.f, specific IgG against *A. fumigatus*. Statistical differences were determined by the Mann-Whitney nonparametric test. The scatter plots show that the levels of sIgE-M.x (A), sIgE-A.f (B), sIgG-A.f (C), and total IgE (D) were higher in the patients with ABPA than in those with SAFS, * $P < 0.01$.

(IQR) for non-normally distributed data, The differences in percentages between two groups were analyzed by the X^2 test. We compared the non-normally distributed data between groups by using the nonparametric Mann-Whitney U test, and Normally distributed data by the unpaired *t*-test. Receiver operating characteristic (ROC) curves were produced using an empirical model. A value of $P < 0.05$ was considered statistically significant (Supplementary Data).

Results

General observations

There were no significant differences in basic indicators such as gender, average age, disease progress, height, body mass index, smoking history, obesity, and sinusitis between the SAFS and ABPA groups. Body weight was significantly lower, while the bronchiectasis rate was significantly higher, in the ABPA group than in the SAFS group (**Table 1**). These results indicated that the patients with ABPA experienced bronchiectasis at a higher rate than those with SAFS and that the bronchial damage was more severe in the former group.

Clinical characteristics

Induced sputum analysis for the two groups showed that eosinophilic inflammation was dominant in both types of patients. The eosinophil percentage (EOS%) in the induced sputum, blood eosinophil counts, and ESR were significantly higher in the ABPA group than in the SAFS group. With respect to pulmonary indicators such as FVC% pred, FEV_1% pred, FEV_1%/FVC, and FEF_{25-75}% pred, there were no statistically significant differences between the ABPA and SAFS groups. The FeNO levels were higher than the normal level (25 ppb) in both groups, suggesting an inflammatory response in these patients; however, there were no statistically significant differences between the two groups (**Table 2**).

Serum immunological indicators

The sIgE-M.x, sIgE-A.f, sIgG-A.f, and serum tIgE levels were significantly higher in patients with ABPA than in those with SAFS (all $P < 0.001$), indicating that the level of sensitization against *A. fumigatus* was significantly higher in the patients with ABPA than in those with SAFS (**Figure 1**).

Analysis of ROC curves of laboratory test indicators

ROC curves were plotted for blood eosinophil counts and sIgE-M.x, sIgE-A.f, and sIgG-A.f lev-

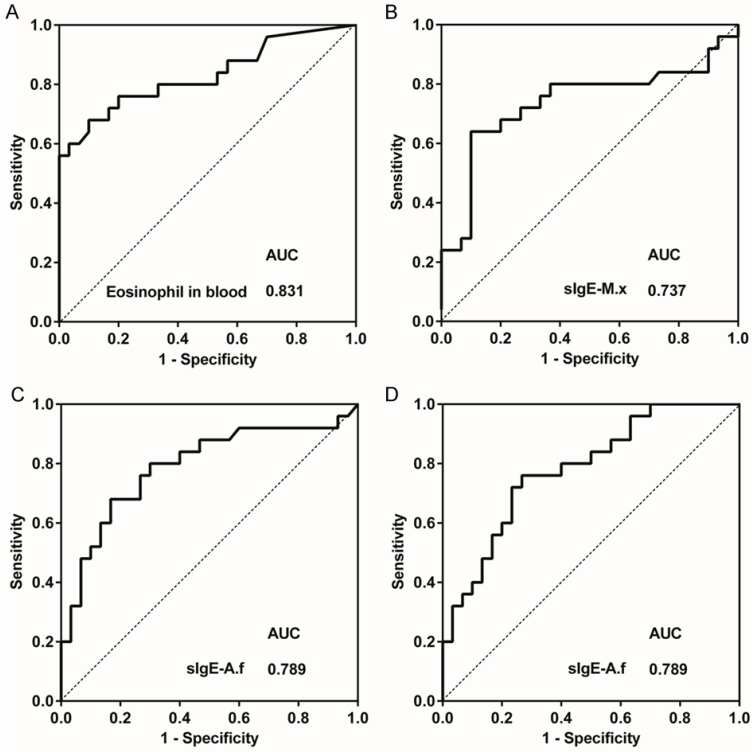

Figure 2. Receiver operating characteristic (ROC) curves of blood eosinophil counts and sIgE-M.x, sIgE-A.f, and sIgG-A.f antibody levels for the diagnosis of ABPA. SIgE-A.f, specific IgE against *Aspergillus fumigatus*; sIgE-M.x, specific IgE against mixed mycosis agents; sIgG-A.f, specific IgG against *A. fumigatus*. Statistical differences were determined by ROC curve analysis. The area under the curve (AUC) for blood eosinophil counts (A) was 0.831, while those for sIgE-M.x (B), sIgE-A.f (C), and sIgG-A.f (D) were 0.737, 0.789, and 0.781, respectively ($P < 0.01$; $P = 0.003$; $P < 0.001$; and $P < 0.001$, respectively).

els in the patients with SAFS and ABPA (**Figure 2**). The areas under the ROC curves (AUCs) of total blood eosinophil counts, sIgE-M.x, sIgE-A.f, and sIgG-A.f were >0.7, with $P < 0.05$ (see **Table 3** for details). The sensitivity levels were >60%; in particular, the sensitivity level of sIgG-A.f was 76.0%. The specificity levels for total blood eosinophil counts, sIgE-M.x, and sIgE-A.f were 90.0%, 90.0%, and 93.0%, respectively. The clinical reference values of total blood eosinophil counts, sIgE-M.x, sIgE-A.f, and sIgG-A.f for differential diagnosis of SAFS and ABPA were 0.41 × 10^9/L, 3.22 kU/L, 3.86 kU/L, and 28.75 mgA/L, respectively. The AUC value for tIgE was 0.626 and not statistically significant ($P = 0.110$). These results demonstrated that the level of sensitivity of sIgG-A.F as a diagnostic tool for ABPA and SAFS was higher than those of the other three indicators, which, however, showed higher specificity. In particular, among the latter three indicators, the use of sIgE-A.f levels enabled the differential diagnosis of ABPA and SAFS with a relatively high specificity.

Discussion

Fungi are a class of organisms that are widespread in nature and closely associated with the lives of humans. Fungal allergens, which are spread via the release of spores and hyphal fragments into the air, may be inhaled to cause severe allergic and bronchopulmonary diseases. Fungal allergens represent independent risk factors for the onset and development of asthma [23]. Fungal sensitization is closely associated with the severity of asthma, frequency of respiratory symptoms and admission into intensive care, and higher mortality rates [24-26]. The relationship between fungal sensitization and asthma has recently received much attention [19]. In particular, the fungal-sensitized asthmatic condition SAFS and the *Aspergillus*-related asthmatic condition ABPA are the focus of intense research [18]. ABPA, a more severe disease with a rapid onset, was first described in 1952 [20]. The first case of ABPA in an adult was reported in the US in 1968 [27]. Subsequently, Wen *et al.* [28] reported three cases of ABPA in China in 1985, and since then, ABPA has been widely recognized as a clinical disorder. ABPA is considered an exacerbated form of *Aspergillus* sensitization, which is likely represents the first step in the development of ABPA. Agarwal *et al.* [29] conducted a meta-analysis of 21 cases of asthma and sensitization by *A. fumigatus* and found that the rate of *A. fumigatus*-induced asthma was 28% in asthmatic patients, while ABPA was observed in 12.9% of asthmatic patients. Almost 40% of *A. fumigatus*-sensitized asthmatic patients meet the diagnostic criteria for ABPA. Therefore, *A. fumigatus*-sensitized asthma is closely associated with ABPA. Although

Table 3. Clinical reference values of blood indicators for the differential diagnosis of SAFS and ABPA

	Clinical reference value	Sensitivity	Specificity	AUC	*P*-value	95% Confidence interval
Eosinophils (10^9/L)	0.41	68.0%	90.0%	0.831	0.001	(0.716-0.945)
sIgE-M.x (kU/L)	3.22	64.0%	90.0%	0.737	0.003	(0.591-0.882)
sIgE-A.f (kU/L)	3.86	68.0%	93.0%	0.789	0.001	(0.663-0.916)
sIgG-A.f (mgA/L)	28.75	76.0%	73.3%	0.781	0.001	(0.661-0.902)

AUC, area under the curve; sIgE-A.f, specific IgE against *Aspergillus fumigatus*; sIgE-M.x, specific IgE against mixed mycosis agents; sIgG-A.f, specific IgG against *A. fumigatus*. Statistical differences were determined by ROC curve analysis. $P < 0.05$ was considered statistically significant.

fungal-sensitized diseases have begun to garner much attention in recent years [18, 30], there is still a lack of studies on SAFS and ABPA among the Chinese population. Chang *et al.* [31] have suggested that specific sensitization to fungal allergens plays a critical role in the pathogenesis of atopic diseases. Zhou *et al.* [32] have published a patient report about ABPA in children and concluded that early diagnosis and initiation of systemic corticosteroids are essential to prevent irreversible damage. In the present work, the clinical and immunological characteristics of SAFS and ABPA were investigated to provide a scientific basis for the clinical standardization and early diagnosis of SAFS and ABPA.

In the present work, over half of the patients from both the SAFS and ABPA groups had sinusitis. Diseases of the upper and lower respiratory tracts are closely associated with each other and often occur concurrently. The rate of bronchiectasis, blood eosinophil counts in induced sputa, ESR, and total blood eosinophil counts were higher in ABPA relative to SAFS. This may be attributed to a higher sensitization level in ABPA as allergenic *A. fumigatus* adheres to and undergoes mycelial growth in the airway epithelium. This fungal activity produces a large amount of harmful substances, such as fumagillin and proteases, which weaken the cilia and destroy the airway, leading to the aggregation of inflammatory cells and induction of allergic responses. Furthermore, the growth of *A. fumigatus* stimulates the differentiation of T cells into type 2 helper T cells and promotes the release of IgE and IgG antibodies. This results in an increase of the number of eosinophils in the airway and peripheral bloodstream, which elicits further pathophysiological changes such as bronchial spasms, mucus accumulation, bronchiectasis, and inflammatory infiltration of the lungs [33].

ABPA differs from SAFS in that it is more difficult to control, and is associated with a poorer prognosis. In addition, ABPA is more likely to exhibit a rapid progression and cause irreversible damage during later stages [34]. However, it is difficult to distinguish ABPA from *A. fumigatus*-sensitized SAFS during clinical diagnosis. Comparison of serum immunological characteristics of the two conditions has shown that the levels of tIgE, sIgE-A.f, and sIgG-A.f antibodies were significantly higher in ABPA than in SAFS [35], and the present findings are consistent with this report. Previous studies have reported that the threshold values for total blood eosinophil counts, tIgE, sIgE-A.f, and sIgG-A.f were 0.507×10^9/L, 2,347 kU/L, 1.91 kU/L, and 40 mgA/L, respectively, for differentiating ABPA from asthma [36, 37]. According to the common diagnostic criteria for ABPA [18], the corresponding values are 0.500×10^9/L, 1,000 kU/L, and 40 mgA/L for the total blood eosinophil counts, tIgE, and sIgG-A.f, respectively. Therefore, there is no definitive diagnostic threshold value for the sIgE-A.f antibody level yet. In this study, comparison of diagnostic thresholds for ABPA and SAFS indicated that the threshold values for total blood eosinophil counts, sIgE-A.f, sIgG-A.f, and sIgE-M.x were 0.410×10^9/L, 3.86 kU/L, 29 mgA/L, and 3.22 kU/L, respectively. The AUCs of the ROC curves for these four indicators were >0.7, and the sensitivity of the sIgG-A.f antibodies and specificity of the sIgE-A.f antibodies were the highest. Therefore, these parameters may be utilized for the differential diagnosis of ABPA and SAFS at the clinical level. The values for total blood eosinophil counts and the levels of sIgE-A.f and sIgG-A.f antibodies obtained in the present study were inconsistent with those reported previously [18]. In addition, to our knowledge, the utility of sIgE-M.x antibodies for the diagnosis of ABPA has not been reported previously. To date, the diagnosis of ABPA in

China has relied on the standards established in other countries. Hence, a variety of factors specific to the Chinese population, such as the ethnic background, climate, population characteristics, host and fungal gene expression, history of drug use, and autoimmune status, have not been considered. Therefore, we believe it is imperative to establish a set of diagnostic criteria specific to the Chinese population in order to better define the characteristics of ABPA and to implement methods for the early diagnosis and timely treatment of the disease.

Since this was a retrospective cohort study, part of the clinical symptom data and detection results were not available. Furthermore, analysis of clinical symptoms and post-treatment follow-up data were omitted from this study. Future studies should aim to obtain all relevant clinical indicators for an in-depth analysis of the clinical and immunological characteristics of SAFS and ABPA.

Conclusions

SAFS is a typical asthma disease with fungal sensitization, and fungi can worsen asthma symptoms. If a specific case of asthma is found to be triggered by fungal sensitization, further testing of total blood eosinophil counts and sIgE-A.f, sIgG-A.f, and sIgE-M.x antibodies is necessary. Comparison of the threshold values with those for ABPA should enable early diagnosis and treatment. Therefore, this study provides an important clinical basis for the differential diagnosis, prevention, and treatment of SAFS and ABPA.

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Disclosure of conflict of interest

None.

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