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Serological analysis of sensitization in allergic bronchopulmonary aspergillosis: a study on allergen components and interspecies relationships

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ABSTRACT

Background: Serological testing for immunoglobulin (Ig) E or IgG is useful for diagnosing allergic bronchopulmonary aspergillosis (ABPA), as it detects type I and III allergic reactions to *Aspergillus* species. However, few reports have investigated the allergen component and cross-reactivity among *Aspergillus* species. We aimed to measure and analyze the levels of IgGs specific to each *Aspergillus* species and investigate the prevalence of IgEs specific to each allergen component of *A. fumigatus* (Af) in ABPA patients.

Methods: Serum samples were collected from 12 ABPA patients who visited our hospital between February and December 2017, and 16 with Af-sensitized asthma and 41 with Af-unsensitized asthma were controls. Immuno-CAP was performed to analyze the IgEs and IgGs specific to Af, *A. niger*, *A. flavus* and *A. terreus*, and IgEs specific to allergen components Asp f 1, 2, 3, 4 and 6.

Results: The ABPA group was significantly more frequently sensitized to Asp f 1 and 2 than the control groups. Af-specific IgEs were significantly positively correlated to the IgEs specific to *A. flavus*, *A. niger* and *A. terreus*. Af-specific IgGs were positively correlated to the IgGs specific to all the other species.

Conclusions: Tests using allergen components were useful for ABPA diagnosis. Both IgE and IgG were highly cross-reactive among the *Aspergillus* species. There were many patients apart from asthmatic patients with ABPA, who displayed high *Aspergillus* IgG values.

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Introduction

The presence of allergic bronchopulmonary aspergillosis (ABPA) was first reported in 1952 by Hinson et al. [1] In this disease, type I and III allergic reactions are caused through the inhalation of *Aspergillus* spores. It is reported to affect 1% to 2% of all adult patients with asthma and is characterized by the presence of recurrent infiltrates and central bronchiectasis. As the repeated onset of this disease can cause irreversible changes, such as bronchiectasis, cystic changes and fibrosis, early diagnosis is important. The known diagnostic criteria for ABPA include those developed by Rosenberg (1977) [2], Greenberger (1988) [3], and the International Society for Human and Animal Mycology working group (2013) [4], all of which emphasize the importance of performing a skin or blood test to confirm the presence of allergic reactions

to *Aspergillus*. In the blood test, the levels of *Aspergillus*-specific immunoglobulin (Ig) E and IgG antibodies or precipitating antibodies are measured for the detection of type I and III allergic reactions, which are major pathological conditions of ABPA.

A recent report indicated that ABPA is not always accompanied by asthma [5]. Patients with ABPA include those with few subjective symptoms and those without an abnormal shadow detectable on the performance of a chest roentgenogram in the early stages. In some cases, necessary tests are not performed promptly; consequently, the diagnosis of ABPA is delayed. Thus, there is a need for specific diagnostic markers that can pathologically differentiate between simple sensitization to *Aspergillus* and ABPA. In recent years, molecular diagnostic techniques using allergen components have been proposed for the treatment of allergic diseases. Regarding ABPA, the Molecular Allergy User's Guide, issued by the

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European Academy of Allergy and Clinical Immunology in 2016, cites a report by Cramer et al. [6] indicating that patients with ABPA are specifically sensitized to Asp f 2, 4 and 6. However, as no large-scale study has focused on the distinction between *Aspergillus*-sensitized asthma and ABPA by allergen component, the guide concludes that this sensitization has still not been verified [7]. Tanimoto et al. reported that as patients with ABPA are frequently sensitized to Asp f 1 and 2, these allergens are useful for the diagnosis of ABPA [8]. Based on this report, Fukutomi et al. also advocate the effectiveness of using allergen components for ABPA diagnosis [9]. Testing for allergen components may allow for the early diagnosis of ABPA before the occurrence of irreversible changes. Thus, we investigated the prevalence of IgEs specific to each allergen component of Af in all ABPA patients treated at our hospital and determined which allergen component they were specifically sensitized to.

Tests to detect type III allergic reactions in ABPA include the measurement of precipitating antibodies and IgG. Agarwal et al. reported that testing for precipitating antibodies to Af has low sensitivity (27.4%) in the diagnosis of ABPA, and that the measurement of *Aspergillus*-specific IgG levels with a cutoff level of 26.9 mg_A/L is useful for ABPA diagnosis (sensitivity of 88% and specificity of 100%) [10].

A Japanese study reported that, with a cutoff Af-specific IgG level of 26.9 mg_A/L, 39 of 53 patients with ABPA (75%) were positive for IgG, and that patients who were positive only for Af-specific IgGs but negative for precipitating antibodies accounted for 27% of all the patients [11].

Based on combined findings from these reports, the measurement of *Aspergillus*-specific IgG levels appears to be important for the detection of type III allergic reactions in the diagnosis of ABPA. Thus, we measured and analyzed the levels of IgGs specific to each *Aspergillus* species in patients with ABPA or asthma, who were treated at our hospital.

We previously reported that the Af-specific IgE levels differed between Immuno-CAP and multiple specific IgE assays [12]. We assumed that this result was attributed to the difference in the amounts of solid-phase allergens in each of those test kits. As allergen components were measured in the present study, we additionally sought to determine which allergen component was responsible for the difference in the measurements between these assays.

Methods

The serum samples used were collected from the following patients who visited Fujita Health University Second

Education Hospital between February and December 2017 and consented to participate in the present study: 12 patients with ABPA, and controls comprising 16 patients with *Aspergillus*-sensitized asthma (with-asp group) and 41 with *Aspergillus*-unsensitized asthma (without-asp group). Bronchial asthma was diagnosed by pulmonologists, according to the 2015 Japanese Asthma Prevention and Management Guidelines [13]. ABPA was diagnosed by pulmonologists, according to the Patterson criteria [3], when the chest imaging studies showed central bronchiectasis or infiltrates. The with-asp group comprised patients with asthma whose *Aspergillus*-specific IgE level was determined to be ≥ 0.35 U_A/mL by Immuno-CAP. The without-asp group comprised patients with bronchial asthma whose *Aspergillus*-specific IgE level was determined to be < 0.35 U_A/mL by Immuno-CAP. Immuno-CAP was performed using serum samples from the patients for the measurement and analyses of the following: IgEs and IgGs specific to *Aspergillus fumigatus* (Af), *A. niger*, *A. flavus* and *A. terreus*; IgEs specific to Asp f 1, 2, 3, 4 and 6; *Malassezia*-specific IgEs; and mite-specific IgEs. The cutoff IgE level was set at 0.35 U_A/mL, and the cutoff IgG level at 26.9 mg_A/L. In patients who underwent multiple specific IgE assays, the association between the *Aspergillus*-specific IgE antibody measurements by these assays and the sensitization to each allergen component were analyzed. The present study was conducted after review and approval by the Medical Research Ethics Committee of Fujita Health University (Approval No. HM16-372).

Statistical analysis

Numerical data are expressed as mean, median and interquartile range (IQR), and categorical data as count and percentage.

Two independent groups were compared using a Mann-Whitney *U* test. The degree of association between two variables was determined using Spearman's rank correlation coefficients. Differences across three or more independent groups were assessed using a Kruskal-Wallis test. Values of $p < 0.05$ were considered statistically significant. Statistical software StatMate version 3.19 (ATMS Co., Ltd., Tokyo, Japan) was used for all statistical analyses.

Results

Patient characteristics

Table 1 presents the patient characteristics, by group. There were 12 patients in the ABPA group, 16 in the with-asp group and 41 in the without-asp group.

Table 1. Patient characteristics in the ABPA, and control (with-asp and without-asp) groups with-asp: patients with bronchial asthma and *Aspergillus*-specific IgE ≥ 0.35 U_A/mL without-asp: patients with bronchial asthma and *Aspergillus*-specific IgE < 0.35 U_A/mL.

	All participants (n = 69)	ABPA (n = 12)	Control		p Value
			With-asp (n = 16)	Without-asp (n = 41)	
All patients n = 69					
Age (year), mean $\pm$ SD	54.7 $\pm$ 18.6	60.9 $\pm$ 14.7	47.5 $\pm$ 19.0	55.8 $\pm$ 19.0	NS
Sex (men:women), n (%)	38:31	4:8	11:5	23:18	<0.01
Disease duration (year), median (IQR)	11(5–29)	15.5(4.8–34)	20.5(12.3–35.5)	10(3–24)	NS
Smoking history (pack-year), median (IQR)	0(0–25)	0(0–25)	0(0–16.25)	10(0–27.5)	NS
Peripheral eosinophil count, median (IQR)	294.5(109–553)	937(552–1359)	336(190–546)	196(88–360)	<0.001
Total IgE (IU/mL), median (IQR)	663(211–1568)	1535(1059–2958)	1659(544.5–2917)	250(73–696)	<0.001
FEV1 (mL), mean $\pm$ SD	2475 $\pm$ 927	1975 $\pm$ 939	2678 $\pm$ 963.7	2555 $\pm$ 924	<0.001
FEV1/FVC (%), mean $\pm$ SD	75.0 $\pm$ 11.8	71.7 $\pm$ 11.8	76.1 $\pm$ 10.76	75.9 $\pm$ 12.32	NS
FVC (mL), mean $\pm$ SD	3070 $\pm$ 929	2835 $\pm$ 941.0	3501 $\pm$ 1061	3314 $\pm$ 892	<0.05

ABPA: allergic bronchopulmonary aspergillosis; IgE: immunoglobulin E; IQR: interquartile range; FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity; SD: standard deviation; NS: not significant.

Table 2. Component-specific IgE (U_A/mL) measured in serum samples and positive rates of patients with *Aspergillus*-specific IgE level > 0.35 U_A/mL (CLASS 1) in the ABPA group and the two control (with-asp and without-asp) groups.

	ABPA (n = 12)	Control	
		With-asp (n = 16)	Without-asp (n = 41)
<i>Aspergillus</i> (M3)-specific IgE	12.25(3.74–16.42)	1.8(0.64–5.96)*	0.05(0.05–0.05)***
Asp f 1 (U _A /mL), median (IQR)	2.50(0.31–9.98)	0.20(0.17–0.24)***	0.14(0.13–0.17)***
Asp f 2 (U _A /mL), median (IQR)	1.64(0.54–4.07)	0.05(0.05–0.23)**	0.05(0.05–0.05)***
Asp f 3 (U _A /mL), median (IQR)	0.74(0.20–21.20)	0.08(0.05–0.68)	0.05(0.05–0.05)***
Asp f 4 (U _A /mL), median (IQR)	0.15(0.05–4.85)	0.13(0.05–0.20)	0.05(0.05–0.05)***
Asp f 6 (U _A /mL), median (IQR)	0.26(0.09–1.87)	0.13(0.05–0.57)	0.05(0.05–0.05)***
Asp f 1-positive rate, n (%)	8(67%)	2(13%) ^{†††}	3(7%) ^{†††}
Asp f 2-positive rate, n (%)	9(75%)	3(19%) ^{†††}	0(0%) ^{†††}
Asp f 3-positive rate, n (%)	8(67%)	5(31%) ^{††}	0(0%) ^{†††}
Asp f 4-positive rate, n (%)	4(33%)	1(6%) [†]	1(2%) ^{†††}
Asp f 6-positive rate, n (%)	6(50%)	5(31%)	1(2%) ^{†††}
<i>A. niger</i> IgE(U _A /mL), median (IQR)	1.14(0.30–4.58)	0.434(0.272–1.195)	0.05(0.05–0.05)***
<i>A. flavus</i> IgE(U _A /mL), median (IQR)	1.46(0.41–3.49)	0.495(0.151–1.605)	0.05(0.05–0.05)***
<i>A. terreus</i> IgE(U _A /mL), median (IQR)	1.57(0.16–4.51)	0.32(0.05–1.833)	0.05(0.05–0.05)***
<i>A. fumigatus</i> IgG (mg _A /L), median (IQR)	70.3(43.25–122.5)	23.75(14.55–54.63)**	34.8(18.9–55.8)**
<i>A. niger</i> IgG (mg _A /L), median (IQR)	35.1(18.86–57.68)	11.7(6.567–27.15)**	15.8(9.37–33.1)*
<i>A. flavus</i> IgG (mg _A /L), median (IQR)	66.05(24.18–107.75)	21.95(10.972–47.875)**	31.6(18.9–48.9)
<i>A. terreus</i> IgG (mg _A /L), median (IQR)	28.65(17.7–56.85)	10.9(6.072–21.325)**	10.8(8.09–18.3)***
Mite (U _A /mL), median (IQR)	1.10(0.36–14.77)	14(2.20–33.3)	0.156(0.05–2.01)*
<i>Malassezia</i> (U _A /mL), median (IQR)	0.23(0.05–1.12)	2.02(0.19–22.93)*	0.05(0.05–0.05)*

IgE (U_A/mL) and IgG (mg_A/L) specific to *Aspergillus fumigatus*, *A. niger*, *A. flavus* and *A. terreus*. ABPA: allergic bronchopulmonary aspergillosis; With-asp: patients with bronchial asthma and *Aspergillus*-specific IgE level ≥ 0.35 U_A/mL; Without-asp: patients with bronchial asthma and *Aspergillus*-specific IgE level < 0.35 U_A/mL; IgE: immunoglobulin E; Af: *Aspergillus fumigatus*; IQR: interquartile range.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; difference compared with values in the ABPA group by a Mann–Whitney *U* test.

[†] $p < 0.05$, ^{††} $p < 0.01$, ^{†††} $p < 0.001$; difference compared with values in the ABPA group by a chi-square test.

Overall, the participants comprised 38 men and 31 women, and their mean age was 54.7 years. The median disease duration was 11 years (IQR: 5–29 years). The median total IgE level was 663 IU/mL (IQR: 211–1568 IU/mL). Compared to the control groups, those in the ABPA group had a significantly higher eosinophil count ($p < 0.001$) and significantly lower forced expiratory volume in 1 s ($p < 0.001$) and forced vital capacity ($p < 0.05$).

Allergen components

IgEs specific to each allergen component (i.e. Asp f 1, 2, 3, 4 and 6) were quantified by Immuno-CAP and

analyzed. Based on the comparison of the quantitative values, the ABPA group was more frequently sensitized to Asp f 1 and 2 than the control groups: these results were significant.

No significant differences were observed in the levels of Asp f 3, 4 or 6 between these groups (Table 2 and Figure 1). Next, with a cutoff level of 0.35 U_A/mL (CLASS 1), the ABPA group showed significantly higher positivity rates for Asp f 1, 2, 3 and 4 than the control groups (Table 2). When the correlation between each allergen component and *Malassezia*-specific IgE was analyzed, only Asp f 6 was significantly positively correlated to *Malassezia*-specific IgEs ($r = 0.3982$, $p < 0.001$).

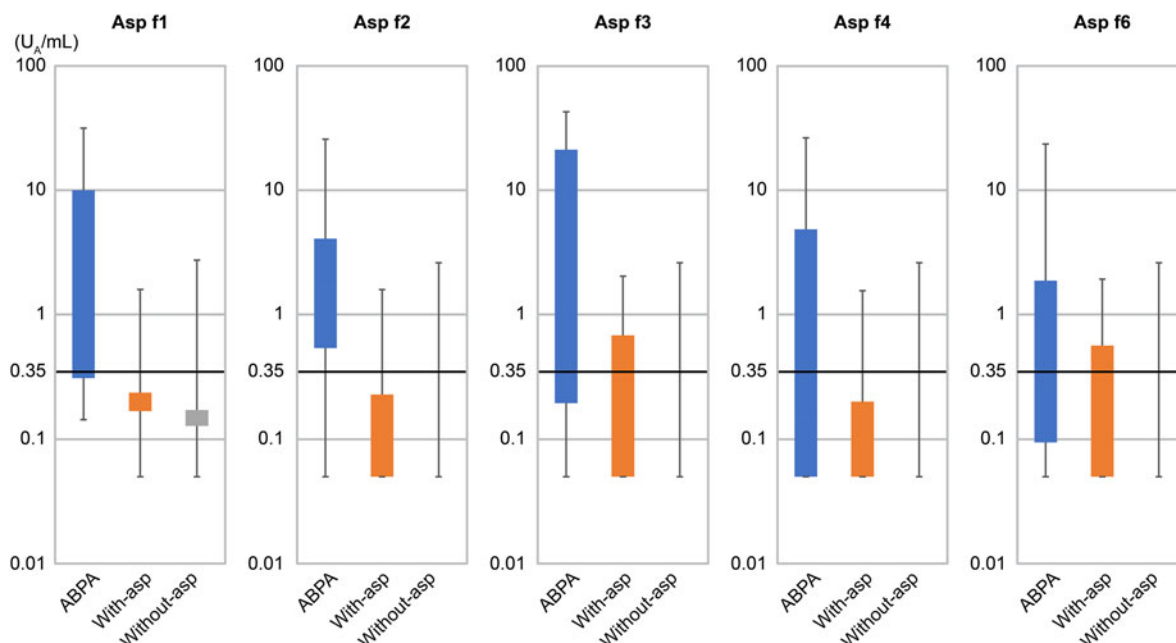


Figure 1. Component-specific immunoglobulin E levels measured in the serum samples obtained from the ABPA group and the two control groups. The vertical axis uses a logarithmic scale. ABPA: allergic bronchopulmonary aspergillosis With-asp: patients with bronchial asthma and *Aspergillus*-specific immunoglobulin E level ≥ 0.35 UA/mL Without-asp: patients with bronchial asthma and *Aspergillus*-specific immunoglobulin E level < 0.35 UA/mL.

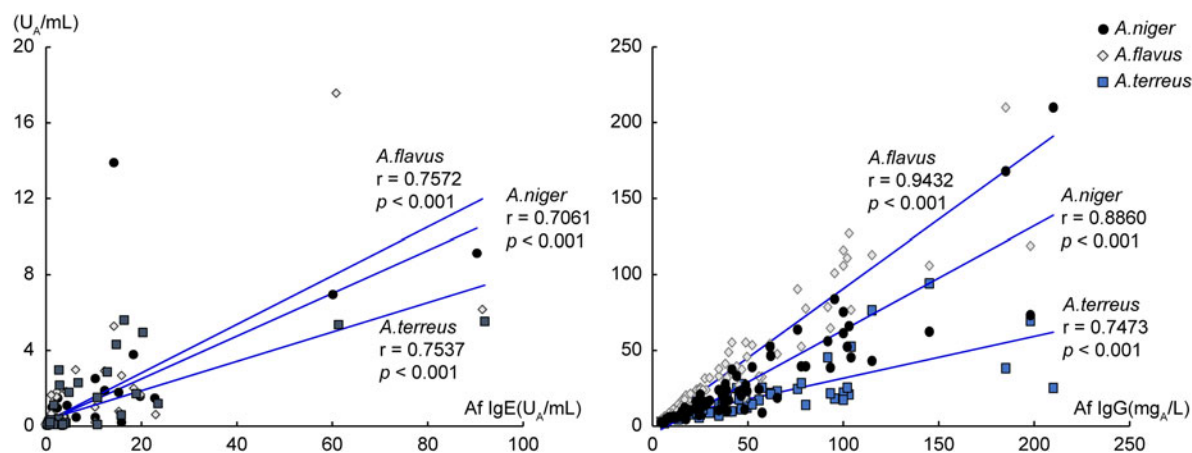


Figure 2. Correlation of the specific immunoglobulin E antibodies between *Aspergillus fumigatus* and the other species (*A. niger*, *A. flavus* and *A. terreus*). Left: Correlation of *A. fumigatus*-specific immunoglobulin E with immunoglobulin E antibodies specific to *A. niger*, *A. flavus* and *A. terreus*. Right: Correlation of *A. fumigatus*-specific immunoglobulin G with immunoglobulin G antibodies specific to *A. niger*, *A. flavus* and *A. terreus*. Af: *Aspergillus fumigatus*. r : Spearman's rank correlation coefficient.

Interspecies association of IgE

Table 2 shows the measurements of the IgEs specific to Af, *A. niger*, *A. flavus* and *A. terreus*. Af-specific IgEs were significantly positively correlated to the IgEs specific to *A. flavus* ($r = 0.7572$, $p < 0.001$), *A. niger* ($r = 0.7061$, $p < 0.001$) and *A. terreus* ($r = 0.7537$, $p < 0.001$) (Figure 2). Af showed nearly the same level of the coefficient as *A. flavus* ($r = 0.7572$). Meanwhile, all patients negative for Af-specific IgEs (< 0.35 U_A/mL) ($n = 41$) were also negative for IgEs specific to *A.*

niger, *A. flavus* and *A. terreus*. In addition, patients positive for IgEs specific to *A. niger*, *A. flavus* and *A. terreus* (> 0.35 U_A/mL) were all determined to be positive for Af-specific IgEs.

Af-specific IgG

The median Af-specific IgG levels (IQR) were 70.3 (43.25–122.5) mg_A/L in the ABPA group, 23.75 (14.55–54.63) mg_A/L in the with-asp group and 34.8

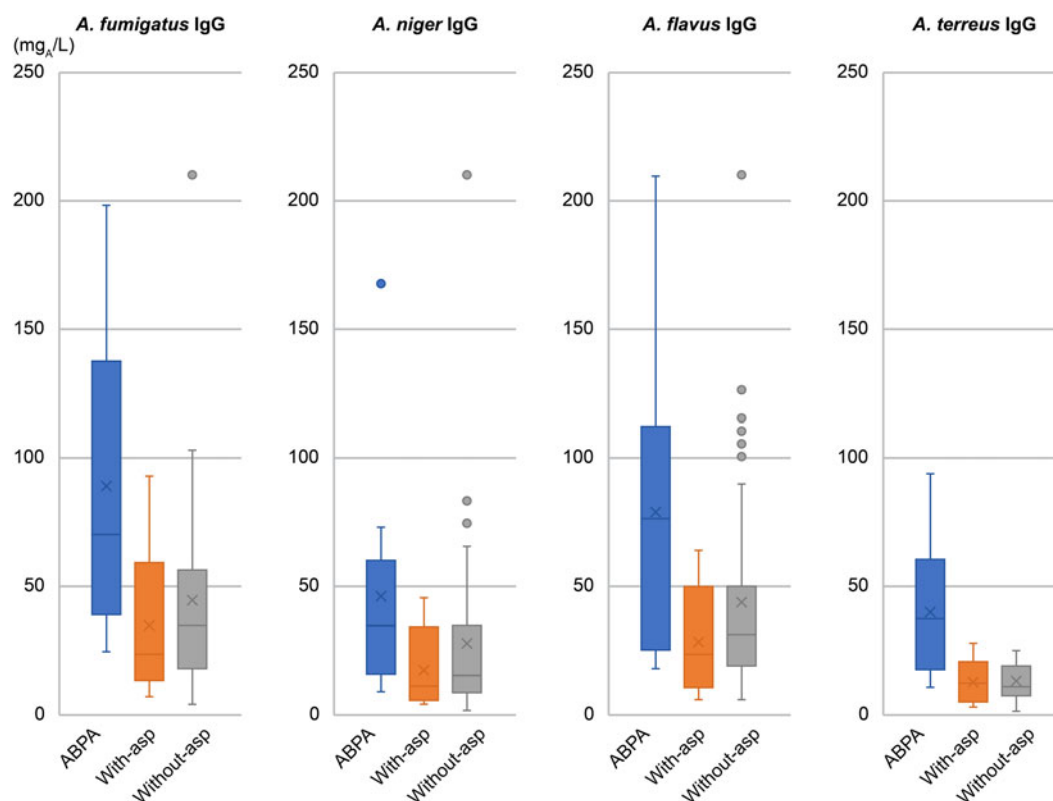


Figure 3. Levels of immunoglobulin G antibodies specific to each *Aspergillus* species (*A. fumigatus*, *A. niger*, *A. flavus* and *A. terreus*) in the ABPA, with-asp and without-asp groups. ABPA: allergic bronchopulmonary aspergillosis; With-asp: patients with bronchial asthma and *Aspergillus*-specific immunoglobulin E level ≥ 0.35 UA/mL; Without-asp: patients with bronchial asthma and *Aspergillus*-specific immunoglobulin E level < 0.35 UA/mL.

Table 3. Correlation of the specific immunoglobulin E and specific immunoglobulin G measured in the serum samples of patients in the ABPA, with-asp, and without-asp groups, among the *Aspergillus* species (*A. fumigatus*, *A. niger*, *A. flavus* and *A. terreus*).

Specific IgE	<i>A. fumigatus</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>A. terreus</i>
<i>A. fumigatus</i>		$r = 0.7061$ $p < 0.001$	$r = 0.7572$ $p < 0.001$	$r = 0.7537$ $p < 0.001$
<i>A. niger</i>			$r = 0.6887$ $p < 0.001$	$r = 0.6992$ $p < 0.001$
<i>A. flavus</i>				$r = 0.7412$ $p < 0.001$
<i>A. terreus</i>				
Specific IgG	<i>A. fumigatus</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>A. terreus</i>
<i>A. fumigatus</i>		$r = 0.8860$ $p < 0.001$	$r = 0.9432$ $p < 0.001$	$r = 0.7473$ $p < 0.001$
<i>A. niger</i>			$r = 0.9419$ $p < 0.001$	$r = 0.4714$ $p < 0.001$
<i>A. flavus</i>				$r = 0.6071$ $p < 0.001$
<i>A. terreus</i>				

r: Spearman's rank correlation coefficient.

(18.9–55.8) mg_A/L in the without-asp group (Table 2 and Figure 3). When the cut-off *Aspergillus*-specific IgG level was set at 26.9 mg_A/L, 83% of the patients in the ABPA group ($n = 10/12$) and 54% in the control groups ($n = 31/57$) showed positivity (sensitivity of 83% and specificity of 46%).

Interspecies associations between IgGs

Next, the associations between specific IgGs among the *Aspergillus* species were analyzed. Table 2 shows the measurements of the IgGs specific to all the *Aspergillus* species, and Table 3 shows the correlations between the species.

Af-specific IgGs were positively correlated to IgGs specific to all the other species (*A. niger*: $r = 0.8860$, $p < 0.001$; *A. flavus*: $r = 0.9432$, $p < 0.001$; and *A. terreus*: $r = 0.7473$, $p < 0.001$); a strong positive correlation was observed particularly between Af and *A. flavus* ($r = 0.9432$, $p < 0.001$).

Multiple specific IgE assays and allergen components

Of 57 patients who underwent multiple specific IgE assays (View Allergy[®] and MAST IV[®]), Table 4 shows the ratio of patients positive for *Aspergillus* crude antigen-specific IgEs ($\geq$ CLASS 1) to those positive for each allergen component (Asp f 1, 2, 3, 4 and 6). All patients positive for any of the components were determined to be positive for *Aspergillus* crude antigen-specific IgEs by View Allergy[®]. MAST IV[®] determined

Table 4. Positivity rates for each component based on multiple specific immunoglobulin E assays.

<i>n</i> = 57	Asp f 1	Asp f 2	Asp f 3	Asp f 4	Asp f 6
View Allergy®	100% (6/6)	100% (6/6)	100% (10/10)	100% (4/4)	100% (10/10)
MAST IV®	50% (3/6)	66.7% (4/6)	60% (6/10)	75% (3/4)	50% (5/10)

The *Aspergillus*-specific IgE levels in the serum samples from 57 patients (including 6 ABPA patients) were measured by View Allergy® (Thermo Fisher Scientific, Uppsala, Sweden) and MAST IV® (Hitachi Chemical Diagnostics, Inc., Mountain View, CA, USA). Results of Class 1 or greater were regarded as positive. This table shows the ratios of patients who were determined to be positive for *Aspergillus*-specific IgE ($\geq$ Class 1) by each multiple specific IgE assay (View Allergy® and MAST IV®) to those who were determined to be positive for each allergen component (Asp f 1, 2, 3, 4 and 6) by Immuno-CAP.

some patients to be negative for *Aspergillus* crude antigen-specific IgEs across all components.

Discussion

The present study revealed that the ABPA group was more frequently sensitized to Asp f 1 and 2 than the with-asp group. This was comparable to the results reported by Tanimoto et al. [8] ABPA presents different clinical features, such as age at onset and total IgE level, depending on the country [5]. This is presumably attributable to the differences in the conditions required for fungal growth (e.g. humidity and temperature), owing to differing climatic conditions and housing environments across countries, as well as the low prevalence of cystic fibrosis – an underlying disease of ABPA – in Japan. Cramer et al. reported that patients with ABPA are frequently sensitized to Asp f 2, 4 and 6 [6], and it seems that the difference in the frequency of sensitization to allergen components may be affected by different patient characteristics and environmental factors. Asp f 1 (Ribotoxin) is a protein specifically expressed in Af within the *Aspergillus* genus [14]. This protein does not exist in spore form in the environment, but Af spores colonize the airway, germinate into hyphae and secrete the protein. Thus, sensitization to Asp f 1 indicates that Af has existed in the body for a certain period and formed colonies. Given this feature of Asp f 1, it appears to be plausible that patients with ABPA, which is caused by the airway colonization of *Aspergillus* species, are frequently sensitized to Asp f 1. Asp f 3 (a peroxisomal-like protein) and Asp f 6 (an Mn superoxide dismutase) are proteins widely expressed in not only *Aspergillus* species but also other fungi [15]. The present study revealed that only Asp f 6 among the allergen components was positively correlated to *Malassezia*-specific IgE antibodies. Moreover, of five patients who were positive for only Asp f six among the components, four patients (80%) were determined to be positive for IgEs specific to the crude extract antigen of Af by Immuno-CAP. These patients may have been sensitized to *Malassezia* or other fungi instead of *Aspergillus*; consequently, they were

determined to be positive for Af-specific IgEs because of cross-reaction. Thus, allergen-specific IgE testing based on the features of each *Aspergillus* allergen component appears to provide information that conventional allergen-specific IgE testing with crude extract antigens does not. Information that can be clinically obtained from the components includes that pertaining to whether a patient is actually sensitized to *Aspergillus* or found positive because of cross-reactivity with other fungi. In addition, before chest radiography provides clear imaging findings, ABPA can be early suspected based on whether a patient is sensitized to Asp f 1 or 2. Fukutomi et al. proposed the measurement of the levels of the IgEs specific to Asp f 1 and 2 in patients with asthma who are sensitized to the crude extract antigens of *Aspergillus*. If they are positive for either Asp f 1 or 2, ABPA should be suspected, and additional tests are recommended [9]. The results of our study also support their proposal. Furthermore, IgE antibodies are known to be cross-reactive among fungi [16], and the cross-reactivity of IgE antibodies among *Aspergillus* species has been reported [17]. The present study confirmed that, in addition to *Aspergillus*-specific IgE antibodies, *Aspergillus*-specific IgG antibodies are also cross-reactive among the species. Both Af-specific IgEs and IgGs were the most strongly correlated to *A. flavus*-specific IgEs and IgGs.

Piechura et al. performed electrophoresis with the antigen components of *Aspergillus* species on serum samples from patients with ABPA, reporting that Af is more cross-antigenic with *A. flavus* than *A. niger* and *A. terreus* [18]. Their results appear to be consistent with ours. *Aspergillus* species existing in the environment include not only Af but also various other species such as *A. niger*, *A. flavus* and *A. terreus*; all these species can cause ABPA. Therefore, when evaluating the results of the *Aspergillus* antibody test, it is necessary to consider immunological interspecies cross-reactivity and simultaneous exposure and sensitization to multiple *Aspergillus* species (i.e. co-sensitization) [19]. Therefore, we did not perform IgE inhibition assays in the present study and patients with co-sensitization were not excluded.

While Af is the most frequently used test *Aspergillus* species in daily clinical practice, our study revealed that all patients who were positive for IgEs specific to *A. niger*, *A. flavus* or *A. terreus* were determined to be positive for Af-specific IgE. This suggests that patients sensitized to Af, *A. niger*, *A. flavus* or *A. terreus* may be determined to be positive for Af-specific IgEs by Immuno-CAP, which is performed in daily clinical practice. It should be kept in mind that patients sensitized to *A. niger*, *A. flavus* or *A. terreus* may be determined to be positive for antibodies against Af due to cross-reactivity.

Markers for the detection of type III allergic reactions in ABPA include *Aspergillus*-precipitating antibodies and IgG. Agarwal et al., in India, reported that the sensitivity of the precipitating antibody test is low (sensitivity of 27.4%) for the diagnosis of ABPA, and that a cutoff *Aspergillus*-specific IgG level of 26.9 mg_A/L is appropriate (sensitivity of 88% and specificity of 100%) and useful for ABPA diagnosis [10]. In Japan, Harada reported that, with a cutoff *Aspergillus*-specific IgG level of 26.9 mg_A/L, 39 of 53 patients with ABPA (75%) were positive for IgG, and that 27% of all patients were positive for only *Aspergillus*-specific IgGs but negative for precipitating antibodies [11].

These reports indicate the high sensitivity of *Aspergillus*-specific IgGs in ABPA diagnosis.

In the present study, the IgG-positivity rate in patients with ABPA was 83% ($n = 10/12$), which was comparable to the rates described in the aforementioned reports. However, even in the control groups (i.e. the with-asp and without-asp groups), 54% of the patients ($n = 31/57$) had an Af-specific IgG level exceeding 26.9 mg_A/L. Although this cutoff Af-specific IgG level for the diagnosis of ABPA is quite similar to that reported by Agarwal et al. (26.9 mg_A/L), it should be noted that the specificity of these cutoff levels may be low.

Limitations

The present study has the shortcoming of a small sample size; i.e. only 12 patients had ABPA. In addition, since IgE assays, which are necessary to show immunological interspecies cross-reactivity, were not performed, it is possible that the present study contained patients who were simultaneously exposed and sensitized to multiple *Aspergillus* species (i.e. co-sensitization).

Conclusion

Patients with ABPA were more frequently sensitized to Asp f 1 and 2 than those with *Aspergillus*-sensitized

asthma. Both IgE and IgG levels were positively correlated among Af, *A. niger*, *A. flavus* and *A. terreus*. There were many patients apart from asthmatic patients with ABPA, who displayed high *Aspergillus* IgG values.

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