

Original Article

Diagnostic Cutoffs and Clinical Utility of Recombinant *Aspergillus fumigatus* Antigens in the Diagnosis of Allergic Bronchopulmonary Aspergillosis

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What is already known about this topic? Although recombinant *Aspergillus fumigatus* (rAsp)-specific antigens are used in diagnosing allergic bronchopulmonary aspergillosis (ABPA), the optimal diagnostic cutoff and clinical utility of rAsp-specific IgE are not known.

What does this article add to our knowledge? The cutoffs derived from receiver-operating characteristic analysis fared better than fixed cutoffs. IgE against rAsp f1 (sensitivity, 89%; specificity, 100%) and the combination of either rAsp f1 or f2 (sensitivity, 100%; specificity, 81%) were most useful in differentiating ABPA from *Aspergillus*-sensitized asthma.

How does this study impact current management guidelines? Our study provides optimal and usable cutoffs to diagnose ABPA using rAsp antigens. The existing guidelines do not incorporate rAsp-specific IgE. We provide evidence to integrate IgE against rAsp f1 and f2 in the diagnosis of ABPA.

BACKGROUND: The clinical utility of IgE against recombinant *Aspergillus fumigatus* (rAsp)-specific antigens in allergic bronchopulmonary aspergillosis (ABPA) remains unclear.

OBJECTIVE: To identify the optimal diagnostic cutoffs of rAsp-specific IgE in differentiating ABPA from *A. fumigatus*-sensitized asthma (ASA), and define their utility in the diagnosis of ABPA.

METHODS: We enrolled consecutive subjects with ASA and ABPA. IgE against rAsp f1, f2, f3, f4, and f6 was assayed in all the subjects. We evaluated 3 fixed cutoffs (0.35, 0.5, and 1.0 kU_A/L) for their diagnostic performance in the entire cohort. We also divided the study population into derivation and validation cohorts. Cutoffs for rAsp-specific IgE were obtained using the receiver-operating characteristic analysis in the derivation cohort. We then evaluated the diagnostic performance of these

cutoffs in the validation cohort. We further correlated rAsp-specific IgE levels in ABPA with asthma control, spirometry, imaging, and immunologic markers.

RESULTS: We included 194 subjects (123 ABPA and 71 ASA). The statistically derived cutoffs proved superior to fixed cutoffs. IgE against rAsp f1 yielded the best combination of sensitivity (89%) and specificity (100%). The sensitivity and specificity of IgE against either rAsp f1 (cutoff, 4.465 kU_A/L) or f2 (cutoff, 1.300 kU_A/L) for diagnosing ABPA were 100% and 81%, respectively. The correlation between rAsp-specific IgE and most clinical parameters of ABPA was weak.

CONCLUSIONS: IgE against rAsp f1 and f2 (using receiver-operating characteristic-derived cutoffs) were found to be the most useful in differentiating ABPA from ASA. Because this study was conducted at a single center, our results require

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Abbreviations used

ABPA- Allergic bronchopulmonary aspergillosis
 ASA- Aspergillus-sensitized asthma
 AUROC- Area under the receiver-operating characteristic
 FVC- Forced vital capacity
 kU_A- Kilounits of antigen-specific antibodies
 rAsp- Recombinant Aspergillus fumigatus
 ROC- Receiver-operating characteristic

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Key words: ABPA; Allergy; Fungal sensitization; Asthma; Allergic bronchopulmonary mycosis

INTRODUCTION

The prevalence of allergic bronchopulmonary aspergillosis (ABPA) in asthma ranges from 8% to 19% in asthma clinics.¹ Isolated sensitization to *Aspergillus fumigatus* is even more prevalent (about 40% in chest clinics).¹ Unlike ABPA, *Aspergillus*-sensitized asthma (ASA) does not require any specific treatment, except in those with severe asthma.^{2,3} Therefore, from a therapeutic standpoint, it is important to differentiate ABPA from ASA. Currently, in the evaluation of ABPA, antigens derived from the crude extracts of *A fumigatus* are used. These antigens are limited by their purity and batch-to-batch variability.⁴ Moreover, the differentiation of ASA from ABPA not only requires several investigations, but reliable discrimination is also sometimes arduous.⁵ For instance, difficult-to-control asthma, elevated serum IgE level (total and *A fumigatus*-specific), and even bronchiectasis may be encountered in both ASA and ABPA.⁶ The limitations of the current immunologic assays in ABPA may be potentially addressed by using pure antigen components.^{7,8} Of the 23 *A fumigatus* antigens listed in the World Health Organization and International Union of Immunological Societies nomenclature,⁹ 5 recombinant *A fumigatus* antigens (rAsp f1, f2, f3, f4, and f6) are available commercially and have been evaluated in the diagnosis of ABPA.¹⁰⁻¹⁴ Unfortunately, the optimal diagnostic cutoffs to be used and the clinical value of these antigens remain unclear.¹⁵ Herein, we explore the utility of recombinant antigens in differentiating ABPA from ASA.

METHODS**Study design and setting**

This was a prospective cohort study conducted between December 2016 and March 2018, in the chest clinic of the Postgraduate Institute of Medical Education and Research. The Institutional Ethics Committee approved the study protocol, and written informed consent was obtained from all the participants.

Study participants

Inclusion criteria. We prospectively enrolled consecutive subjects with ASA or ABPA who were aged 12 years or older.

Definitions. *Asthma* was diagnosed if the subject satisfied 2 of the following criteria: (1) recurrent episodes of cough, chest tightness, or

breathlessness (in the absence of an alternate explanation for these symptoms); (2) wheeze on auscultation of the chest; and (3) obstructive defect on spirometry (FEV₁ to forced vital capacity [FVC] ratio less than the lower limit of normal) with significant bronchodilator reversibility (increase in FEV₁ or FVC by at least 12% and 200 mL, after inhalation of 400 µg of salbutamol), at any point of time during the illness.¹⁶

ASA was diagnosed in subjects of asthma with *A fumigatus*-specific IgE level of greater than or equal to 0.35 kilounits of antigen-specific antibodies (kU_A)/L, and not fulfilling the criteria for the diagnosis of ABPA. ABPA was diagnosed in subjects with asthma according to the modified criteria proposed by "ABPA in asthmatics" working group of the International Society of Human and Animal Mycology: (1) serum *A fumigatus*-specific IgE level of greater than or equal to 0.35 kU_A/L; and (2) serum total IgE level of greater than or equal to 1000 international units/mL; and 2 of the following: (1) *A fumigatus*-specific IgG level of greater than or equal to 27 mg of antigen-specific antibodies/L; (2) peripheral blood eosinophil count of greater than or equal to 500 cells/µL; and (3) imaging consistent with ABPA.¹⁷

Exclusion criteria. Participants were excluded if any of the following was present: (1) comorbidities or immunosuppressive states (chronic liver disease, chronic renal failure, cytotoxic therapy, uncontrolled diabetes mellitus, and others); (2) pregnancy; (3) previous diagnosis of chronic obstructive pulmonary disease; (4) treatment with systemic glucocorticoids or antifungal azoles for more than 1 month at any time point; and (5) lack of informed consent.

Study procedure

We obtained a detailed history (including the duration of asthma, presence of atopy, allergic rhinitis, and control of asthma¹⁶) from the study participants. Sera from the study participants were collected and stored at -80°C, till the antibodies were measured. We performed the following investigations in all the included subjects.

Serum *A fumigatus*-specific IgG. Serum *A fumigatus*-specific IgG level was measured using the M3 ImmunoCap with the commercial ImmunoCAP system (Phadia 100, Phadia, Uppsala, Sweden). The antigen used was a crude extract derived from the conidia and mycelia of *A fumigatus*. Diluted (1 in 100) serum samples were used, and the results were expressed as milligrams of antigen-specific antibodies/L.¹⁸

Serum *A fumigatus*-specific IgE. Serum *A fumigatus*-specific IgE level was estimated in diluted serum samples using the M3 ImmunoCap.¹⁹⁻²¹ The results were expressed as kilounits of antigen-specific antibodies (kU_A)/L.

Serum total IgE. Serum total IgE level was measured using the ImmunoCap system,¹⁹⁻²¹ and the results were expressed as international units/mL.

Total eosinophil count. Total eosinophil count was determined in the peripheral blood using an autoanalyzer (LH-750, Beckman Coulter, Brea, Calif, USA). The differential leukocyte count was assessed by counting 100 white blood cells on a peripheral blood smear, and the total eosinophil count was obtained as a product of the percentage of eosinophils and the total leucocyte count.

TABLE I. Baseline clinical features and investigations of subjects with ASA and ABPA

Parameter	ASA (n = 71)	ABPA (n = 123)	Total (n = 194)	P value
Clinical features				
Age (y)*	42 (24-57)	35 (25-46)	38.5 (24-50)	.14
Sex: male	44 (62)	69 (56.1)	113 (58.4)	.45
Duration of asthma (mo)*	60 (24-120)	120 (24-180)	84 (24-168)	.06
Nonsmokers	68 (95.8)	120 (97.6)	188 (96.9)	.67
Cough	70 (98.6)	122 (99.2)	192 (99)	1.00
Expectoration	22 (31)	63 (51.2)	85 (43.8)	.001
Dyspnea	71 (100)	119 (96.7)	190 (97.9)	.30
Wheeze	71 (100)	121 (98.4)	192 (99)	.53
Hemoptysis	3 (4.2)	10 (8.2)	13 (6.7)	.38
Expectoration of mucus plugs	5 (7)	9 (7.3)	14 (7.2)	1.00
Allergic rhinitis	23 (32.9)	39 (31.7)	62 (32.1)	.87
Allergic conjunctivitis	5 (7)	13 (10.6)	18 (9.3)	.45
Family history of asthma	21 (29.6)	48 (39)	69 (35.6)	.19
History of ATT intake	6 (8.5)	21 (17.1)	27 (13.9)	.13
Height (cm)*	160 (154.7-168)	160 (153.5-168)	160 (154-168)	.46
Weight (kg)*	60.2 (47.3-70.5)	56 (45-65.5)	57 (46.6-67.4)	.06
Asthma control at presentation				
Daytime symptoms >twice a week	26 (36.6)	45 (36.8)	71 (36.8)	1.00
Any nocturnal symptoms	27 (38)	43 (35)	70 (36.1)	.76
Any limitation in activities of daily life	17 (23.9)	39 (31.7)	56 (28.9)	.32
FEV ₁ <80% predicted	54 (76.1)	87 (70.7)	141 (72.7)	.50
Inadequately controlled asthma†	34 (47.9)	56 (45.5)	90 (46.4)	.77
Imaging				
Fleeting opacities on chest radiograph	1 (1.4)	5 (4)	6 (3.1)	.30
Bronchiectasis	19 (26.8)	96 (78)	115 (59.3)	.0001
No. of lobes involved*	0 (0-0)	1 (0-2)	0 (0-2)	.001
No. of segments involved*	0 (0-2.25)	5 (2-8)	3 (0-6)	.0001
Presence of mucus plug	1 (1.4)	31 (25.2)	32 (16.5)	.001
Aspergilloma	4 (5.6)	12 (9.8)	16 (8.2)	.42
Presence of HAM	0	47 (38.2)	47 (24.2)	.0001
Spirometry*				
FEV ₁ (L)	1.81 (1.36-2.49)	1.89 (1.28-2.62)	1.88 (1.29-2.60)	.65
FVC (L)	2.57 (1.96-3.90)	2.66 (1.97-3.43)	2.60 (1.97-3.47)	.39
FEV ₁ /FVC	71.8 (58.8-80.7)	70.6 (61.3-77.6)	70.9 (60.4-78.5)	.51
Immunologic investigations*				
<i>A fumigatus</i> —specific IgE (kU _A /L)	1.79 (0.64-7.74)	28.4 (12-45.7)	15.1 (1.49-33.75)	.0001
<i>A fumigatus</i> —specific IgG (mg _A /L)	19 (9.5-41.5)	99.2 (62.7-176.3)	71.4 (23.8-139.5)	.0001
Total IgE (international units/mL)	741 (443-1230)	5000 (2421-10430)	2638 (994-5520)	.0001
Total eosinophil count (cells/μL)	310 (177-500)	800 (560-1280)	600 (316-1000)	.0001
IgE against rAsp antigens* (kU_A/L)				
rAsp f1	0.21 (0.11-0.53)	17.2 (6.94-35.4)	6.55 (0.23-22.73)	.0001
rAsp f2	0.49 (0.09-3.79)	4.92 (2.66-12.5)	3.75 (0.83-8.25)	.0001
rAsp f3	0.67 (0.33-3.59)	10.7 (2.82-33.1)	6.15 (0.57-20.93)	.0001
rAsp f4	0.18 (0.05-0.51)	1.92 (0.58-14.13)	0.82 (0.19-4.54)	.0001
rAsp f6	0.27 (0.07-0.81)	4.08 (1.34-9.76)	1.95 (0.27-6.73)	.0001

ATT, Antitubercular therapy; mg_A, milligrams of antigen-specific antibodies.

Values are presented as numbers (percentages), unless otherwise indicated.

*Median (interquartile range).

†Inadequately controlled asthma (presence of daytime symptoms more than twice a week or any nighttime symptom or limitation in activities of daily life or FEV₁% predicted < 80% in the preceding 4 wk), as defined by the Indian Asthma guidelines.¹⁶

Spirometry. Spirometry was performed on a dry rolling seal spirometer to determine the lung function measurements and bronchodilator reversibility, as previously described.²²

Computed tomography of the chest. Computed tomography of the chest was performed using a multidetector computed tomography scanner with a matrix size of 512 × 512. We

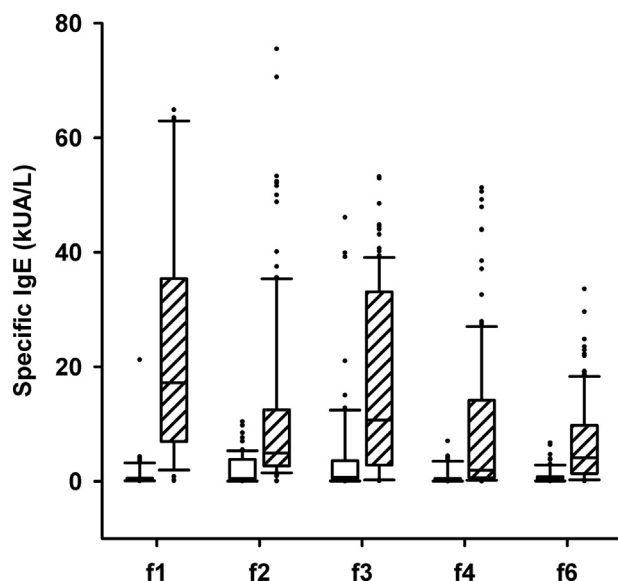


FIGURE 1. Vertical box plots showing the median (with interquartile range) of serum rAsp-specific IgE levels (kUA/L) in subjects with *A fumigatus*-sensitized asthma (white box) and ABPA (shaded box). The whiskers below and above the box denote the 10th and 90th percentiles, respectively. The dots represent outliers.

recorded the presence of bronchiectasis and high-attenuation mucus (HAM), using the criteria described previously.²³⁻²⁶

Serum IgE against recombinant *A fumigatus* antigens. Levels of serum IgE against rAsp f1, f2, f3, f4, and f6 were determined using the same ImmunoCap system (allergen code: m218, m219, m220, m221, and m222 for rAsp f1, f2, f3, f4, and f6, respectively), and the results were expressed as kUA/L.

All the immunologic investigations were performed by an experienced technician (H.C.) blinded to the other study details. The diagnosis of ABPA and ASA was made by clinicians who were unaware of the results of rAsp-specific IgE.

Outcomes

The *primary objective* of this study was to ascertain the diagnostic performance (sensitivity and specificity) of rAsp-specific IgE in differentiating ABPA from ASA at various cutoffs.

Because the cutoffs for interpreting rAsp-specific IgE remain unknown, we evaluated the following methods: (1) *Fixed cutoffs*: The manufacturer-recommended cutoff (0.35 kUA/L) and 2 arbitrary cutoffs (0.50 and 1.0 kUA/L) in the entire cohort ($n = 194$); and (2) *Cutoffs derived from the receiver-operating characteristic (ROC) curve*²⁷: The entire study population was randomly divided into 2 halves (derivation and validation cohorts). In the derivation cohort ($n = 102$), we determined the best cutoff value for IgE against each of the rAsp antigens to differentiate ABPA from ASA, using the Youden J statistic (or the Youden index), calculated as sensitivity + specificity - 1.²⁸ The cutoffs so obtained were then used to ascertain the diagnostic performance in the validation cohort ($n = 92$).

We evaluated the performance of IgE against individual antigens. Furthermore, the IgE positivity against 2 or more of the rAsp

TABLE II. Sensitivity and specificity of rAsp-specific IgE against individual antigens and their combinations using a fixed cutoff level of 0.35 kUA/L, to differentiate ASA from ABPA ($n = 194$)

rAsp antigens	Sensitivity (95% CI)	Specificity (95% CI)
IgE against individual antigens		
rAsp f1	91.1 (84.6-95.5)	66.2 (53.9-77)
rAsp f2	97.5 (92.9-99.5)	34.9 (23.5-47.6)
rAsp f3	89.3 (82.5-94.2)	25.7 (16-37.6)
rAsp f4	84.4 (76.8-90.4)	67.6 (55.5-78.2)
rAsp f6	88.6 (81.6-93.6)	56.3 (44.1-68.1)
Positive IgE against more than 1 rAsp antigen		
rAsp f1 or f2 or f3 or f4 or f6	100 (97-100)	11.3 (4.9-21)
rAsp f1 or f2 or f3 or f4	100 (97-100)	11.3 (4.9-21)
rAsp f1 or f2 or f4	100 (97-100)	0
rAsp f1 or f2 or f3	100 (97.1-100)	12.7 (5.9-22.7)
rAsp f1 or f2	99.2 (95.6-99.9)	32.4 (21.8-44.6)
rAsp f1 or f3	100 (97.1-100)	19.7 (11.2-30.9)
rAsp f4 or f6	95.1 (89.7-98.2)	49.3 (37.2-61.4)
Arithmetic mean of serum levels of rAsp-specific IgEs		
rAsp f1 + f2 + f3 + f4 + f6	100 (96.9-100)	21.5 (12.3-33.5)
rAsp f1 + f2 + f3 + f4	100 (96.9-100)	24.6 (14.8-36.9)
rAsp f1 + f2 + f4	99.2 (95.5-99.9)	37.9 (26.2-50.7)
rAsp f1 + f2 + f3	100 (96.9-100)	24.6 (14.8-36.9)
rAsp f1 + f2	99.2 (95.5-99.9)	37.9 (26.2-50.7)
rAsp f1 + f3	100 (97-100)	27.1 (17.2-39.1)
rAsp f4 + f6	91.8 (85.4-96)	57.1 (44.8-68.9)

antigens was analyzed as follows: (1) f1 or f2 or f3 or f4 or f6; (2) f1 or f2 or f3 or f4; (3) f1 or f2 or f3; (4) f1 or f2 or f4; (5) f1 or f2; (6) f4 or f6; and (7) f1 or f3. We also interpreted the mean IgE against rAsp antigens for the following combinations: (1) [f1 + f2 + f3 + f4 + f6]/5; (2) [f1 + f2 + f3 + f4]/4; (3) [f1 + f2 + f3]/3; (4) [f1 + f2 + f4]/3; (5) [f1 + f2]/2; (6) [f4 + f6]/2; and (7) [f1 + f3]/2. For both these approaches, the fixed as well as the ROC-derived cutoffs (as described above) were used.

The *secondary objectives* were to investigate the association of rAsp-specific IgE with (1) asthma control (adequate or inadequately controlled)¹⁶; (2) immunologic parameters (peripheral blood eosinophil count, serum total IgE, *A fumigatus*-specific IgE, and IgG); (3) lung function (FEV₁ and FVC); and (4) imaging findings (presence of bronchiectasis or HAM).

Statistical analyses

Statistical analyses were performed using a commercial statistical software (Statistical Package for Social Sciences [SPSS] for MS Windows, version 22.0, IBM, Armonk, NY). Descriptive data are presented as median (interquartile range) and numbers (percentages). The differences between the study groups were compared using Mann-Whitney U test for continuous variables and the χ^2 test for categorical variables. The association between clinical parameters and rAsp-specific IgE was assessed using the Spearman rank-order (rho) test. A P value of less than .05 was considered statistically significant. The diagnostic performance (sensitivity and specificity) of the rAsp-specific IgE at different cutoffs was computed using the DAG_stat application.²⁹

TABLE III. Sensitivity and specificity of rAsp-specific IgE against individual antigens and their combinations interpreted at a cutoff of 0.5 kU_A/L, to differentiate ASA from ABPA (n = 194)

rAsp antigens	Sensitivity (95% CI)	Specificity (95% CI)
IgE against individual antigens		
rAsp f1	91.1 (84.6-95.5)	74.7 (62.9-84.2)
rAsp f2	97.5 (92.9-99.5)	54.6 (41.8-66.9)
rAsp f3	88.5 (81.5-93.6)	44.3 (32.4-56.7)
rAsp f4	78.9 (70.4-85.6)	74.7 (62.9-84.2)
rAsp f6	85.4 (77.9-91.1)	64.8 (52.5-75.8)
Positive IgE against more than 1 rAsp antigen		
rAsp f1 or f2 or f3 or f4 or f6	100 (97.1-100)	14.1 (6.9-24.4)
rAsp f1 or f2 or f3 or f4	100 (97.1-100)	16.9 (9.1-27.7)
rAsp f1 or f2 or f4	99.2 (95.6-99.9)	38.0 (26.7-50.3)
rAsp f1 or f2 or f3	100 (97.1-100)	22.5 (13.5-34)
rAsp f1 or f2	99.2 (95.6-99.9)	46.5 (34.6-58.7)
rAsp f1 or f3	100 (97.1-100)	30.9 (20.5-43.1)
rAsp f4 or f6	91.9 (85.6-96)	57.8 (45.4-69.4)
Arithmetic mean of serum levels of rAsp-specific IgEs		
rAsp f1 + f2 + f3 + f4 + f6	100 (96.9-100)	29.7 (18.9-42.4)
rAsp f1 + f2 + f3 + f4	100 (96.9-100)	30.8 (19.9-43.4)
rAsp f1 + f2 + f4	99.2 (95.4-99.9)	48.5 (35.9-61.1)
rAsp f1 + f2 + f3	100 (96.9-100)	33.9 (22.6-46.7)
rAsp f1 + f2	99.2 (95.5-99.9)	54.6 (41.9-66.9)
rAsp f1 + f3	100 (97-100)	37.7 (26.3-50.2)
rAsp f4 + f6	90.2 (83.5-94.8)	64.8 (52.5-75.8)

RESULTS

Baseline characteristics

We enrolled 123 subjects with ABPA and 71 subjects with ASA during the study period. The median age of the study population (58.4% men) was 38.5 years. Most (96.9%) were nonsmokers. Asthma was inadequately controlled at presentation in 90 (46.4%) subjects. A history of allergic rhinitis and a family history of asthma was noted in 62 (32.1%) and 69 (35.6%) subjects, respectively. The baseline demographic and clinical characteristics were similar in both ABPA and ASA groups (Table I), except that productive cough was more common in subjects with ABPA (51.2% vs 31%; $P = .001$). Bronchiectasis was observed more frequently in subjects with ABPA than in subjects with ASA (78% vs 26.8%; $P = .0001$). HAM was exclusively noted in subjects ($n = 47$ [38.2%]) with ABPA. The lung functions (FEV₁, FVC, and FEV₁/FVC) were similar in both the study groups. The immunologic markers (total IgE, eosinophil count, *A fumigatus*-specific IgE, and IgG) as well as rAsp-specific IgE (f1, f2, f3, f4, and f6) levels were significantly higher in the ABPA group than in the ASA group (Figure 1 and Table I).

PRIMARY OBJECTIVE

Diagnostic performance of rAsp-specific IgE using fixed cutoffs

Cutoff of 0.35 kU_A/L (Table II)

Individual antigens. The IgE against rAsp f2 followed by rAsp f1 was the most sensitive (97.5% and 91.1%, respectively) in differentiating ABPA from ASA. However, the specificity of each of these antigens was poor and ranged from 25.7% (for rAsp f3) to 67.6% (for rAsp f4).

TABLE IV. Sensitivity and specificity of rAsp-specific IgE against individual antigens and their combinations interpreted at a cutoff of 1.0 kU_A/L, to differentiate ASA from ABPA (n = 194)

rAsp antigens	Sensitivity (95% CI)	Specificity (95% CI)
IgE against individual antigens		
rAsp f1	90.2 (83.6-94.9)	78.9 (66.6-87.7)
rAsp f2	95.9 (90.6-98.6)	68.2 (55.6-79.1)
rAsp f3	86.1 (78.6-91.7)	52.9 (40.6-64.9)
rAsp f4	66.4 (57.3-74.7)	83.1 (72.3-90.9)
rAsp f6	79.7 (71.5-86.4)	76.1 (64.5-85.4)
Positive IgE against more than 1 rAsp antigen		
rAsp f1 or f2 or f3 or f4 or f6	100 (97.1-100)	23.9 (14.6-35.5)
rAsp f1 or f2 or f3 or f4	100 (97.1-100)	26.8 (16.9-38.6)
rAsp f1 or f2 or f4	99.2 (95.6-99.9)	50.7 (38.6-62.8)
rAsp f1 or f2 or f3	100 (97.1-100)	32.4 (21.8-44.6)
rAsp f1 or f2	99.2 (95.6-99.9)	56.3 (44.1-68.1)
rAsp f1 or f3	100 (97.1-100)	40.9 (29.3-53.2)
rAsp f4 or f6	86.9 (79.7-92.4)	41.7 (25.5-59.2)
Arithmetic mean of serum levels of rAsp-specific IgEs		
rAsp f1 + f2 + f3 + f4 + f6	100 (96.9-100)	52.3 (39.5-64.9)
rAsp f1 + f2 + f3 + f4	100 (96.9-100)	47.7 (35.2-60.5)
rAsp f1 + f2 + f4	99.2 (95.4-99.9)	57.6 (44.8-69.7)
rAsp f1 + f2 + f3	100 (96.9-100)	47.7 (35.2-60.5)
rAsp f1 + f2	99.2 (95.5-99.9)	56.1 (43.3-68.3)
rAsp f1 + f3	100 (97-100)	48.6 (36.4-60.8)
rAsp f4 + f6	77.9 (69.5-84.9)	74.7 (62.9-84.2)

Combination of antigens. The combination of IgE positivity (>0.35 kU_A/L) against 1 or more rAsp antigens increased the sensitivity (Table II), but the specificity declined further (11.3%-49.3% for the various combinations). The mean of IgE against multiple rAsp antigens (interpreted at a cutoff of 0.35 kU_A/L) was also associated with a poor specificity for all the possible combinations (<60%), even though the sensitivity was high (91.8%-100%).

Fixed cutoff of 0.5 kU_A/L and 1.0 kU_A/L (Tables III and IV)

Individual antigens. At both these cutoffs, the IgE against individual rAsp antigens had a high sensitivity (range, 79%-98% at cutoff 0.5 kU_A/L; 66%-96% at cutoff 1 kU_A/L) to diagnose ABPA. At a cutoff of 0.5 kU_A/L, rAsp f2 IgE followed by rAsp f1 provided the highest sensitivities (97.5% and 91.1%, respectively). Although the specificity to diagnose ABPA with IgE against individual antigen was higher at these 2 cutoffs than at the 0.35 kU_A/L cutoff, it was still below 80% (except for rAsp f4 IgE at 1.0 kU_A/L, 83%).

Combination of antigens. The combination of antigens as well as the mean of IgE against rAsp antigens also yielded a poor specificity (<75%), despite high sensitivities (>95%).

Diagnostic performance using the best cutoffs obtained from the ROC curve (Table V)

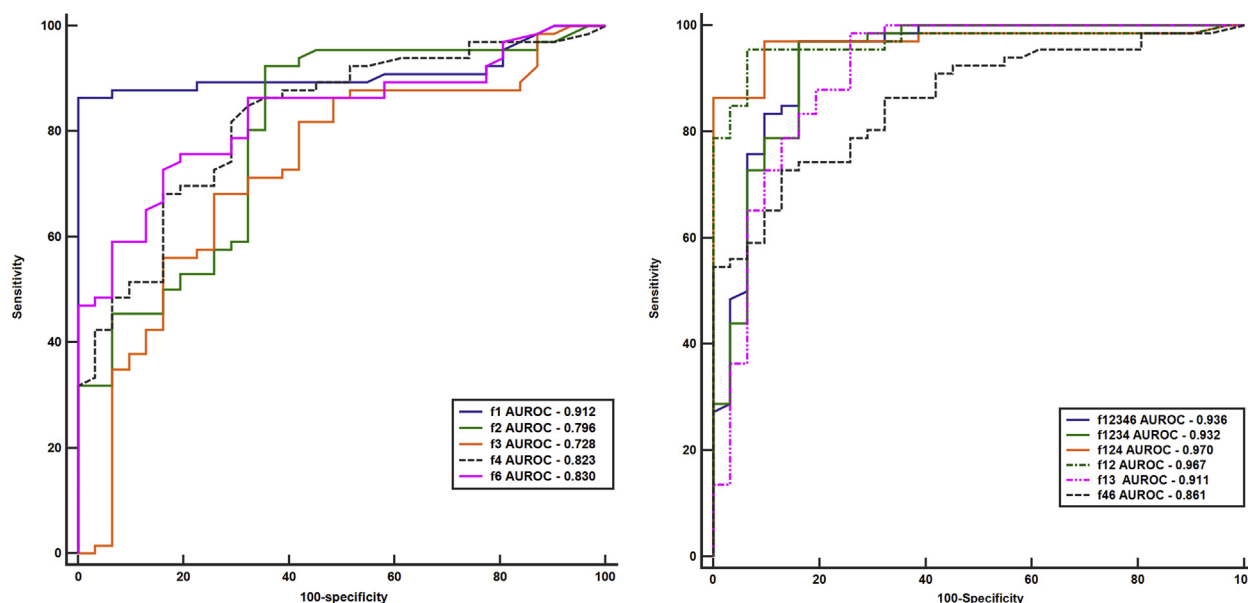
Identification of cutoff for individual antigens. The derivation cohort ($n = 102$) comprised 67 and 35 subjects with ABPA and ASA, respectively. The cutoffs were derived for each

TABLE V. Sensitivity and specificity of rAsp-specific IgE against individual antigens and their combinations using the cutoff derived from Youden index, to differentiate ASA from ABPA

rAsp antigens	AUROC curve (95% CI)	The best rAsp-specific IgE cutoff* (kU _A /L) (sensitivity, specificity in the derivation cohort)	Sensitivity in validation cohort (95% CI)	Specificity in validation cohort (95% CI)
IgE against individual antigens				
rAsp f1	0.912 (0.851-0.973)	4.465 (86.4%, 100%)	89.3 (78.1-95.9)	100 (90.3-100)
rAsp f2	0.796 (0.699-0.893)	1.300 (92.4%, 64.5%)	98.2 (90.3-99.9)	79.4 (62.1-91.3)
rAsp f3	0.728 (0.617-0.838)	5.990 (68.2%, 74.2%)	72.7 (59-83.9)	88.9 (73.9-96.9)
rAsp f4	0.823 (0.737-0.909)	0.435 (81.8%, 71%)	80 (67-89.6)	72.2 (54.8-85.8)
rAsp f6	0.830 (0.750-0.911)	1.960 (72.7%, 83.9%)	69.6 (55.9-81.2)	91.7 (77.5-98.3)
Positive IgE against more than 1 rAsp antigen interpreted in combination†				
rAsp f1 or f2 or f3 or f4 or f6			100 (93.6-100)	63.9 (46.2-79.2)
rAsp f1 or f2 or f3 or f4			100 (93.6-100)	63.9 (46.2-79.2)
rAsp f1 or f2 or f4			100 (93.6-100)	63.9 (46.2-79.2)
rAsp f1 or f2 or f3			100 (93.7-100)	80.6 (63.9-91.2)
rAsp f1 or f2			100 (93.7-100)	80.6 (63.9-91.2)
rAsp f1 or f3			96.4 (87.7-99.6)	88.9 (73.9-96.9)
rAsp f4 or f6			83.9 (71.7-92.4)	72.2 (54.8-85.8)
Arithmetic mean of serum levels of rAsp-specific IgEs				
rAsp f1 + f2 + f3 + f4 + f6	0.936 (0.877-0.995)	3.331 (97%, 83.9%)	98.1 (88.9-99.9)	88.2 (72.6-96.7)
rAsp f1 + f2 + f3 + f4	0.932 (0.870-0.993)	3.540 (97%, 83.9%)	98.1 (89.9-99.9)	91.2 (76.3-98.1)
rAsp f1 + f2 + f4	0.967 (0.932-1.000)	4.1267 (86.4%, 100%)	85.2 (72.9-93.4)	100.0 (89.7-100)
rAsp f1 + f2 + f3	0.919 (0.851-0.987)	3.795 (97%, 80.6%)	98.2 (90.1-99.9)	91.2 (76.3-98.1)
rAsp f1 + f2	0.967 (0.932-1.000)	3.20 (95.5%, 93.5%)	98.2 (90.3-99.9)	88.2 (72.6-96.7)
rAsp f1 + f3	0.911 (0.838-0.983)	3.145 (98.5%, 74.2%)	96.4 (87.5-99.6)	86.1 (70.5-95.3)
rAsp f4 + f6	0.861 (0.790-0.933)	1.9325 (72.7%, 87.1%)	70.9 (57.1-82.4)	86.1 (70.5-95.3)

*The cutoffs were obtained from the derivation cohort (n = 102; 67 ABPA and 35 ASA) using the Youden *J* statistic. The sensitivity and specificity in the derivation cohort (n = 92; 56 ABPA, 36 ASA) are provided in parentheses.

†Individual rAsp-specific IgE was interpreted using the following cutoffs: 4.465 kU_A/L (f1), 1.300 kU_A/L (f2), 5.990 kU_A/L (f3), 0.435 kU_A/L (f4), and 1.960 kU_A/L (f6).

**FIGURE 2.** ROC curves demonstrating the utility of (A) rAsp-specific IgE against individual antigens (rAsp f1, f2, f3, f4, and f6) and (B) arithmetic mean of various rAsp-specific IgE combinations.

rAsp antigen by constructing ROC curves (Figure 2). The area under the ROC (AUROC) curve was the highest for rAsp f1 IgE (0.912), followed by rAsp f6 IgE (0.830) and rAsp f4-specific IgE (0.823), whereas rAsp f3-specific IgE had the poorest

performance (AUROC curve, 0.728). The best cutoffs identified in the derivation cohort were 4.465 kU_A/L (rAsp f1 IgE), 1.300 kU_A/L (rAsp f2 IgE), 5.990 kU_A/L (rAsp f3 IgE), 0.435 kU_A/L (rAsp f4 IgE), and 1.960 kU_A/L (rAsp f6 IgE).

TABLE VI. Association of rAsp-specific IgE levels in serum with asthma control and radiological features in subjects with ABPA (n = 123)

Antigen-specific IgE (kU _A /L)	Asthma control			Bronchiectasis			HAM		
	Adequate	Inadequate	P value	Present	Absent	P value	ABPA-HAM	No HAM	P value
rAsp f1	15.25 (8.41-32.0)	18.30 (6.31-48.5)	.58	17.05 (8.03-33.90)	22.6 (5.78-54.9)	.91	18.3 (10.90-31.60)	17.05 (6.22-46.83)	.73
rAsp f2	3.5 (1.5-8.01)	5.31 (2.87-14.98)	.014	4.84 (2.63-12.10)	6.99 (2.71-15.40)	.83	4.93 (2.80-12.53)	4.87 (2.66-12.60)	.59
rAsp f3	8.42 (2.75-31.20)	11.7 (4.06-34.20)	.41	10.0 (1.78-32.0)	11.80 (6.62-35.40)	.18	6.17 (1.15-31.63)	11.70 (6.67-34.43)	.017
rAsp f4	1.67 (0.52-4.93)	3.55 (0.65-15.25)	.25	1.88 (0.69-11.50)	4.22 (0.24-23.95)	.91	1.81 (0.68-8.70)	3.56 (0.43-16.35)	.84
rAsp f6	2.91 (1.11-7.43)	5.56 (1.95-12.00)	.022	4.09 (1.62-10.09)	3.46 (0.71-9.30)	.55	4.09 (1.88-11.50)	4.05 (1.32-9.06)	.62

Values in bold indicate statistical significance.

On applying these cutoffs to the validation cohort (n = 92; 56 ABPA, 36 ASA), rAsp f1 IgE provided the highest specificity (100%), while rAsp f2 IgE was the most sensitive (98.2%), followed by rAsp f1 IgE (89.3%). The best combination of sensitivity and specificity (89.3% and 100%) to diagnose ABPA was observed with rAsp f1 IgE.

Performance of rAsp-specific IgE in combination. All the combinations where rAsp f1 or f2 IgE was positive (with or without positivity to other antigens) had a 100% sensitivity. However, these combinations were less specific (<90%) than IgE against individual antigens (particularly rAsp f1 IgE [100% specific]).

Mean of rAsp-specific IgE. When interpreted at statistically derived cutoffs, the mean of IgE against rAsp f1 + f2 and rAsp f1 + f2 + f4 had the highest AUROC curve (0.967) for diagnosing ABPA. The best sensitivity (98.2%) was seen with a mean of IgE rAsp f1 + f2 and rAsp f1 + f2 + f3, while their corresponding specificities were 88.2% and 91.2%, respectively (Table V). The mean of IgE against rAsp f1 + f2 + f4 yielded the highest specificity (100%) at a cutoff of 4.1267 kU_A/L.

SECONDARY OBJECTIVE

Correlation of rAsp-specific IgE with clinical, radiological, and immunologic parameters in subjects with ABPA (n = 123)

Asthma control and radiological features. Asthma control and radiological features showed few significant associations with rAsp IgE levels. The median IgE against rAsp f2 and rAsp f6 was significantly higher in subjects with inadequately controlled asthma at baseline (Table VI). The rAsp-specific IgE levels were not significantly different between subjects with and without bronchiectasis. Subjects with ABPA-HAM had a significantly lower median level of rAsp f3 IgE than did those without HAM.

Correlation of lung function and immunologic markers. Correlation of lung function and immunologic markers with rAsp-specific IgE was also poor (Table VII). The only few significant but weak correlations were that of rAsp f2 IgE with FEV₁ and FVC (r, -0.205 and -0.249, respectively). There was also a negligible positive correlation of rAsp f4 IgE with serum total IgE (r = 0.193).

DISCUSSION

We found that the cutoffs of IgE against rAsp antigens derived using the ROC curves performed better than the fixed values. In fact, raised IgE against a single antigen, rAsp f1 IgE (cutoff, 4.465 kU_A/L), provided a high sensitivity (89.3%) and specificity (100%) in diagnosing ABPA. Although the correlation of rAsp-specific IgE with clinical and radiological severity was weak, these tests have a potential role in differentiating ABPA from ASA. To our knowledge, the present study is the largest study evaluating the role of rAsp antigens, and the first to provide usable cutoffs in the diagnosis of ABPA-complicating asthma.

Of the commercially available *A fumigatus*-specific antigens, rAsp f1, f2, and f4 are considered highly specific, whereas rAsp f3 and f6 cross-react with other fungi.¹¹ A recent systematic review and meta-analysis of studies evaluating rAsp antigens found the combination of rAsp f1 and f3 to be the most sensitive (pooled

TABLE VII. Correlation of rAsp-specific IgE with lung functions (FEV₁ and FVC) and immunologic markers in subjects with ABPA (n = 123)

Antigen	FEV ₁	FVC	FEV ₁ /FVC	Blood eosinophil count	Serum total IgE	<i>A fumigatus</i> –specific IgE	<i>A fumigatus</i> –specific IgG
rAsp f1	0.126	0.144	–0.011	0.068	0.159	0.065	0.090
rAsp f2	–.205*	–.249†	–0.121	0.028	0.152	0.018	0.065
rAsp f3	–0.104	–0.118	–0.075	–0.061	0.166	0.088	0.006
rAsp f4	0.004	0.033	–0.074	0.071	0.193*	0.043	0.061
rAsp f6	0.117	0.123	–0.010	0.040	0.170	0.033	0.037

The correlation coefficients are provided in the columns of the table. A negative correlation is indicated by the “–” sign.

*Correlation is significant at the .05 level (2-tailed).

†Correlation is significant at the .01 level (2-tailed).

sensitivity, 97%) test in diagnosing ABPA among those with asthma, whereas the combination of rAsp f4 and f6 was the most specific (pooled specificity, 99%).¹⁰ However, most of the included studies were small, and the quality of evidence was low.^{13,15,30–34} On the contrary, a recent large study from Japan found rAsp f1 and f2 to be the most useful in diagnosing ABPA.¹⁵ In the present study also, the diagnostic performance of rAsp f1 IgE was the best (AUROC curve, 0.912), whereas the nonspecific allergen rAsp f3 had the poorest performance (AUROC curve, 0.728). The highly specific rAsp antigens (f1, f2, and f4) interpreted at ROC-derived cutoff provided the best sensitivity and specificity, consistent with the concepts of molecular allergy–based diagnostics.^{7,8}

In the recent Japanese study, rAsp-specific IgE were interpreted at various arbitrary cutoffs (0.35 kU_A/L, 0.70 kU_A/L, and 1.0 kU_A/L). The diagnostic performance of rAsp f1 or rAsp f2 IgE was only modest even at the highest evaluated cutoff (sensitivity 74% and specificity 91% at a cutoff of 1.0 kU_A/L).¹⁵ The major drawback of this study was the lack of identification of an optimal cutoff to define a positive test result. We observed that the optimal cutoff to diagnose ABPA varied widely for each of these antigens (ranging from 0.435 for rAsp f4 to 4.465 for rAsp f1 IgE). Not surprisingly, using the same fixed cutoff or the manufacturer's recommendation uniformly for interpreting all the rAsp-specific IgE is likely to result in poor diagnostic performance. By including a large number of subjects with ABPA (n = 123) and statistically deriving the optimal cutoff, we were able to demonstrate the usefulness of rAsp-specific IgE.

Despite a notable role of rAsp-specific IgE in the diagnosis of ABPA, their correlation with most of the parameters of disease severity was weak. This weak association is similar to a recent study, where rAsp-specific IgE had no relationship with lung function and only a weak correlation with total IgE, eosinophil count, and *A fumigatus*–specific IgE.³⁵ In the same study, a higher level of rAsp f3 and f4 IgE was observed in subjects with bronchiectasis.³⁵ In another study, allergic sensitization to rAsp f1 and f3 was an independent predictor of bronchiectasis in subjects with chronic obstructive pulmonary disease.³⁶ On the contrary, we observed that rAsp f3 IgE was significantly lower in subjects with HAM, a pathognomonic radiological manifestation of ABPA.

What are the clinical implications of our study? The diagnosis of ABPA and its differentiation from ASA currently involve performing several investigations, including serum *Aspergillus*–specific IgE and IgG, serum total IgE, computed tomography, and others. The use of IgE against rAsp antigens has the potential to simplify the diagnosis of ABPA. The data from our study

provide evidence to refine the existing guidelines and incorporate *A fumigatus*–specific recombinant antigens (rAsp f1, f2, and f4) in the diagnostic algorithm of ABPA. We suggest interpreting rAsp-specific IgE at statistically derived cutoffs. Furthermore, in subjects of asthma with aspergillus sensitization, we suggest initially using rAsp f1 IgE because it identifies nearly 90% of the subjects with ABPA with 100% specificity. The remaining 10% can be identified by performing rAsp f1 (>4.465 kU_A/L) and f2 (>1.300 kU_A/L) IgE (100% sensitive, if either of them is positive). However, the specificity of the combination of either rAsp f1 or f2 at these cutoffs is about 80%. Hence, further confirmation, if required, may be done using the mean of rAsp f1 + f2 + f4 (100% specificity at a cutoff of 4.1267 kU_A/L). The misclassification rate with the aforementioned approach is only 7% (4 of the 56 patients in the validation cohort). However, this requires further validation.

Our study has a few limitations, the most important being a single-center study conducted at a tertiary referral center. The included population, thus, may not be representative of the community. The results do not apply to ABPA-complicating cystic fibrosis. We have evaluated the rAsp-specific IgE only in treatment-naïve patients with ABPA. Whether excluding such potential patients introduced bias in the study remains unclear. Previous studies have shown that the levels of total IgE and *A fumigatus*–specific IgE change after treatment. However, the behavior of rAsp antigens after treatment remains unknown. Because the present study was aimed at deriving a usable cutoff, we recruited only treatment-naïve subjects with ABPA, to maintain homogeneity. Thus, the dynamics and diagnostic utility of rAsp-specific IgE during follow-up or in previously treated individuals remain to be seen. The major advantage of the rAsp antigens over crude antigen is its superior specificity for *A fumigatus*, and the lack of cross-reactivity to other fungi, as shown in several studies. However, we did not evaluate this aspect in the present study. Finally, we measured the rAsp-specific IgE only once (because of financial constraints), and hence, reproducibility could not be assessed.

CONCLUSIONS

IgE against rAsp antigens (f1 and f2) are useful in differentiating ABPA from ASA, when the diagnostic cutoffs derived by the Youden index are used, rather than arbitrary cutoffs. The rAsp f1 IgE appears to be the single most useful test to diagnose ABPA among individuals with *A fumigatus*–sensitized asthma. The results of our study require further validation in a multi-center study.

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