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Original Article

Concordance between *Aspergillus*-specific precipitating antibody and IgG in allergic bronchopulmonary aspergillosisKazuki Harada ^a, Tsuyoshi Oguma ^{a,*}, Akemi Saito ^b, Yuma Fukutomi ^b, Jun Tanaka ^a, Katsuyoshi Tomomatsu ^a, Masami Taniguchi ^b, Koichiro Asano ^a^a Division of Pulmonary Medicine, Department of Medicine, Tokai University School of Medicine, Kanagawa, Japan^b Clinical Research Center for Allergy and Rheumatology, Sagami National Hospital, Kanagawa, Japan

ARTICLE INFO

Article history:

Received 24 January 2018

Received in revised form

2 April 2018

Accepted 21 April 2018

Available online xxx

Keywords:

ABPA

Aspergillus fumigatus

concordance

IgG

precipitin

Abbreviations list:

ABPA allergic bronchopulmonary aspergillosis

CF complement fixation

CIE counterimmunoelectrophoresis

FVC forced vital capacity

OCS oral corticosteroids

ABSTRACTS

Background: Several serological tests for specific precipitin or IgG are available to demonstrate type III hypersensitivity reactions to *Aspergillus* species and are essential for infectious fungal disease diagnosis. These assays are also important for allergic bronchopulmonary aspergillosis (ABPA) diagnosis; however, their concordance in ABPA was not well studied.

Methods: Fifty-two ABPA patients diagnosed based on ISHAM criteria were enrolled. Precipitins and IgG specific to *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, or *Aspergillus terreus* were measured using Ouchterlony double immunodiffusion tests and ImmunoCAP method, respectively. *A. fumigatus*-specific IgG was also determined using complement-fixation (CF) method.

Results: Forty-eight percent of cases were double-positive for *A. fumigatus*-specific precipitin and IgG (ImmunoCAP), whereas 3 (6%) and 14 (28%) cases were positive for precipitin or IgG alone, respectively. Kappa coefficient between these measurements was 0.32, suggesting poor concordance. Double-positive cases were more likely to present: *Aspergillus* sp. in sputum culture, lower pulmonary functions, peripheral blood eosinophilia, higher total IgE and *A. fumigatus*-specific IgG titer than precipitin-negative cases. *A. fumigatus*-specific IgG (CF) was positive only in 8 (15%) cases. The presence of *A. fumigatus*-specific precipitin or IgG was associated with antibodies specific for other *Aspergillus* spp., suggesting cross-reactivity.

Conclusions: Positive rate of *A. fumigatus*-specific precipitin or IgG (ImmunoCAP) was superior to IgG (CF), but relatively poor concordance was noted between precipitin and IgG (ImmunoCAP). Positive precipitin for *A. fumigatus* suggests more active diseases. Cross-reactivity may exist between antibodies to different *Aspergillus* spp. Therefore, the type III hypersensitivity results in ABPA diagnosis should be carefully evaluated.

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Introduction

Allergic bronchopulmonary aspergillosis (ABPA) is an airway disease characterized by peripheral blood eosinophilia, elevated serum IgE, pulmonary infiltrates, bronchiectasis, and mucoid impaction in the central bronchi. For the diagnosis of ABPA, serological tests to demonstrate type I and III hypersensitivity reactions to *Aspergillus* species are essential.

Assay of specific IgE in the serum or immediate skin reaction using crude *Aspergillus fumigatus* antigen is the commonly-

employed tests for type I hypersensitivity. Measurement of IgE specific for *A. fumigatus* allergen components is available and might minimize the cross-reaction among different fungal species, which tend to occur when crude extracts of allergen are used.¹ We have previously demonstrated the usefulness of measuring specific IgE for allergen components of *A. fumigatus* such as Asp f 1 and Asp f 2 in the diagnosis of ABPA.²

There are also several methods to evaluate type III hypersensitivity to *A. fumigatus* including precipitating antibody or specific IgG to the crude antigen. The optimal cut-off value of *A. fumigatus*-specific IgG measured by ImmunoCAP method has recently been reported in the diagnosis of ABPA.³ In our nationwide survey of ABPA in Japan, *Aspergillus*-specific precipitin was examined in 67% of cases while *Aspergillus*-specific IgG measurement with either ImmunoCAP or complement fixation (CF) assay was performed in

* Corresponding author. Division of Pulmonary Medicine, Department of Medicine, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan.

E-mail address: tyoguma@tokai-u.jp (T. Oguma).

Peer review under responsibility of Japanese Society of Allergy.

<https://doi.org/10.1016/j.alit.2018.04.009>

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16% of cases.⁴ The positive rates of *Aspergillus*-specific precipitin and IgG were 73% and 74%, respectively. However, there are few studies that directly compared the diagnostic utility of *Aspergillus*-specific precipitin and IgGs measured by these different methods.^{5,6} We therefore evaluated the concordance of *Aspergillus*-specific precipitin and IgG measured using two different assays in the serum of patients with ABPA.

Methods

Study subjects

Patients with ABPA, aged 16 years or older, were enrolled from 10 institutes in Japan between April 2013 and June 2016. Clinical data were retrospectively collected from medical records. ABPA was diagnosed based on the International Society of Human and Animal Mycology (ISHAM) criteria,⁷ which included two essential features: 1) positive immediate cutaneous hyper-reactivity or specific IgE to *A. fumigatus*; 2) elevated total IgE levels in serum ≥ 1000 IU/mL with three other features: presence of specific precipitin or IgG against *A. fumigatus*, the existence of radiographic findings in the lungs consistent with ABPA, and total eosinophil counts in peripheral blood ≥ 500 cells/ μ L. At least two out of the three criteria are needed for the diagnosis of ABPA. This study was approved by the Institutional Review Board of Tokai University Hospital (#13R-107),

and implemented in compliance with the Declaration of Helsinki. All the patients gave written informed consent.

Measurement of *Aspergillus*-specific precipitating antibodies and IgG

At enrollment, peripheral blood was obtained from the study subjects, and serum was frozen at -80°C for the measurement of serum antibodies. Precipitating antibodies in the serum were evaluated by Ouchterlony double immunodiffusion testing using commercial crude extract of *A. fumigatus*, *Aspergillus niger*, *Aspergillus terreus* (Hollister–Stier, WA, USA), or *Aspergillus flavus* (Greer Laboratories, Lenoir, NC). An extract of *A. fumigatus* from a different supplier (Torii Pharmaceuticals, Tokyo, Japan) was also examined. Specific IgG concentrations for *A. fumigatus* (m3), *A. niger* (m207), *A. terreus* (m36), or *A. flavus* (m228) were measured with ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden). The levels of *A. fumigatus* specific IgG (ImmunoCAP) ≥ 26.9 mg_A/L were considered as positive. *A. fumigatus*-specific IgG was also determined using CF method.⁸

Statistical analysis

Numerical data were expressed as median and interquartile range while categorical data were presented as numbers with their

Table 1
Demographic data at diagnosis of ABPA.

	All subjects	Treated with OCS at enrollment		p [*]
	n = 52	yes n = 22	no n = 30	
Age	66 (50–73)	67 (52–74)	64 (50–73)	0.79
Age at onset of ABPA	60 (50–68)	58 (48–66)	61 (50–69)	0.45
Female	23 (44)	7 (32)	16 (53)	0.05
History of asthma	40 (77)	19 (86)	21 (70)	0.20
Laboratory data at diagnosis				
Peripheral blood eosinophil counts, μ L	1218 (687–1953) [n = 51]	1388 (877–2313)	1136 (648–1793) [n = 29]	0.43
Total serum IgE, IU/mL	2528 (1348–5298)	3368(1726–6651)	2291(1173–4262)	0.07
Specific IgE or immediate skin reaction to <i>A. fumigatus</i> antigen	51 (100) [n = 51]	22 (100)	29 (100) [n = 29]	0.99
<i>A. fumigatus</i> -specific precipitin or IgG	35 (76) [n = 46]	11 (58) [n = 19]	24 (89) [n = 27]	0.03
Positive fungus culture in sputum or bronchial washing	[n = 51]	[n = 21]	[n = 30]	
<i>Aspergillus</i> sp.	13 (25)	5 (24)	8 (27)	0.99
Any fungi	31 (60)	12 (55)	19 (63)	0.77
Pulmonary function	[n = 31]	[n = 12]	[n = 19]	
FEV ₁ , %predicted	81 (62–99)	77 (57–98)	88 (71–100)	0.29
FEV ₁ /FVC, %	73 (63–81)	73 (66–89)	74 (61–80)	0.46
Chest Images				
Central bronchiectasis	39 (75)	18 (82)	21 (70)	0.52
Mucus plugs	45 (87)	15 (68)	30 (100)	0.001
High attenuation mucus	29 (56)	8 (36)	21 (70)	0.02
Pulmonary opacities	46 (88)	21 (95)	25 (83)	0.23
Treatment				
Oral corticosteroids	36 (69)	22 (100)	14 (47)	
Anti-fungal agents	21 (40)	11 (50)	10 (33)	0.26
Anti-IgE antibody	2 (4)	1 (5)	1 (3)	0.99
Laboratory data at enrollment				
Peripheral blood eosinophil counts, μ L	329 (189–795) [n = 51]	395 (198–738) [n = 22]	317 (185–964) [n = 29]	0.99
Total serum IgE, IU/mL	1842 (627–2971) [n = 44]	1722 (624–3006) [n = 19]	1962 (624–2725) [n = 25]	0.85
<i>A. fumigatus</i> -specific antibody				
Precipitin	28 (54)	12 (55)	16 (53)	0.99
IgG CF	8 (15)	6 (27)	2 (7)	0.06
IgG ImmunoCAP, m _A /mL	52 (22–112)	44 (25–130)	55 (21–104)	0.86

Values are medians (interquartile range) or number (proportion) of patients in each study groups.

ABPA, allergic bronchopulmonary aspergillosis; OCS, oral corticosteroids; FVC, forced vital capacity; CF, complement fixation.

* Treated with OCS at enrollment, yes vs. no.

percentages. Numerical data were analyzed using the Mann–Whitney's *U* test or Kruskal–Wallis test while categorical data were analyzed using the Fisher's exact test. In trend data, Jonckheere–Terpstra analysis or chi-square test for trend was used. Correlation between specific IgG for *A. fumigatus* and the other *Aspergillus* species were determined using Spearman's rank correlation analysis. The statistical analyses were performed using the IBM SPSS statistics ver.24 (IBM, Chicago, IL, USA) and GraphPad prism ver.5.0 (GraphPad Software, La Jolla, CA, USA). A *p* value < 0.05 was considered statistically significant.

Results

Data on a total of 52 cases that met the diagnostic criteria of ABPA were analyzed. Demographic data of these patients are shown in Table 1. Median age at the onset of ABPA was 60 years, and 12 (23%) of the patients had no history of asthma (one of the predisposing conditions in the ISHAM criteria). Forty-one (79%) cases showed positive *A. fumigatus*-specific precipitin and/or IgG, while 56% of the cases had high attenuation mucus on their CT findings, which were consistent with the finding in our nationwide survey.⁴ Thirty-six cases had been treated with systemic corticosteroids and 22 cases were still under the treatment with oral corticosteroids (OCS) at the time of enrollment.

Precipitating and specific IgG for *A. fumigatus*

When *A. fumigatus*-specific precipitating antibody and IgG (ImmunoCAP) were compared, both were positive and negative in 25 (48%) and 10 (19%) cases, respectively (Fig. 1). In 17 (33%) cases, there was a disagreement between the results of precipitating antibody and IgG (ImmunoCAP), including 14 cases positive for IgG (ImmunoCAP) alone and 3 cases positive for precipitating antibody alone (Fig. 1). Kappa coefficient between these measurements was 0.32, suggesting poor concordance. *A. fumigatus*-specific IgG (CF) was positive only in 8 (15%) cases, most of which were positive for both precipitating antibody and IgG (ImmunoCAP) (Fig. 1).

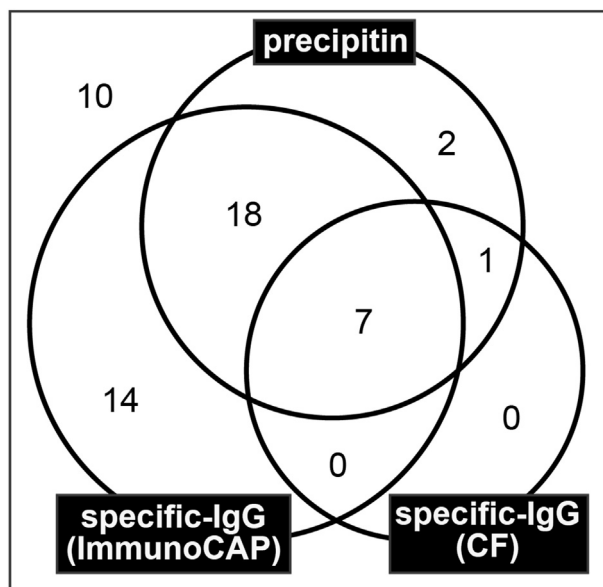


Fig. 1. Concordance between different assays for type III hypersensitivity to *A. fumigatus* in patients with ABPA. The Venn diagram shows the numbers of cases positive for *A. fumigatus*-specific precipitin, specific IgG determined by ImmunoCAP method, and the specific IgG determined by the complement fixation method.

In addition, we compared the clinical characteristics among ABPA patients who were double-positive for *A. fumigatus*-specific precipitin and IgG (ImmunoCAP), positive for IgG (ImmunoCAP) alone, and double-negative (Table 2). In double-positive cases, peripheral blood eosinophil counts and the levels of serum total IgE were higher than those in the single-positive or double-negative cases, even though the proportion of the subjects treated with corticosteroid or anti-fungal agents were higher than in the other groups. Fungi of *Aspergillus* species were more frequently isolated from sputum in the double-positive cases, while the positive rates of fungus in the sputum culture were similar among the groups.

Precipitating and specific IgG for *Aspergillus* species other than *A. fumigatus*

To evaluate species specificities, we examined precipitin and IgG (ImmunoCAP) for *A. fumigatus* and three other *Aspergillus* species (*A. flavus*, *A. niger*, and *A. terreus*). In 28 patients positive for specific precipitin to *A. fumigatus*, 19 (68%) cases were also positive for precipitins in one or more other *Aspergillus* spp., including 16 cases both positive for *A. fumigatus* and *A. flavus* (Fig. 2A). The levels of *A. fumigatus*-specific IgG (ImmunoCAP) was significantly correlated with the levels for *A. flavus*, *A. niger*, or *A. terreus* ($\rho = 0.92, 0.88, 0.93$, respectively, Fig. 2B).

Titers of *Aspergillus*-specific IgG (ImmunoCAP) were compared between the precipitin-positive and -negative groups for each *Aspergillus* species. The titer of *A. fumigatus*- or *A. flavus*-specific IgG was significantly higher in the precipitin-positive cases, but no such relationship was observed between *A. niger*- or *A. terreus*-specific IgG (ImmunoCAP) and their respective precipitin (Fig. 3).

Discussion

In the present study, we assessed the concordance among three tests that evaluated type III hypersensitivity to *A. fumigatus* in patients with ABPA. The performance of *A. fumigatus*-specific precipitin and IgG (ImmunoCAP) were superior to those of *A. fumigatus*-specific IgG (CF) because of their relatively higher sensitivity. However, the Kappa coefficient between *A. fumigatus*-specific precipitin and IgG (ImmunoCAP) was below 0.40, suggesting poor agreement.

Positive rate of *A. fumigatus*-specific IgG (CF) in patients with ABPA was only 15% in the present cohort. *A. fumigatus*-specific IgG (CF) is often measured for the diagnosis of chronic pulmonary aspergillosis, because of its reasonable sensitivity with lower costs.^{9,10} On the other hand, there were few evidences demonstrating the usefulness of *A. fumigatus*-specific IgG (CF) in the diagnosis of ABPA.^{11,12} Our data suggest that *A. fumigatus*-specific IgG (CF) might not be useful in the diagnosis of ABPA due to its low sensitivity.

A. fumigatus-specific precipitins have been widely used in the diagnosis of ABPA, but their positive rates in cases with ABPA ranged widely from 27% to 87%.^{3,13,14} In our nationwide survey in Japan, precipitins were examined in 67% of patients with ABPA and its positive rate was 73%.⁴ In the present study, the positive rate of precipitin (54%) at enrollment into the study was lower than those reported in previous studies. One of the possible reasons for this discrepancy is that the treatment with systemic corticosteroids might have lowered the positive rate of precipitins. However, the positive rate for *Aspergillus*-specific precipitin in cases treated with or without systemic corticosteroids at enrollment were not different (Table 1). The other possibility was the difference in the antigen used in measuring precipitins in this study. We therefore also checked *A. fumigatus*-specific precipitin using the other antigen of *A. fumigatus* (Torii, Tokyo, Japan) or those measured in a

Table 2Differences in the clinical characteristics of the groups classified according to the results of the two assays for type III *A. fumigatus* allergy.

	<i>A. fumigatus</i> -specific precipitating antibody			<i>p</i>
	(−)	(−)	(+)	
	<i>A. fumigatus</i> -specific IgG ≥26.9 mg _A /mL			
	(−)	(+)	(+)	
n	10	14	25	
Age	67 (56–72)	67 (57–75)	63 (49–74)	0.83
Age at onset of ABPA	62 (55–69)	61 (46–67)	58 (49–69)	0.70
Female	6 (60)	4 (29)	11 (44)	0.60
History of asthma	6 (60)	11 (79)	20 (80)	0.26
Laboratory data at diagnosis				
Peripheral blood eosinophil counts, μL	929 (506–1314) [n = 9]	1177 (651–1623)	1729 (940–2750)	0.01
Total serum IgE, IU/mL	2528 (1956–5192)	2760 (1173–7465)	2421 (1197–4461)	0.61
Specific IgE or Immediate skin reaction to <i>A. fumigatus</i> antigen	9 (100)	14 (100)	24 (100)[n = 24]	0.99
Positive fungus culture in sputum or bronchial washing	[n = 9]	[n = 13]	[n = 25]	
<i>Aspergillus</i> sp.	1 (11)	3 (23)	10 (40)	0.06
Any fungi	5 (56)	6 (46)	13 (52)	0.94
Pulmonary function	[n = 7]	[n = 9]	[n = 18]	
FEV ₁ , % predicted	99 (96–117)	87 (76–99)	75 (53–95)	0.01
FEV ₁ /FVC, %	80 (75–83)	73 (70–80)	70 (61–82)	0.11
Chest Images				
Central bronchiectasis	9 (90)	9 (64)	19 (76)	0.58
Mucus plugs	7 (70)	13 (93)	22 (88)	0.27
High attenuation mucus	6 (60)	8 (57)	14 (56)	0.61
Pulmonary opacities	7 (70)	13 (93)	23 (92)	0.12
Treatment				
Oral corticosteroids	6 (60)	8 (57)	20 (80)	0.16
Anti-fungal agents	3 (30)	3 (21)	14 (56)	0.07
Anti-IgE antibody	0 (0)	0 (0)	2 (8)	0.20
Laboratory data at enrollment				
Peripheral blood eosinophil counts, μL	218(99–743)	317(215–762)[n = 13]	515(240–1041)	0.07
Total serum IgE, IU/mL	1056(268–2568)	1631(818–2846)[n = 13]	2487(1018–3300)[n = 18]	0.07
<i>A. fumigatus</i> -specific antibody				
IgG CF	0 (0)	0 (0)	7 (28)	0.01
IgG ImmunoCAP, m _A /mL	14 (11–21)	47 (39–62)	114 (83–141)	<0.001

Values are medians (interquartile range) or number (proportion) of patients in each study groups.

ABPA, allergic bronchopulmonary aspergillosis; FVC, forced vital capacity; CF, complement fixation.

commercial laboratory (SRL, Tokyo, Japan). The positive rates in the two approaches (46% and 58%, respectively) were similar with that of using the antigen of *A. fumigatus* from Hollister–Stier in this study. Kappa coefficient between these measurements were 0.69–0.85, suggesting good reproducibility.

The positive rate of *A. fumigatus*-specific IgG (ImmunoCAP) with a 26.9 mg_A/L cut-off value was the highest among the three methods examined in the present study. This cut-off value was adopted based on a study performed in India, demonstrating the diagnostic usefulness of *A. fumigatus*-specific IgG (ImmunoCAP) with a 26.9 mg_A/L cut-off value and a sensitivity and specificity of 88% and 100%, respectively for ABPA.³ Different cut-off values have been suggested for *Aspergillus*-associated asthma (12.8 mg_A/L) and chronic pulmonary aspergillosis (20–40 mg_A/L).^{6,15} If a 40 mg_A/L cut-off value was applied, the positive rate of *A. fumigatus*-specific IgG (ImmunoCAP) would have been 63% in our cases. In a Japanese general population aged 20–59 years who donated blood for Japanese Red Cross, however, 36% and 23% of the subjects demonstrated *A. fumigatus*-specific IgG (ImmunoCAP) ≥ 26.9 mg_A/L and ≥ 40 mg_A/L, respectively (unpublished data). Further study is necessary for the evaluation of specificity of *A. fumigatus*-specific IgG (ImmunoCAP) in diagnosis of *Aspergillus*-associated lung diseases.

There are few studies that have examined the concordance among *Aspergillus*-specific precipitin and IgG.^{5,6} Baxter *et al.*⁵ demonstrated that two methods of ELISA including ImmunoCap

assay had good agreement with each other and showed better sensitivity for the diagnosis of ABPA compared to counter-immunoelectrophoresis (CIE) assay, which was an alternative method for Ouchterlony double immunodiffusion testing. However, the reported rate of CIE in ABPA was 15%, which was lower than our results or previous reports, even though their ABPA group included several cases with severe asthma with fungal sensitization.⁵

In the present study, we compared the clinical characteristics of patients with double-positive for *A. fumigatus*-specific precipitin and IgG (ImmunoCAP) and positive for IgG alone to clarify the reason of the discordance in the results between *A. fumigatus*-specific precipitin and IgG. Peripheral blood eosinophil counts, total IgE levels in the serum, impairment of pulmonary functions, and the frequency of *Aspergillus* species isolated in the sputum cultures were higher in the double-positive patients. These results suggest that the disease activity of ABPA and the magnitude of exposure to *Aspergillus*-associated antigen might be related to the discordances between them.

Since crude antigens were used for the analysis, cross-reactive antibodies among *Aspergillus* species might have developed. We therefore evaluated precipitin and IgG (ImmunoCAP) for four *Aspergillus* spp., including *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus*. There was substantial overlap in the *A. fumigatus* and other *Aspergillus*-specific precipitin positive cases, especially between precipitins to *A. fumigatus* and *A. flavus*. Furthermore, the titers of

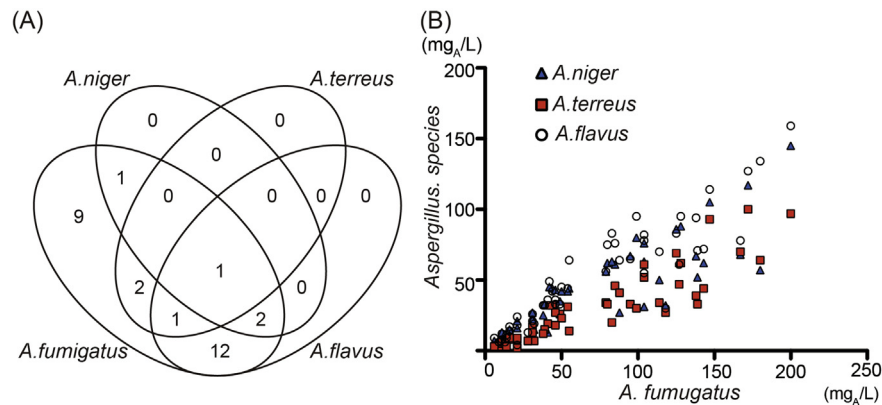


Fig. 2. (A) Specific precipitins for four *Aspergillus* spp. in patients with ABPA. The Venn diagram shows the numbers of cases with positive precipitin for *A. fumigatus*, *A. flavus*, *A. niger*, or *A. terreus*. (B) Correlation between the titers of *A. fumigatus*-specific IgG (ImmunoCAP) and IgGs specific for *A. flavus* (blue triangle), *A. niger* (red square), or *A. terreus* (open circle).

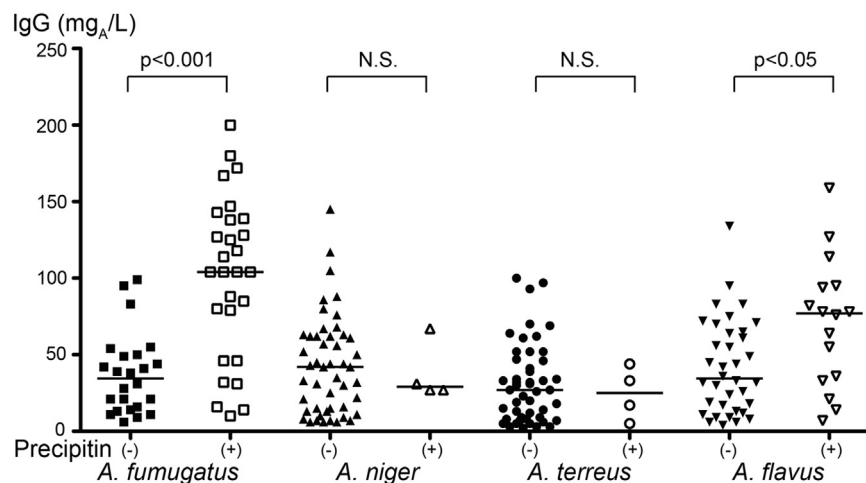


Fig. 3. Titers of *Aspergillus*-specific IgG according to the presence or absence of precipitin. The titers of specific IgG for *A. fumigatus*, *A. flavus*, *A. niger*, or *A. terreus* measured by ImmunoCAP method were compared according to the presence or absence of respective precipitin. N.S., not significant.

IgG (ImmunoCAP) specific for *A. fumigatus* were significantly correlated with those for *Aspergillus* species other than *A. fumigatus*. These results suggest that there were cross-reactivities between *A. fumigatus* and other *Aspergillus* species; although it is still possible that sensitization to multiple *Aspergillus* species may have occurred simultaneously or sequentially.

There are several limitations in the present study. First, there was no data on healthy controls or other disease control subjects with fungal-sensitized asthma. Therefore, the specificity of these assays could not be assessed in this study. Second, this study did not follow the patients longitudinally; therefore, we could not examine the reproducibility of the assays. Third, there are other methods for detecting specific IgG such as CIE or hemagglutination,¹⁰ which we did not examine in the present study.

In conclusion, the positive rate of *A. fumigatus*-specific precipitin and IgG (ImmunoCAP) were higher than that of *A. fumigatus*-specific IgG (CF). However, there are substantial disagreements between the results of *A. fumigatus*-specific precipitin and IgG (ImmunoCAP) especially in cases with the lower disease activity. In addition, there might be a substantial cross-reactivity among antibodies to different *Aspergillus* spp. Therefore, we should carefully evaluate the results of the assays for type III hypersensitivity to *Aspergillus* sp. in the diagnosis of ABPA.

Acknowledgements

We thank the members of Japan ABPM research program: Terufumi Shimoda, Katsuhiko Kamei, Hiroto Matsuse, Akira Hebisawa, Noboru Takayanagi, Satoshi Konno, Koichi Fukunaga, and Shigeharu Ueki. We also thank Ms. Naoko Yatabe and Mafumi Kwarabata for their excellent technical contributions to this study. This work was supported by Research Grant on Allergic Disease and Immunology from the Japan Agency for Medical Research and Development under Grant Number JP18ek0410026.

Conflict of interest

KA has received lecture fees from Boehringer Ingelheim and AstraZeneca. MT has received lecture fees from AstraZeneca. The rest of the authors have no conflict of interest.

Authors' contributions

TO and KA has complete access to all the data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis. KH and TO contributed to the conception and design of the study, acquisition of data, analysis and interpretation of data, and drafting the manuscript and are guarantors of the paper. AS and YF measured and evaluated precipitins. YF, JT, KT, and MT contributed to the acquisition and interpretation of data and revised the manuscript. KA contributed to the conception and design of the study, the supervision of all activities related to the conduct of the study, writing, and final approval of the

manuscript. All the authors have agreed to be accountable for all aspects of the study and have approved the final version of the manuscript.

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