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Original Article

Optimal *Aspergillus fumigatus* and Asp f 1 serum IgG cut-offs for the diagnosis of allergic bronchopulmonary aspergillosis

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ABPA, allergic bronchopulmonary aspergillosis; Abs, antibodies; Af, *Aspergillus fumigatus*; AUC, area under the curve; EIA, enzyme immunoassay; HC, healthy controls; ISHAM, International Society for Human and Animal Mycology; ROC, receiver operating characteristic

ABSTRACT

Background: The presence of IgG antibodies (Abs) to *Aspergillus fumigatus* (Af) is a crucial diagnostic criterion for allergic bronchopulmonary aspergillosis (ABPA). Although precipitation is traditionally used to document IgG Abs, anti-Af serum IgG levels can also be measured by enzyme immunoassay (EIA). However, there are insufficient data on the optimal cut-offs to assess diagnostic performance of the EIA method. This study aimed to determine cut-off levels of IgG binding crude Af extracts or recombinant Asp f 1 (by ImmunoCAP®) and to compare their efficacy for ABPA diagnosis with Af-precipitating Abs.

Methods: The age distribution of levels of IgG to crude extracts of Af (Af-IgG) and recombinant Asp f 1 (Asp f 1-IgG) was established using sera from 694 healthy controls (HC). Receiver operating characteristic analysis for Af-IgG and Asp f 1-IgG levels for the purpose of ABPA diagnosis was performed in 306 Af-sensitized asthma patients (including 49 ABPA), and cut-offs were determined.

Results: An age-dependent decline in the levels of Af-IgG was observed in HC. Thus, cut-offs for Af-IgG levels were determined separately by age as 60 mg/L for patients aged <55 years, and 45 mg/L for those aged ≥55 years. For Asp f 1-IgG, 6.6 mg/L was set as the cut-off regardless of age. Although such IgG testing by EIA allowed a sufficiently good diagnostic performance, Af-precipitating Abs had better diagnostic applicability for ABPA.

Conclusions: We determined cut-offs for Af-IgG and Asp f 1-IgG measured by EIA, which can be useful in clinical settings where precipitating Abs are unavailable.

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Introduction

Allergic bronchopulmonary aspergillosis (ABPA) manifests as severe asthma characterized by elevated IgE levels to *Aspergillus fumigatus* (Af), elevated serum IgE levels, eosinophilia, lung infiltration, bronchiectasis, and the presence of IgG antibodies (Abs) specific for Af. Indeed, "Presence of precipitating or IgG Abs to Af" is

one of the diagnostic criteria for ABPA as proposed by Rosenberg and Patterson,¹ Greenberger,² and the International Society for Human and Animal Mycology (ISHAM) working group.³ The precipitating Abs method has been traditionally performed for the detection of IgG Abs to Af. Its clinical significance has been well-described in the literature, but the method has some disadvantages, including low sensitivity and poor reproducibility. Furthermore, it is challenging to interpret the results objectively.^{4–6} These drawbacks are due to the nature of the qualitative test that requires special techniques and time to implement. Additionally, in some countries (including Japan), this test is not covered by health insurance.

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On the other hand, serum IgG levels to Af can be measured by enzyme immunoassay (EIA), which is highly quantitative and technically easy to perform. Nevertheless, it is not clear whether the quantification of levels of IgG to Af by EIA has a diagnostic performance equivalent to or better than the precipitating Abs technique. There are only a few studies on appropriate cut-offs for Af-IgG levels as assessed by the EIA method for the diagnosis of ABPA.^{4,7}

Recent advances in molecular biology have made it possible to quantify IgGs specific for various allergen components of crude Af. The levels of IgG Abs to recombinant Asp f 1, f 2, f 3, f 4, and f 6 can be quantified currently using a commercially-available ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden). Asp f 1 is a highly species-specific major allergen of Af which is not present in spores but is produced only after germination and growth of the fungi.⁸ Evidence of sensitization to Asp f 1 indicates that the patient has genuinely been exposed to and has responded to Af.^{9–11} In our previous study on the diagnosis of ABPA, we performed receiver operating characteristic curve (ROC) analyses for levels of IgG to crude Af extracts and Af-components, and found that the area under the curve (AUC) for Asp f 1-IgG was higher than for other Af-components.¹¹ However, that study focused on the diagnostic significance of IgE binding to Af-components, and not on IgG Abs to Af allergens. Measuring IgG Abs to specific allergen components may improve diagnostic performance because it can exclude the possibility of cross-reactivity between fungal allergen extracts.¹² However, these issues remained to be fully investigated. Hence, the present study aimed to analyze serum IgG levels measured by the EIA method in ABPA cases, disease controls, and healthy controls (HC) to determine optimal diagnostic cut-offs. Furthermore, we aimed to compare the diagnostic performance of the precipitating Abs method with the results obtained by measuring IgG Abs to crude-Af extracts or recombinant Asp f 1 as determined by EIA.

Methods

Study design

We accessed serum samples from 694 HC to 306 patients with Af-sensitized asthma. Serum samples from HC were used to determine the age distribution of specific IgG levels in the general population. Serum samples from Af-sensitized asthma patients were used for the determination of optimal cut-offs for levels of specific IgG for the diagnosis of ABPA and the comparison of diagnostic performance between the optimal cut-offs of specific IgG and the precipitating Abs method. The study protocol (No. 2016-027) was approved by the local research ethics committees in National Hospital Organization Sagamihara National Hospital, Tokai University School of Medicine, and the Japanese Red Cross Society.

Samples and participants

Blood specimens from HC were obtained from donors to the Japanese Red Cross Society, and had been kept frozen for 11 years for retrospective studies on the early detection and treatment of transfusion-transmitted infectious disease. The 800 serum samples from subjects aged 20–59 years, which comprised 50 men and 50 women in each age group at 20–24, 25–29, 30–34, 35–39, 40–44, 45–49, 50–54, and 55–59 years of age, were randomly selected from blood samples donated in Tokyo in October 2005. Among these 800 HC, there were 93 with some current or past medical history on self-reported health history questionnaires and 13 others with no medical history but who were positive for serum IgE Abs to Af (≥ 0.35 kUA/L). These subjects were not included in the

study, in order to exclude any possible influence on the presence of Af-IgG of current or past medical history or IgE sensitization to Af. Thus, finally, 694 HC samples were studied.

Serum samples from the Af-sensitized asthma patients had already been used in our previous study, and their collection has been described.¹¹ In brief, patients with ABPA and Af-sensitized asthma were recruited from a cohort of sequential adult outpatients (≥ 16 years of age) at Sagamihara National Hospital from 2002 to 2013. All cases had a) asthma diagnosed by pulmonologists and allergists according to American Thoracic Society criteria,¹³ and b) elevated IgE Abs to Af (≥ 0.35 kUA/L). Medical chart review was performed to extract information such as peak total IgE levels, X-ray findings matching ABPA (central bronchodilation, infiltration shadow, mucus plug), peak peripheral blood eosinophil counts, age, and gender.

Diagnostic criteria for ABPA, and classification

ABPA diagnosis was based on the ISHAM working group criteria.³ ABPA in asthma was diagnosed when both the following “obligatory criteria” (i) and “other criteria” (ii) were met, as follows: (i) obligatory criteria [both (a) and (b) should be present] – (a) Af-specific IgE ≥ 0.35 kUA/L and (b) total IgE levels ≥ 1000 IU/mL; and (ii) other criteria [at least two of the following three (a) to (c)] – (a) presence of precipitating/IgG Abs to Af, (b) total eosinophil count ≥ 500 cells/ μ L, and (c) radiographic lung opacities consistent with ABPA. Furthermore, according to the criteria of the ISHAM working group, even if total IgE values were <1000 IU/mL, the diagnosis of ABPA was made if all items of the other criteria were present. In our study, all cases met the criteria of Af-specific IgE ≥ 0.35 kUA/L, because it is one of the inclusion criteria of this study. The results of precipitating Abs (not levels of IgG Abs) were used for the criterion “presence of precipitating/IgG Abs to Af” because the main concern was to evaluate the diagnostic performance of levels of IgG Abs by EIA. Patients with asthma who did not meet these diagnostic criteria were treated as non-ABPA. Moreover, patients with ABPA were divided into two subgroups: (i) those who had all variables for both “obligatory criteria” and “other criteria” and (ii) those who did not have all of them.

Genuine ABPA determined by component-resolved diagnostics

Because the ISHAM diagnostic criteria for ABPA do not include repeated detection of Af from sputum and the mucus plug, ABPA diagnosed by these criteria may include allergic bronchopulmonary mycosis (ABPM) caused by fungi other than Af.^{10,11} Furthermore, IgE to crude extracts of Af can be present even if the fungus causing disease in an individual patient is not Af, because of cross-reactivity between fungal allergen extracts. According to the concept of component-resolved allergy diagnostics, sensitization to specific allergen components from Af (Asp f 1 and f 2) can verify genuine sensitization to Af.^{10,14} Therefore, we subdivided the ABPA group into two, namely ABPA with genuine Af sensitization (patients group with elevated IgE (≥ 0.70 kUA/L) to Asp f 1 and/or Asp f 2) and ABPA without proven genuine Af sensitization (patients group without IgE to either Asp f 1 or Asp f 2). We used the cutoff of 0.70 kUA/L for the definition of elevation in IgE to Asp f 1 and f 2 instead of 0.35 kUA/L, because the former can define patients with genuine Af sensitization more specifically.^{10,11}

Serological analysis

Serum samples were stored at ≤ -20 °C until assayed. IgE/IgG Abs binding crude Af, IgG/IgE Abs binding recombinant Asp f 1 and IgE Abs binding recombinant Asp f 2 were measured using a

commercially-available ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden). According to the manufacturer's recommendations, levels of IgE Abs ≥ 0.35 kUA/L were considered positive.

Precipitating Abs to Af were assessed by Ouchterlony double immunodiffusion using the commercially-available crude extract of Af (HollisterStier, WA, USA)¹⁵ testing sera from Af-sensitized asthma patients.

External validation

The dataset of Harada *et al.*'s prospective cohort study ($n = 52$)¹⁶ was used to examine the external validity of the cut-offs for Af-IgG determined in this study. Therefore, after excluding 6 cases enrolled from Sagamiara National Hospital to eliminate the possibility of case duplication, data from 46 cases with ABPA were used for the external validation study.

Statistical analysis

Mann–Whitney U-testing or Kruskal–Wallis testing was performed for continuous variables. Fishers' test or the Chi-square test was used for nominal variables. A logistic regression model was used to evaluate the association of IgG Abs to crude Af (Af-IgG), IgG Abs to Asp f 1 (Asp f 1-IgG), and the presence of Af-precipitating Abs with each item of the ISHAM criteria, and odds ratios (OR) and 95% confidence intervals (CIs) were calculated. ROC analysis, which included the calculation of ROC, AUC, and sensitivities and specificities at each cut-off value, was used to evaluate the diagnostic ability of Af-IgG and Asp f 1-IgG levels. It was performed to delineate cut-offs with the highest sensitivity at different specificities ($>80\%$, $>85\%$, and $>90\%$). In these statistical analyses, Af-IgG or Asp f 1-IgG levels <2.0 mg/L were assigned the value 1.0 mg/L. $P < 0.05$ was considered statistically significant. R software packages (V.3.4.1; R Development Core Team) were used for analysis. ROC and AUC calculations were performed with the pROC package.¹⁷

Results

Participants' characteristics

Characteristics of the Af-sensitized asthma patients ($n = 306$) and HC ($n = 694$) are shown in Table 1. Of the 306 Af-sensitized

asthma patients, 49 (16%) met the ISHAM ABPA diagnostic criteria. There were significant differences between ABPA patients and non-ABPA patients in age, atopic dermatitis, blood eosinophil count, total IgE levels, Af-IgE levels, Af-IgG levels, Asp f 1-IgG levels, presence of Af-precipitating Abs, and central bronchodilation or infiltrative shadow.

Associations of levels of Af-IgG and Asp f 1-IgG with age

Associations between age and levels of IgG to crude extracts of Af (Af-IgG) or recombinant Asp f 1 (Asp f 1-IgG) were evaluated separately in HC, non-ABPA, and ABPA groups (Fig. 1, Supplementary Table 1). Af-IgG levels showed a significant age-dependent decline, but only in the HC group ($P < 0.01$) (Supplementary Table 1). In the non-ABPA group, Af-IgG levels also tended to show an age-dependent decline, but this did not reach statistical significance ($P = 0.20$). On the other hand, there were no trends towards associations between age and Asp f 1-IgG levels either in HC ($P = 0.77$), non-ABPA ($P = 0.66$), or ABPA groups ($P = 0.24$).

We next assessed associations between different five-year age groups and Af-IgG. We found that the levels of these antibodies in the HC group aged 55–59 years were significantly lower than in the other age groups <55 years old (Supplementary Fig. 1). Therefore, subjects were subdivided into two groups, one <55 and the other ≥ 55 years of age (Fig. 2). Levels of Af-IgG in the HC group <55 years of age were significantly higher than in those ≥ 55 years old ($P < 0.001$, Fig. 2A), and tended to be higher in non-ABPA patients ($P = 0.068$, Fig. 2B). Considering these age-dependent differences in the distribution of Af-IgG levels among HC and non-ABPA, we propose that ROC analysis of Af-IgG levels for determining the cut-offs for ABPA diagnosis should be performed separately for patients aged <55 and ≥ 55 years.

ROC analysis and cut-offs of Af-IgG and Asp f 1-IgG for diagnosing ABPA among the Af-sensitized asthma patients

The results of the ROC analysis are shown in Figure 3. The AUC for Af-IgG in patients aged <55 vs ≥ 55 years were 0.708 (95% CI: 0.583–0.833) and 0.785 (95% CI: 0.691–0.879), respectively. This value for Asp f 1-IgG was 0.691 (95% CI: 0.590–0.792). The cut-offs for levels of Af-IgG and Asp f 1-IgG as predictors of ABPA at specificities of 80%, 85%, and 90% are shown in Table 2. The cut-offs of Af-

Table 1
Characteristics of studied subjects.

	Af-sensitized asthma ($n = 306$)			HC ($n = 694$) [†]
	ABPA ($n = 49$)	Non ABPA ($n = 257$)	P	
Age (years), mean $\pm$ SD	55 $\pm$ 12.8	45 $\pm$ 16.4	<0.001	40 $\pm$ 11.5
Female sex, no. (%)	21 (42.9)	145 (56.4)	0.112	350 (50.4)
Atopic dermatitis, no. (%)	13 (26.5)	110 (42.8)	0.049	N.A.
Blood eosinophil count (cells/ μ L), median [range]	1250 [330–8330]	450 [10–5840]	<0.001	N.A.
>500 (cells/ μ L), no. (%)	47 (95.9)	111 (43.2)	<0.001	N.A.
Total IgE (IU/mL), median [range]	3040 [356–38,400]	1100 [15–43,400]	<0.001	N.A.
>1000 (IU/mL), no. (%)	45 (91.8)	137 (53.3)	<0.001	N.A.
Af-IgE (kUA/L), median [range]	19.3 [0.50–100.00]	1.2 [0.35–80.81]	<0.001	0.03 [0.01–0.33]
Af-IgG (mgA/L) [‡] , median [range]	51.4 [9.11–184.22]	23.3 [2.03–198.00]	<0.001	18.9 [1.00–220.48]
Asp f 1-IgG (mgA/L) [§] , median [range]	6.9 [1.00–113.4]	4.0 [1.00–16.34]	<0.001	4.1 [1.00–85.75]
Af precipitating Abs, no. (%)	36 (76.6)	5 (5.9)	<0.001	N.A.
Central bronchiectasis or consolidation, no. (%)	47 (95.9)	21 (8.2)	<0.001	N.A.
ABPA meeting all items in ISHAM criteria, no. (%) [¶]	28 (9.2)	N.A.	N.A.	N.A.

ABPA, allergic bronchopulmonary aspergillosis; Abs, antibodies; Af, *Aspergillus fumigatus*; HC, healthy controls; ISHAM, The International Society for Human and Animal Mycology; N.A., not assessed.

[†] HC consisted of adults (20–59 years of age) who donated blood to the Japanese Red Cross.

[‡] The reference range of Af-IgG was 2.17–120.3 (mgA/L) at the 2.5th–97.5th percentiles.

[§] The reference range of Asp f 1-IgG was 2.42–12.9 (mgA/L) at the 2.5th–97.5th percentiles.

[¶] Meeting all obligatory and other criteria in the ISHAM diagnostic classification.

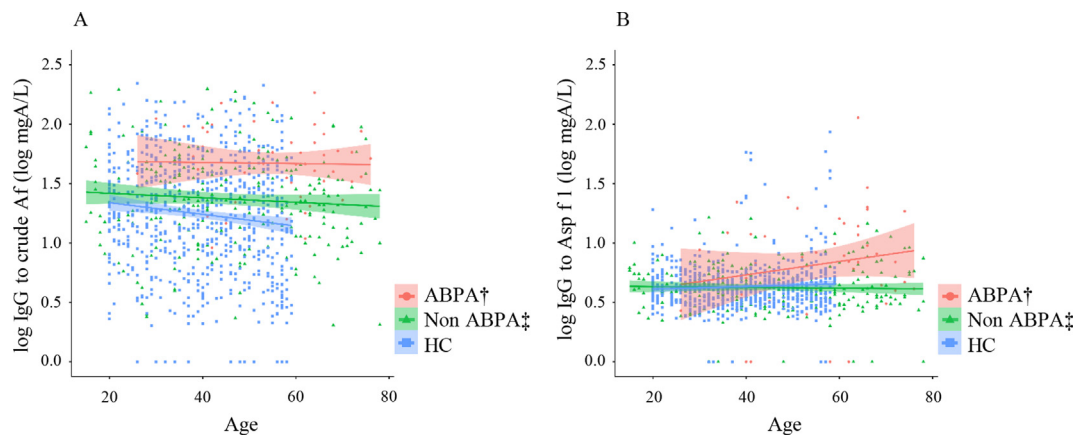


Fig. 1. Association of age with log-transformed levels of IgG to crude extracts of Af (A) or recombinant Asp f 1 (B) in ABPA patients, non-ABPA patients, and HC. The scatterplots are shown with the 95% confidence intervals and regression lines. [†]Patients with ABPA meeting ISHAM criteria. [‡]Af-sensitized patients without ABPA meeting ISHAM criteria.

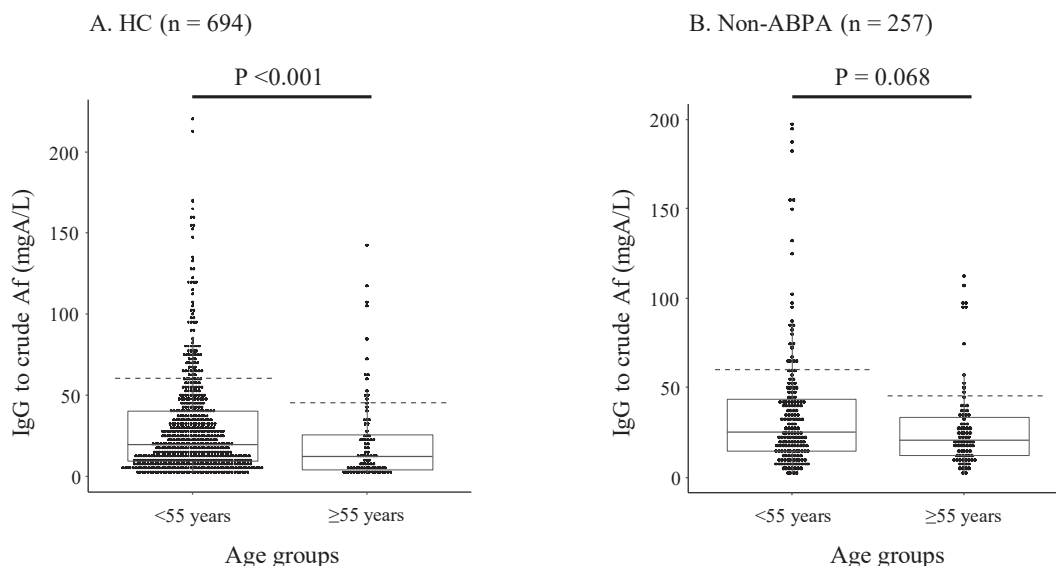


Fig. 2. Levels of IgG to crude extracts of Af by age group (<55 vs ≥55 years) in HC (A) and non-ABPA (B). The dashed line shows cut-offs for Af-IgG levels at 60 mg/L for patients aged <55 years and 45 mg/L for those aged ≥55 years.

IgG in patients aged <55 vs ≥55 years were significantly different, supporting the proposal that it is appropriate to determine age-specific cut-offs.

Table 3 shows the frequencies of positivity for Af-IgG and Asp f 1-IgG as assessed at different cut-offs corresponding to specificities of 80%, 85%, and 90% in the ABPA, non-ABPA, and HC groups. This table also shows the frequencies as assessed by the presence of Af-precipitating Abs in each group. The frequencies of Af-IgG and Asp f 1-IgG in ABPA patients, especially those meeting all ISHAM criteria (suggesting that they were more typical ABPA cases), were higher than in the non-ABPA and HC groups.

From the results shown in Tables 2 and 3, we selected a cut-off at an 85% specificity as optimal because this resulted in a sufficiently high frequency of positivity among ABPA patients but relatively low frequencies in HC (about 10%), as well as the non-ABPA group. This cut-off for Af-IgG was 60 mg/L for patients <55 years of age and 45 mg/L for those ≥55 years, whereas the cut-off for Asp f 1-IgG was 6.6 mg/L regardless of age. The overall sensitivities of Af-IgG and Asp f 1-IgG for diagnosing ABPA at these cut-offs were about 51% and 53%, respectively.

Supplementary Table 2 shows the frequencies of Af-IgG and Asp f 1-IgG compared with Af-precipitating Abs in ABPA patients positive (≥0.7 kUA/L) for IgE to Asp f 1 and/or Asp f 2 (i.e. ABPA with genuine Af sensitization) and those who were IgE-negative (<0.7 kUA/L) to both (i.e. ABPA without proven genuine Af sensitization). The frequencies of Af-IgG, Asp f 1-IgG, and Af-precipitating Abs in the former group (ABPA with genuine Af sensitization) were significantly higher than in the latter.

External validation of cut-offs for Af-IgG levels

For external validation, we accessed a dataset from an independent prospective cohort study conducted in Japan on ABPA meeting the ISHAM diagnostic criteria.¹⁶ Among the ABPA patients in that study, we investigated the proportion with Af-IgG using the optimal cut-off as determined in our study. There was very good agreement between frequencies of Af-IgG-positivity in that study (25/46, 54%) (Supplementary Table 3), and our study (51%).

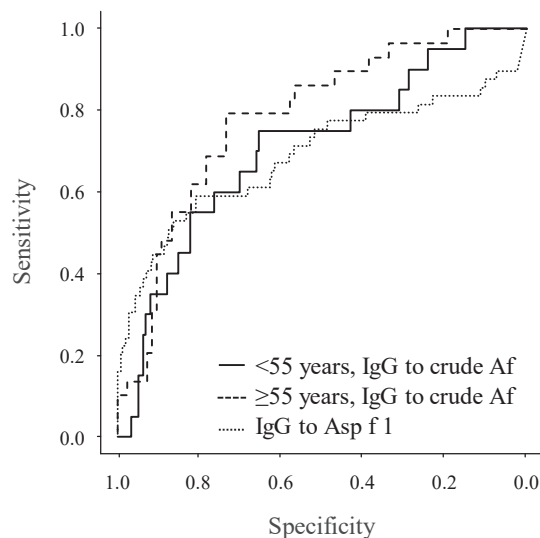


Fig. 3. ROC curves for the levels of IgG to crude extracts of Af (separately for patients aged <55 and ≥55 years) and Asp f 1 to predict ABPA among the Af-sensitized asthma patients.

Comparison between the diagnostic performance of Af-IgG, Asp f 1-IgG, and Af-precipitating Abs

Associations of Af-IgG, Asp f 1-IgG, and Af-precipitating Abs with the ISHAM diagnostic criteria are shown in Table 4. There were some significant associations of Af-IgG and Asp f 1-IgG with a few of these items. On the other hand, data from Af-precipitating Abs were significantly associated with all of the diagnostic criteria at higher odds ratios than yielded by Af-IgG and Asp f 1-IgG.

We compared the diagnostic ability of Af-IgG, Asp f 1-IgG, and Af-precipitating Abs for predicting ABPA as defined without using the criterion of “precipitating/IgG Abs to Af”, i.e. these Abs were considered negative in all patients (Table 5). For this, we reclassified all Af-sensitized patients into those with and those without ABPA, on the assumption that all failed to meet the criterion “presence of precipitating/IgG Abs to Af”. All statistical measures still indicated that the diagnostic performance of Af-precipitating Abs was better than Af-IgG and Asp f 1-IgG levels.

Discussion

In the present study, we determined the cut-offs for EIA-based measurement of IgG-levels to crude extracts of Af (Af-IgG) and recombinant Asp f 1 (Asp f 1-IgG) as predictors of ABPA, relative to precipitating Abs, for its diagnosis. One of the strengths of this study is that we analyzed serum samples from a large number of ABPA cases and controls after recruiting consecutive outpatients with Af-sensitized asthma at a tertiary hospital. We additionally analyzed a large number of serum samples of healthy controls, used for the evaluation of age-dependent differences in IgG-levels and the frequency of positivity for specific IgG among HC at the cut-offs determined in this study. Although EIA-based quantification of Af-IgG and Asp f 1-IgG also showed excellent diagnostic performance, measurement of Af-precipitating Abs was a superior predictor of ABPA than EIA-based measurement of IgG. Nonetheless, the results of this study could be useful in a clinical setting where precipitating Ab testing is not available.

The cut-off for IgG levels in our study was determined so as to retain high specificity rather than high sensitivity for the diagnosis of ABPA because the “Presence of precipitating/IgG Abs to Af” is

Table 2

Cut-offs for the levels of IgG to crude Af (Af-IgG) and Asp f 1 (Asp f 1-IgG) as predictors of ABPA at specificities of 80, 85 and 90%.

	Cut-off (mgA/L)	Sensitivity (%)	Specificity (%)
Specificity >80%			
Af-IgG			
<55 yrs	51	55.0	80.5
≥55 yrs	38	62.1	80.7
(Overall) [†]		59.2	80.5
Asp f 1-IgG	6.0	57.1	80.9
Specificity >85%			
Af-IgG			
<55 yrs	60	45.0	85.1
≥55 yrs	45	55.2	85.5
(Overall) [†]		51.0	85.2
Asp f 1-IgG	6.6	53.1	85.6
Specificity >90%			
Af-IgG			
<55 yrs	80	35.0	90.2
≥55 yrs	54	44.8	90.4
(Overall) [†]		40.8	90.3
Asp f 1-IgG	7.2	44.9	90.3

ABPA, allergic bronchopulmonary aspergillosis; Af, *Aspergillus fumigatus*; yrs, years.

[†] Sensitivity and specificity calculated in the population including both patients aged <55 and ≥55 years together using age-specific cut-offs.

usually considered as an item with high specificity among the ABPA diagnostic criteria.^{3,18} Actually, according to the ISHAM criteria, “Presence of precipitating/IgG Abs to Af” is one of the “other criteria”, and is not obligatory for diagnosis. High sensitivity is not necessary because precipitating Abs or IgG Abs to Af have not been used as screening tests for the diagnosis of ABPA.^{3,18} Therefore, it is considered inappropriate to use the Youden index, where equal weight is given to sensitivity and specificity,¹⁹ for establishing an optimal cut-off to diagnose ABPA among the Af-sensitized asthma patients.

One possible reason for the relatively low frequencies of positivity for Af-IgG and Asp f 1-IgG (about 50%) among ABPA patients in our study may relate to the fact that fungi causing disease are not always Af. That is, patients meeting the diagnostic criteria for ABPA can include those with ABPM caused by fungi other than Af.¹⁰ According to the concept of component-resolved diagnostics,^{20,21} patients with genuine Af sensitization (positive for IgE to Asp f 1 and/or f 2) are regarded as those with ABPM caused by Af, not by fungi other than Af. As shown in Supplementary Table 2, when we limited the analysis to ABPA patients with genuine Af sensitization, frequencies of positivity for Af-IgG and Asp f 1-IgG were higher (64.9% and 70.3%, respectively). Therefore, relatively low frequencies of positivity for Af-IgG and Asp f 1-IgG among ABPA patients observed in our study can be partially explained by the presumption that the real causal fungi for some minority of the criteria-defined ABPA patients were fungi other than Af. This finding also suggests high concordance between positivity for Af-IgG and Asp f 1-IgG, and genuine ABPA as determined by component-resolved diagnostics.

To the best of our knowledge, this is the first study to evaluate the presence of Asp f 1-IgG (to one of the major allergens of Af) in healthy subjects. We found that Asp f 1-IgG levels were not affected by age in HC, non-ABPA, or ABPA. However, Af-IgG levels seemed to be negatively associated with age in HC. Considering the lack of age-dependent decline of Asp f 1-IgG, the age-dependent decrease in Af-IgG may reflect cross reactivity between Af and other environmental fungal allergens, with higher levels of IgG in young adults than in older adults.

Various cut-offs for Af-IgG have been reported in different studies. In the UK study, a cut-off of 40 mg/L was used,^{6,22} but this was determined based on unpublished observations.^{6,23} In a

Table 3

Frequency of positivity for Af-IgG and Asp f 1-IgG at different cut-offs, and frequency of Af-precipitating antibodies.

	Af-sensitized asthma		HC (n = 694)		P
	ABPA		Non ABPA (n = 257)		
	All criteria [†] (n = 28)	Not all criteria [†] (n = 21)			
Positive for Af-IgG at different cut-offs (mgA/L)[‡]					
51 (<55 yrs)/38 (≥55 yrs)	19 (68)	10 (48)	50 (19)	119 (17)	<0.001
60 (<55 yrs)/45 (≥55 yrs)	17 (61)	8 (38)	38 (15)	90 (13)	<0.001
80 (<55 yrs)/54 (≥55 yrs)	14 (50)	6 (29)	25 (10)	49 (7)	<0.001
Positive for Asp f 1-IgG at different cut-offs (mgA/L)[‡]					
6.0	20 (71)	8 (38)	49 (19)	83 (12)	<0.001
6.6	19 (68)	7 (33)	37 (14)	63 (9)	<0.001
7.2	15 (54)	7 (33)	25 (10)	47 (7)	<0.001
Positive for Af-precipitating Abs					
	28 (100) [¶]	8 (38)	5 (2)	N.A.	<0.001

ABPA, allergic bronchopulmonary aspergillosis; Abs, antibodies; Af, *Aspergillus fumigatus*; HC, healthy controls; ISHAM, The International Society for Human and Animal Mycology; N.A., not assessed; yrs, years.

[†] Patients meeting all items of the ISHAM criteria (both obligatory and other criteria).

[‡] Patients not meeting all items of the ISHAM criteria.

[§] Cut-offs corresponding to specificities of 80, 85, and 90% determined after the analysis shown in Table 2.

[¶] The frequencies of Af-precipitating Abs in ABPA patients meeting all ISHAM criteria were 100%, by definition, because the ISHAM diagnostic criteria for ABPA used in this study included the presence of precipitating Abs to Af.

Table 4

Associations of positivity for Af-IgG, Asp f 1-IgG, and Af-precipitating antibodies with criteria of the ISHAM ABPA scheme.

	IgE ≥1000 (IU/mL)	Blood eosinophil count ≥500 (cells/μL)	Central bronchiectasis or consolidation
Positive for Af-IgG [†]	1.14 (0.64–2.01)	1.69 (0.96–2.99)	4.06 (2.22–7.41)**
Positive for Asp f 1-IgG [‡]	1.17 (0.67–2.07)	2.97 (1.63–5.41)**	3.58 (1.97–6.51)**
Positive for Af-precipitating Abs	3.84 (1.64–8.97)*	8.44 (3.21–22.2)**	33.0 (13.6–80.3)**

*P < 0.01.

**P < 0.001.

ABPA, allergic bronchopulmonary aspergillosis; Abs, antibodies; Af, *Aspergillus fumigatus*; ISHAM, The International Society for Human and Animal Mycology; yrs, years.

Odd ratios and 95% confidence intervals are shown.

[†] ≥60 (mgA/L) for patients aged <55 yrs and ≥45 (mgA/L) for patients aged ≥55 yrs.

[‡] ≥6.6 (mgA/L).

Table 5

Diagnostic performance of Af-IgG, Asp f 1-IgG and Af-precipitating antibodies as predictors of ABPA defined not using the criterion “precipitating/IgG antibodies to Af”.

Sensitivity (%)	Specificity (%)	Accuracy (%)	Positive likelihood ratio	Negative likelihood ratio
Positive for Af-IgG[†]				
51.2	84.2	76.5	3.2	0.58
Positive for Asp f 1-IgG[‡]				
51.2	83.8	74.8	3.2	0.58
Positive for Af-precipitating Abs				
68.3	95.1	91.5	13.9	0.33

ABPA, allergic bronchopulmonary aspergillosis; Abs, antibodies; Af, *Aspergillus fumigatus*; yrs, years.

[†] ≥60 (mgA/L) for patients aged <55 yrs and ≥45 (mgA/L) for patients aged ≥55 yrs.

[‡] ≥6.6 (mgA/L).

prospective cohort study in India, a cut-off of 26.9 mg/L for Af-IgG in ABPA was determined by comparing patients with ABPA with total IgE levels ≥1000 IU/mL to patients with Af-associated asthma with total IgE levels <1000 IU/mL.⁷ We consider that the primary reason for the discrepancy in cut-offs for Af-IgG between previous studies and our present study relates to differences in the selection of control patients. In our study, optimal cases and disease controls, reflecting patients in real clinical settings, were recruited because we studied all consecutive asthma outpatients with Af sensitization regardless of total IgE levels.

We found that the diagnostic performance of Af-precipitating Abs was better than EIA-based measurement of Af-IgG or Asp f 1-IgG. This may be due to the different antibody-antigen binding properties in EIA vs precipitation. In EIA, especially the

ImmunoCAP® test, as there are more antigens present than serum-specific Abs in the assay, the excess of allergen immobilized allows specific Abs to completely bind to their targets,²⁴ which contributes to high sensitivity. On the other hand, the formation and precipitation of antibody-antigen complexes requires polyvalent Abs with moderate to high affinities and antigens at the appropriate ratio,²⁵ which contributes to the low sensitivity but high specificity of this approach.

This study has some limitations. First, it was conducted at a single tertiary referral center in Japan, and there may be a problem with general validity. However, the frequency of precipitating Abs in ABPA patients' sera in the present study was about 74%, close to a previous report.²⁶ Moreover, the presence of Af-IgG in 51% of patients at the cut-off selected in our study was very similar to the 54% in an independent prospective Japanese cohort study. Thus, our Af-IgG cut-off is likely to be externally valid, at least in Japan. However, further studies will be needed to verify the generalizability of the cut-offs not only for Af-IgG but also for Asp f 1-IgG.

In conclusion, the present study determined optimal cut-offs for levels of Af-IgG and Asp f 1-IgG for predicting ABPA as measured by EIA methods. Although Af-precipitating Abs had a better diagnostic performance than Af-IgG or Asp f 1-IgG, the findings of the present study are useful in clinical settings where precipitating Ab methods cannot be applied.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2020.07.006>.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

All authors contributed to this study. YF, TO, KA, and MT contributed to the conception and the design of the study and interpretation of the data; YF, AS, KW, YK, KS, TN, KH, YS, and TO were responsible for data acquisition. YH and EN conducted the statistical analyses; YH wrote the draft manuscript; YF, TO, and KA, MT contributed to critical revision of the manuscript.

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Supplementary Tables

Supplementary Table 1. Association of age with log Af-IgG and Asp f 1-IgG

ABPA	Dependent variable: log Af-IgG			
	Independent variable	Slope	S.E.	P
	Age	-4.8e-04	3.5e-03	0.89
	Dependent variable: log Asp f 1-IgG			
	Independent variable	Slope	S.E.	P
	Age	5.7e-03	4.7e-03	0.24
Non-ABPA	Dependent variable: log Af-IgG			
	Independent variable	Slope	S.E.	P
	Age	-1.9e-03	1.5e-03	0.20
	Dependent variable: log Asp f 1-IgG			
	Independent variable	Slope	S.E.	P
	Age	-3.3e-04	7.5e-04	0.66
HC	Dependent variable: log Af-IgG			
	Independent variable	Slope	S.E.	P
	Age	-4.9e-03	1.5e-03	<0.01
	Dependent variable: log Asp f 1-IgG			
	Independent variable	Slope	S.E.	P
	Age	1.2e-03	6.6e-04	0.77

ABPA, allergic bronchopulmonary aspergillosis; Af, Aspergillus fumigatus; HC, healthy

controls; S.E., standard error.

Supplementary Table 2. Frequency of Af-IgG, Asp f 1-IgG and Af-precipitating antibodies positivity among ABPA patients positive (≥ 0.7 kUA/L) for Asp f 1-IgE and/or Asp f 2-IgE or negative (< 0.7 kUA/L) for both.

	ABPA		P
	Positive for Asp f 1 and/or f 2-IgE (n = 37)	Negative for both Asp f 1 and f 2-IgE (n = 12)	
Positive for Af-IgG ≥ 60 (mgA/L) for patients aged < 55 yrs. ≥ 45 (mgA/L) for patients aged ≥ 55 yrs.	24 (64.9)	1 (8.3)	< 0.001
Positive for Asp f 1-IgG ≥ 6.6 (mgA/L)	26 (70.3)	0 (0)	< 0.001
Positive for Af-precipitating Abs	31 (83.8)	5 (41.7)	< 0.01

ABPA, allergic bronchopulmonary aspergillosis; Abs, antibodies; Af, Aspergillus

fumigatus; yrs, years.

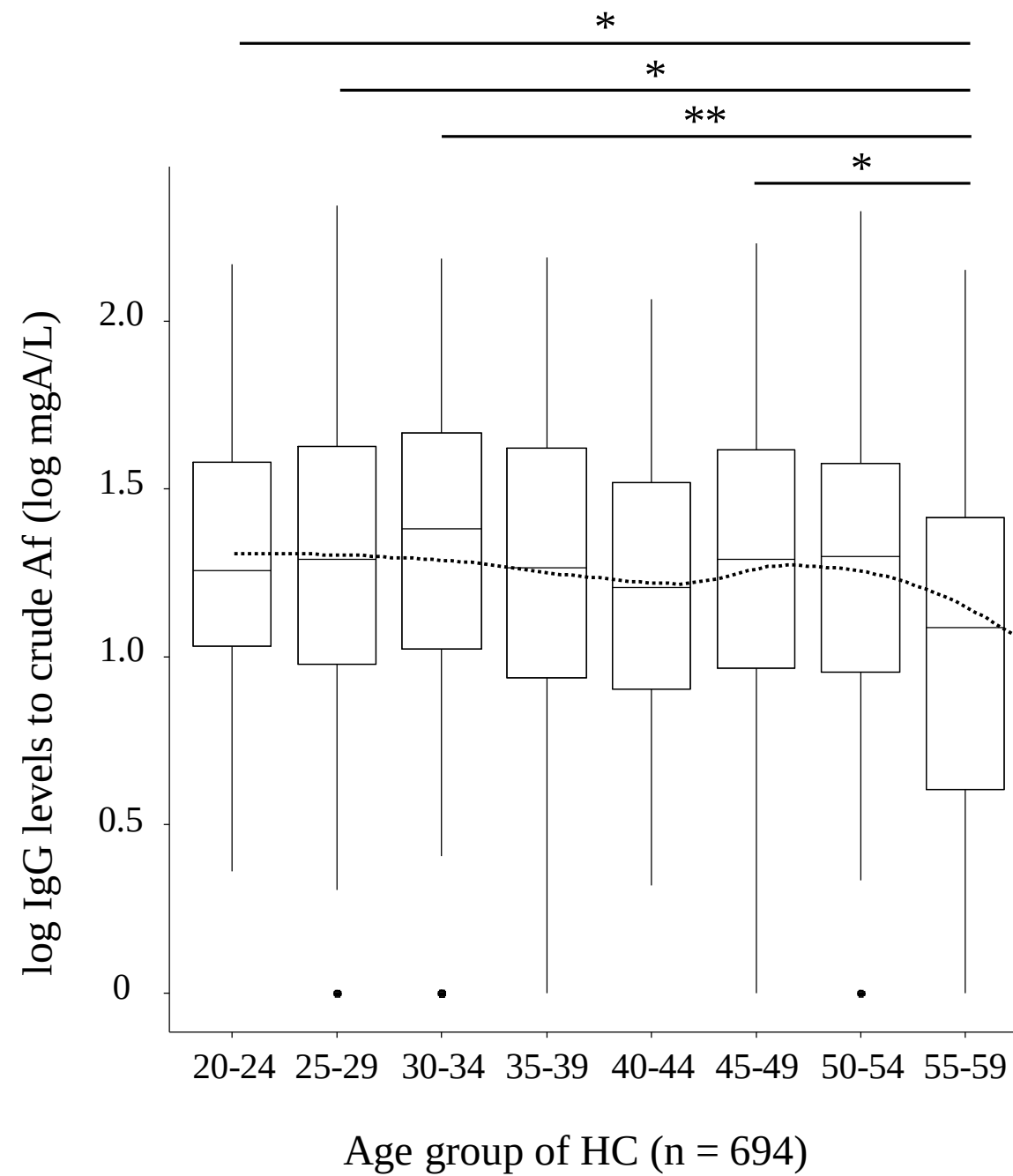
Supplementary Table 3. External validation of the cut-offs determined in this study

	ABPA (n = 46)†
Positive for Af-IgG	25 (54)
≥60 (mgA/L) for patients aged <55 yrs.	
≥45 (mgA/L) for patients aged ≥55 yrs.	

† ABPA from Harada’s study after excluding 6 patients who were recruited in

Sagamihara National Hospital.

ABPA, allergic bronchopulmonary aspergillosis; Af, Aspergillus fumigatus; yrs, years.



Supplementary Figure 1. Levels of HC Af-IgG by 5-year age groups

P value adjustment method: Bonferroni.

Locally estimated scatterplot smoothing (loess) curve is denoted by a dashed line.

* P < 0.05.

** P < 0.01.