



Characteristics of severe asthma with fungal sensitization

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ARTICLE INFO

Article history:

Received for publication April 25, 2017.

Received in revised form June 11, 2017.

Accepted for publication July 5, 2017.

ABSTRACT

Background: Some patients with severe asthma also have fungal sensitization and are considered to have severe asthma with fungal sensitization. However, there is limited information on the clinical features of SAFS.

Objective: To investigate the clinical characteristics of severe asthma with fungal sensitization.

Methods: The present study enrolled 124 patients with severe asthma. We evaluated clinical aspects, such as various serum cytokines, fractional exhaled nitric oxide, pulmonary function, and serum immunoglobulin E (IgE). Fungal sensitization was assessed by determining serum levels of IgE specific to fungal allergens (*Aspergillus*, *Alternaria*, *Candida*, *Cladosporium*, *Penicillium*, and *Trichophyton* species and *Schizophyllum commune*). The protocol was registered at a clinical trial registry (www.umin.ac.jp/ctr/index-j.htm; UMIN 000002980).

Results: Thirty-six patients (29%) showed sensitization to at least 1 fungal allergen. The most common species were *Candida* (16%), *Aspergillus* (11%), and *Trichophyton* (11%). The rate of early-onset asthma (<16 years of age) was higher in patients with fungal sensitization than in those without fungal sensitization (45% vs 25%; $P = .02$). Interleukin-33 levels were higher in patients with fungal sensitization than in those without fungal sensitization. Of patients with atopic asthma, Asthma Control Test scores were worse in patients with multiple fungal sensitizations than in patients with a single fungal sensitization or those without fungal sensitization.

Conclusion: Severe asthma with fungal sensitization is characterized by early onset of disease and high serum levels of interleukin-33. Multiple fungal sensitizations are associated with poor asthma control.

Trial Registration: UMIN Clinical Trials Registry (UMIN-CTR; www.umin.ac.jp/ctr/index-j.htm): UMIN 000002980.

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Disclosures: Dr Izuhara is a scientific advisor of Chugai Pharmaceutical Company and a collaborator of Shino-test Co, Ltd. Other authors have nothing to disclose.

<http://dx.doi.org/10.1016/j.anai.2017.07.008>

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Funding Sources: This study was supported in part by a Research Grant on Allergic Disease and Immunology from the Japan Agency for Medical Research and Development (Koichiro Asano, MD) and a Grant-in-Aid from GlaxoSmithKline, PLC.

Introduction

Asthma is an airway inflammatory disease characterized by bronchial hyperresponsiveness and reversible airflow limitation. Although asthma has been considered a single disease, recent studies have identified its heterogeneity.¹ Airway inflammation can generally be controlled with inhalation of corticosteroids. However, patients with severe asthma can continue to experience respiratory symptoms that are refractory to the highest tolerable doses of inhaled corticosteroids and antiasthma drugs, including long-acting bronchodilators and leukotriene receptor antagonists.² Because the heterogeneous characteristics of severe asthma prevents control with simple antiasthma drugs, such as inhaled corticosteroids, it is essential to consider the asthma phenotype.³ Treatment for patients with severe asthma should be based on the specific molecular mechanism identified in each patient. For example, serum periostin level has been shown to be a reliable biomarker of interleukin (IL)-13 activity in vivo, reflecting persistent eosinophilic inflammation in the airways.⁴

Atopic status is a prominent characteristic of severe asthma, and many allergens, including indoor and outdoor fungi, can exacerbate the disease.^{5,6} As many as 20% of patients with severe asthma will have evidence of fungal sensitization by the skin prick tests or in vitro tests for fungus-specific immunoglobulin (Ig) E.⁷ Fungal sensitization is associated with increased asthma severity, symptoms, and morbidity and decreased pulmonary function.^{5,8,9} Severe asthma with fungal sensitization (SAFS) has been described as a specific phenotype in patients with severe asthma.^{10,11} Although an association between fungal sensitization and increased morbidity is apparent, whether such an association is causal remains unconfirmed.^{12,13}

The present study aimed to clarify the characteristics of patients with SAFS and to investigate potential predictive biomarkers for new therapeutic strategies in these patients.

Methods

Study Population

We enrolled 124 Japanese patients at least 20 years old who presented with severe asthma from April 1, 2010 through December 31, 2012.¹⁴ Severe asthma was defined by criteria of the 2015 version of the Global Initiative for Asthma (GINA). Asthma requiring step 4 or 5 treatment actions (such as inhaled corticosteroids, long-acting β -agonists or muscarinic antagonists, and anti-IgE drugs) to achieve optimum control was considered severe asthma.¹⁵ Patients with malignant tumors or widespread lung disease were excluded from the study. Patients with positive *Aspergillus*-precipitating antibodies or with bronchiectasis at computed tomography were excluded. The living environment of the patients registered in this study was similar to that of patients who lived in nearly the same area (urban area around Tokyo in Japan). Therefore, there was no major difference in climate, environment, etc, among patients. The study protocol (number 2009-9-5) was approved by the institutional review board of each participating institution. The protocol was implemented in compliance with the Declaration of Helsinki. All participants provided written informed consent. The protocol was registered at a clinical trial registry (UMIN 000002980; www.umin.ac.jp/ctr/index-j.htm).

Collection of Clinical Information

At registration, clinical information was obtained using a self-completed questionnaire. The control status of asthma and disease-specific quality of life were determined using Japanese versions of the Asthma Control Test (ACT)¹⁶ and the Asthma Quality of Life Questionnaire,^{17,18} respectively. Laboratory data and

medication use were obtained from medical records. Poor adherence was defined as skipping inhaled corticosteroids more than once per week or nonrecognition of the existence of controller therapy.

Serum Levels of Cytokines and Periostin

Serum levels of IL-5, IL-13, IL-17, IL-23, IL-33, and thymic stromal lymphopoietin were measured using Quantikine Enzyme-Linked Immunosorbent Assay kits (R&D Systems, Minneapolis, Minnesota). Serum levels of IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12, interferon- γ , and tumor necrosis factor- α were measured using the Bio-Plex Suspension Array System (Bio-Rad Laboratories, Hercules, California). Serum periostin level was measured using the Human Periostin Enzyme-Linked Immunosorbent Assay kit (Shino-Test Corporation, Sagamihara, Japan) as previously reported.¹⁹

Evaluation of Sensitization and Atopic Status

Total serum IgE and IgE specific for allergens, including fungi (*Aspergillus*, *Alternaria*, *Candida*, *Cladosporium*, *Penicillium*, and *Trichophyton* species); house dust mites, cat and dog dander, and insects, were measured using fluorescence-enzyme immunoassay (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). Levels of IgE specific for the protein identified as the major allergen of *Schizophyllum commune* were measured by purified glucoamylase Sch c 1.²⁰ Atopic asthma was defined as levels of IgE specific for at least 1 allergen higher than 0.35 UA/mL or high IgE titers against Sch c 1.

Pulmonary Function Tests

Pulmonary function, during stable asthma, was measured using a CHESTAC-9800 spirometer (Chest, Tokyo, Japan), which accommodates criteria of the American Thoracic Society. The predicted values of forced expiratory volume in 1 second for a Japanese population were calculated using the lambda-mu-sigma method proposed by the Japanese Respiratory Society.²¹ The fraction of exhaled nitric oxide was measured using a Sievers Nitric Oxide Analyzer (GE Healthcare Japan, Tokyo, Japan).

Statistical Analysis

Data are expressed as mean $\pm$ SD, median and interquartile range, or percentage. Categorical data were analyzed using the χ^2 , Mann-Whitney, Kruskal-Wallis, or Jonckheere-Terpstra test, as appropriate. A statistically significant difference was defined as a 2-tailed *P* value less than .05. All statistical analyses were performed using SPSS 22.0 for Windows (IBM Corporation, Armonk, New York).

Results

Characteristics of Study Patients

One hundred twenty-four patients (48% men) with severe asthma were enrolled in the study. The median age was 61 years (range 21–85). Using the GINA 2015 criteria,¹⁵ 71 patients received step 4 treatment and 53 received step 5 treatment (Table 1).

Frequencies of Sensitization to Fungal Antigens in Severe Asthma

As presented in Table 2, the frequencies of sensitization to fungal allergens were 16% for *Candida* species (*n* = 20), 11% for *Aspergillus* species (*n* = 14), 11% for *Trichophyton* species (*n* = 14), 8% for *Penicillium* species (*n* = 10), 4% for *Cladosporium* species (*n* = 5), 2% for *Alternaria* species (*n* = 3), and 2% for *S commune* (*n* = 3). Thirty-six patients (29%) showed sensitization to at least 1 fungal allergen. The most common allergen was house dust mite (48%), and frequencies of sensitization to cat dander, dog dander, cockroach,

Table 1
Patient Characteristics^a

	GINA step 4 (n = 71)	GINA step 5 (n = 53)	Overall (n = 124)
Men (%)	55	38	48
Age (y)	61 (26–84)	63 (21–85)	61 (21–85)
Age at onset of asthma (y)	39 (0–74)	26 (0–83)	33 (0–83)
Early onset (%)	25	42	32
Body mass index (kg/m ²)	23.9 ± 4.0	24.6 ± 5.8	24.2 ± 4.8
History of smoking (%)	52	42	48
Smoking (pack-years)	12.0 ± 19.4	7.0 ± 17.6	9.8 ± 18.7
Eosinophils/ μ L of blood	499 ± 496	465 ± 616	484 ± 549
Eosinophils (%)	7.5 ± 6.6	6.2 ± 7.7	7.0 ± 7.1
Total serum IgE (IU/mL)	380 (5–22,000)	250 (6–2,295)	319 (5–22,000)
Serum periostin (ng/mL)	80.2 ± 42.2	80.0 ± 37.5	80.0 ± 40.1
FEV ₁ /FVC (%)	64.8 ± 13.0	65.1 ± 16.1	65.0 ± 14.3
Predicted FEV ₁ (%)	77.7 ± 21.1	69.3 ± 21.3	74.1 ± 21.5
Fractional exhaled nitric oxide (ppb)	51.0 ± 58.9	44.8 ± 32.1	48.9 ± 51.0
ACT score	20.5 (5–25)	20 (11–25)	20 (5–25)
ACT score < 20 (%)	40	51	45
AQLQ score (overall)	4.91 ± 1.16	4.58 ± 1.16	4.78 ± 1.16

Abbreviations: ACT, Asthma Control Test; AQLQ, Asthma Quality of Life Questionnaire; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; GINA, Global Initiative for Asthma; IgE, immunoglobulin E.

^aValues represent proportions of patients in each study group, mean ± SD, or median (minimum–maximum).

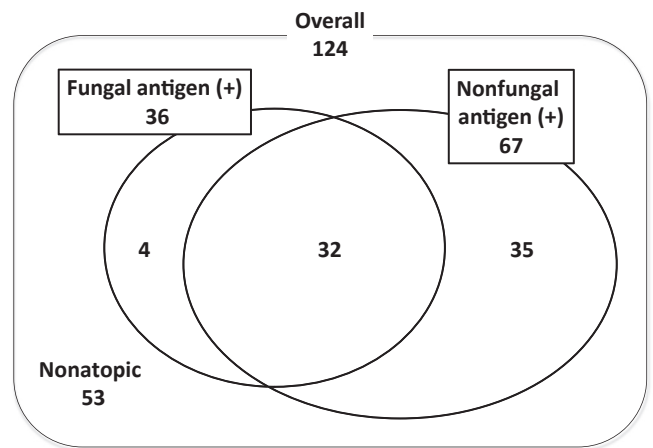
moss, and *Chironomidae* species were 15%, 15%, 12%, 24%, and 14%, respectively. A Venn diagram was used to identify the distribution of fungal and/or nonfungal sensitization (Fig 1). Four patients (3%) had only fungal sensitization, 35 (28%) had nonfungal sensitization, and 32 (26%) had fungal and nonfungal sensitization.

Clinical Features of Patients With and Without Fungal Sensitization

To clarify the characteristics of patients with SAFS, we compared the clinical features of patients with fungal sensitization (n = 36) with those without fungal sensitization (n = 88; Table 3). Age at onset was younger in patients with fungal sensitization (Fig 2), and the rate of early onset (<16 years of age) was higher in patients with fungal sensitization (47% vs 26%; $P = .02$). However, there were no significant differences in body mass index, smoking history, and treatment intensity. Although the ACT scores of the SAFS group were worse than those of the non-SAFS group, there were no significant differences between groups. Biomarkers, such as eosinophil count, fraction of exhaled nitric oxide, total IgE, periostin, and pulmonary function test results, were not different between patients with and without fungal sensitization.

Table 2
Frequency of Sensitization to Each Allergen

	n	%
Atopic type	71	57
Fungal antigen	36	29
<i>Candida</i> sp	20	16
<i>Aspergillus</i> sp	14	11
<i>Trichophyton</i> sp	14	11
<i>Penicillium</i> sp	10	8
<i>Cladosporium</i> sp	5	4
<i>Alternaria</i> sp	3	2
<i>Schizophyllum commune</i>	3	2
Nonfungal antigen	67	54
House dust mite	60	48
Moss	30	24
Cat	19	15
Dog	19	15
Chironomidae	17	14
Cockroach	15	12

**Figure 1.** Venn diagram illustrates overall rate and interrelations between fungal and nonfungal sensitization to at least 1 allergen.

We measured cytokine levels to determine the effect of fungal sensitization on serum cytokine production (Fig 3). Serum IL-33 levels were significantly higher in patients with fungal sensitization ($768 \pm 1,281$ pg/mL) than in those without fungal sensitization (307 ± 812 pg/mL). There were no differences between the 2 groups in the level of other cytokines (IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12, interferon- γ , and tumor necrosis factor- α ; data not shown).

Clinical Characteristics of Patients With Severe Atopic Asthma and Multiple Fungal Sensitizations, Patients With a Single Fungal Sensitization, and Those With Nonfungal Sensitization

As shown in Figure 1, 71 patients had atopic asthma with IgE production for at least 1 antigen. Of patients with atopy, 16 showed sensitization to multiple fungal antigens, 20 showed sensitization to a single fungal antigen, and 35 showed sensitization to only non-fungal antigens. The clinical characteristics of patients with atopic asthma, according to the number of fungal sensitization, are presented in Table 3. There were no significant differences in demographic and clinical characteristics; however, the ACT score was significantly worse in patients with multiple fungal sensitizations (Fig 4). Multiple fungal sensitizations were associated with poor asthma control.

Discussion

Fungal sensitization has been associated with increased asthma severity and mortality.^{5,8} Fukutomi and Taniguchi²² investigated the prevalence of positivity to fungal allergens in 1,288 adult patients with asthma and reported that the prevalences were approximately 45% for *Candida* species, 10% for *Aspergillus* species, 10% for *Alternaria* species, 10% for *Penicillium* species, 7% for *Trichophyton* species, and 5% for *Cladosporium* species. In our study, the prevalence of fungal sensitization was approximately 30%, which was higher than sensitization to animal dander and insects, although fungal sensitization was not associated with the GINA treatment steps.

Patients with SAFS had a younger onset compared with patients without fungal sensitization. Early onset is a typical characteristic of atopic asthma. House dust mites and animal dander are common allergens for patients with early-onset asthma. Sensitization and exposure to fungal allergens pose a significant risk for increased morbidity in children with asthma.⁶ Peat et al²³ reported that not only house dust mites, but also *Alternaria* species, can increase bronchial responsiveness in sensitized children, especially in dry, rural regions. Castanhinha et al²⁴ described the relation between

Table 3
Clinical Features of Patients With and Without Fungal Sensitization

	With fungal sensitization (n = 36)	Without fungal sensitization (n = 88)	P value
GINA steps 4/5	21/15	50/38	—
Men (%)	58	43	.13
Age (y)	53 (26–77)	63 (21–85)	<.01
Age at onset of asthma (y)	25 (0–73)	39 (0–83)	.03
Early onset (%)	47	26	.02
Body mass index (kg/m ²)	24.3 ± 4.8	24.1 ± 4.9	.90
History of smoking (%)	50	47	.73
Smoking (pack-years)	11.0 ± 18.7	9.3 ± 18.8	.66
Eosinophils/μL of blood	570 ± 707	449 ± 468	.27
Eosinophils (%)	7.8 ± 8.4	6.6 ± 6.5	.43
Total serum IgE (IU/mL)	1,018 (380–8,400)	205 (5–2,053)	.03
Serum periostin (ng/mL)	80.6 ± 55.1	79.7 ± 32.4	.91
FEV ₁ /FVC (%)	64.6 ± 15.2	65.1 ± 14.0	.87
Predicted FEV ₁ (%)	75.0 ± 21.8	73.8 ± 21.6	.79
Fractional exhaled nitric oxide (ppb)	50.9 ± 50.1	47.8 ± 52.2	.83
ACT score	17 (5–25)	20 (5–25)	.15
ACT score < 20 (%)	54	41	.19
AQLQ score (overall)	5.0 ± 1.1	4.7 ± 1.2	.29
Daily dose of inhaled corticosteroids (μg) ^a	781 ± 334	735 ± 274	.44
Daily oral corticosteroids (%)	35	40	.62
Omalizumab (%)	17	9	.29
Long-acting β ₂ -agonists (%)	86	86	.97
Leukotriene receptor antagonists (%)	81	82	.87
Long-acting muscarinic antagonists (%)	25	22	.82
Theophylline (%)	47	60	.19

Abbreviations: ACT, Asthma Control Test; AQLQ, Asthma Quality of Life Questionnaire; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; GINA, Global Initiative for Asthma; IgE, immunoglobulin E.

^aDose of inhaled corticosteroids is presented as fluticasone propionate equivalent.

fungal sensitization and severe pediatric asthma and reported that children with SAFS had earlier symptoms, higher total IgE, and non-fungal inhalant allergen-specific IgE. These studies indicate the importance of fungal allergens in patients with early-onset asthma.

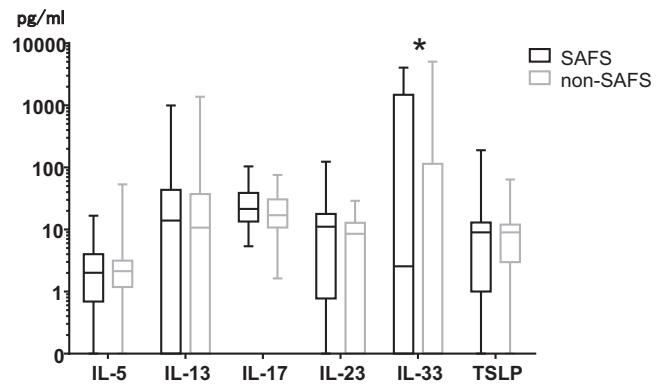
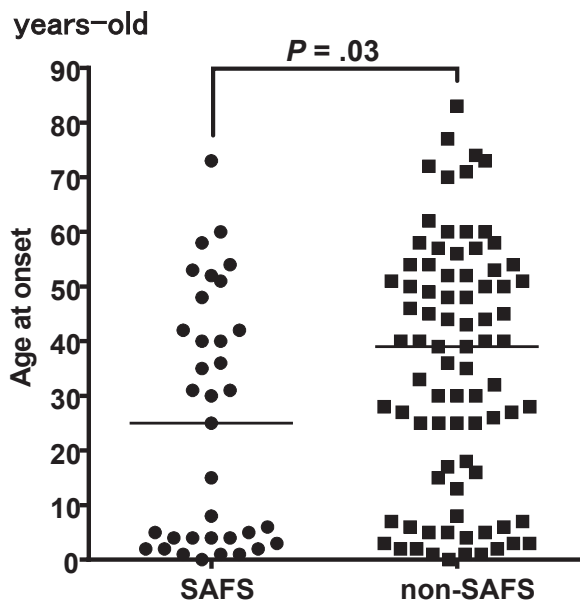


Figure 3. Boxplot illustrates serum cytokine levels of patients. Levels of serum IL-33 were significantly higher in patients with fungal sensitization than in those without fungal sensitization. IL, interleukin; SAFS, severe asthma with fungal sensitization; TSLP, thymic stromal lymphopoietin.

For the effect of fungal sensitization on other allergens, a previous study in mice reported that sensitization to multiple allergens could result in chronic asthma. A combination of ragweed and *Aspergillus* species influenced dendritic cell signaling and T-cell stimulation and prevented the development of tolerance to a single allergen,²⁵ suggesting that young patients without fungal sensitization might not develop severe asthma. In our data of patients with atopy, 35 patients were classified as having non-SAFS and 36 were classified as having SAFS. However, because our study was a cross-sectional study, we could not identify the causal relation between early-onset asthma and fungal sensitization.

Epidemiologic studies have associated fungal sensitization with exacerbation and severity of asthma, and the effect of fungal sensitization on disease etiology and basic experiments has been reported.^{5,6,8,9,23,25} Our data suggest that an increased number of fungal sensitizations worsens asthma control, and our study is the first to report an association between the number of fungal sensitizations and disease control.

Patients with fungal sensitization had high serum IL-33 levels. IL-33 is required for protease allergen-induced airway inflammation and is a crucial activator of type 2 innate lymphoid cells in allergic inflammation.²⁶ Matsuwaki et al²⁷ reported that eosinophils are equipped with an innate cellular activation machinery that responds to an extracellular aspartate protease secreted by *Alternaria* species. IL-33 is involved in the pathogenesis of severe steroid-resistant asthma.²⁸ Kabata et al²⁹ reported that corticosteroid resistance in the IL-33 and type 2 innate lymphoid cells axis

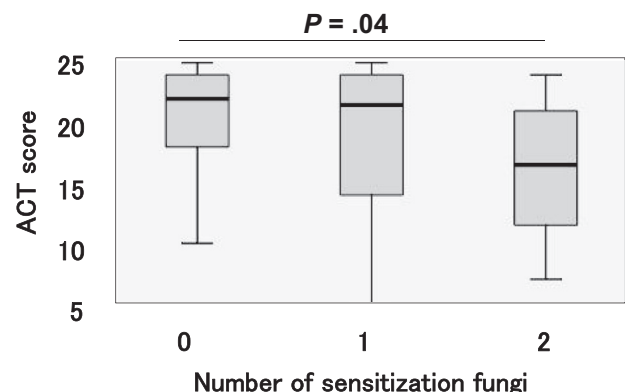


Figure 4. Boxplot illustrates that the ACT score was significantly worse in patients with multiple fungal sensitizations than in other patients. ACT, Asthma Control Test.

was triggered by thymic stromal lymphopoietin through control of STAT5 phosphorylation. Guo et al.³⁰ measured higher serum IL-33 levels in patients with asthma than in controls. Severe asthma in children with fungal sensitization has been associated with high serum IL-33 levels.^{24,31} Castanhinha et al.²⁴ reported that SAFS was associated with high airway IL-33 levels and hypothesized that IL-33 might be a novel therapeutic target for patients with SAFS. After long-term exposure to house dust mite in an adult murine model, IL-33 was found to contribute to the development of fungal exacerbation of allergic airway diseases.³² Fungi could induce IL-33 production and worsen disease control. Our results support the hypothesis that blockade of IL-33 might be a novel therapeutic strategy in severe asthma.

In our study, clinical results, such as concomitant disorders, were based on answers to the self-administered questionnaire and not on objective assessments. In addition, this was a cross-sectional study. Therefore, patients with asymptomatic concomitant diseases might have been missed in the analyses. From the viewpoint of cross-reactions to fungal antigens (Fig 1), positive IgE test reaction does not represent genuine sensitization, and the diagnosis of sensitization made by investigation of allergen-specific components, such as Asp f 1 for *Aspergillus fumigatus* sensitization, is more accurate.³³

In conclusion, SAFS shows early onset and high serum levels of IL-33. Blockade and neutralization of IL-33 might be effective in patients with SAFS. However, further studies are needed to confirm this effectiveness.

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