New clinical diagnostic criteria for allergic bronchopulmonary aspergillosis/mycosis and its validation

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Background: There are several clinical diagnostic criteria for allergic bronchopulmonary aspergillosis (ABPA). However, these criteria have not been validated in detail, and no criteria for allergic bronchopulmonary mycosis (ABPM) are currently available.

Objective: This study proposes new diagnostic criteria for ABPA/ABPM, consisting of 10 components, and compares its sensitivity and specificity to existing methods.

Methods: Rosenberg-Patterson criteria proposed in 1977, the International Society for Human and Animal Mycology (ISHAM) criteria proposed in 2013, and this new criteria were applied to 79 cases with pathological ABPM and the control population with allergic mucin in the absence of fungal hyphae (n = 37), chronic eosinophilic pneumonia (n = 64), *Aspergillus*-sensitized severe asthma (n = 26), or chronic pulmonary aspergillosis (n = 24). These criteria were also applied to the 179 cases with physician-diagnosed ABPA/ABPM in a nationwide Japanese survey.

Results: The sensitivity for pathological ABPM with Rosenberg-Patterson criteria, ISHAM criteria, and this new criteria were 25.3%, 77.2%, and 96.2%, respectively. The sensitivity for physician-diagnosed ABPA/ABPM were 49.2%, 82.7%, and 94.4%, respectively. The areas under the curve for the receiver-operating characteristic curves were 0.85, 0.90, and 0.98, respectively. The sensitivity for ABPM cases that were culture-positive for non-*Aspergillus* fungi were 13.0%, 47.8%, and 91.3%, respectively.

Conclusions: The new diagnostic criteria, compared with existing criteria, showed better sensitivity and specificity for diagnosing ABPA/ABPM. (J Allergy Clin Immunol 2021;147:1261-68.)

Key words: *Allergic bronchopulmonary aspergillosis, allergic bronchopulmonary mycosis,* Aspergillus*, diagnosis, eosinophils, fungus, IgE, mucus plugs, severe asthma with fungal sensitization*

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This study was funded by the Research Grant on Allergic Disease and Immunology, from the Japan Agency for Medical Research and Development, under the grant numbers JP18ek0410026 and JP20ek0410055.

Disclosure of potential conflict of interest: K. Asano reports grants from Japan Agency for Medical Research and Development; personal fees from Thermo Fisher Diagnostics KK during the conduct of the study; personal fees from AstraZeneca KK GlaxoSmithKline KK, and Teijin Pharma Ltd; grants and personal fees from Nippon Boehringer Ingelheim Co, Ltd and Novartis Pharma KK; and grants and personal fees from Sanofi SA outside the submitted work. A. Hebisawa reports grants from Japan Agency for Medical Research and Development during the conduct of the study. J. Suzuki reports grants from Japan Agency for Medical Research and Development during the conduct of the study. Y. Fukutomi reports grants from Japan Agency for Medical Research and Development during the conduct of the study; personal fees from Thermo Fisher Diagnostics KK, Torii Pharmaceutical Co, Ltd, and Kyorin Pharmaceutical Co, Ltd; grants and personal fees from Novartis Pharma KK; and grants from GlaxoSmithKline KK outside the submitted work. S. Ueki reports grants and personal fees from Novartis Pharma KK, AstraZeneca KK, and Sanofi; grants from Japan Allergy Foundation, Charitable Trust Laboratory Medicine Research Foundation of Japan, Japan Society for the Promotion of Science Kakenhi, Maruho Co, Ltd, Mochida Memorial Foundation for Medical and Pharmaceutical Research, and Japanese Society of Laboratory Medicine Fund for Promotion of Scientific Research; grants from Japan Agency for Medical Research and Development during the conduct of the study; and personal fees from GlaxoSmithKline KK. K. Fukunaga reports grants from Japan Agency for Medical Research and Development during the conduct of the study. S. Konno reports grants from Japan Agency for Medical Research and Development during the conduct of the study; grants from AstraZeneca KK, Novartis Pharma KK, Nippon Boehringer Ingelheim Co, Ltd, and Japan Allergy Foundation; and grants from Kyorin Pharmaceutical Co, Ltd, outside the submitted work. H. Matsuse reports grants from Japan Agency for Medical Research and Development, during the conduct of the study; personal fees from Novartis, MSD KK, AstraZeneca KK, Astellas Pharma Inc, Nippon Boehringer Ingelheim Co, Ltd, and GlaxoSmithKline KK; and personal fees from Kyorin Pharmaceutical Co, Ltd, outside the submitted work. K. Kamei reports grants from Japan Agency for Medical Research and Development, during the conduct of the study. M. Taniguchi reports grants from Japan Agency for Medical Research and Development during the conduct of the study. T. Shimoda reports grants from Japan Agency for Medical Research and Development during the conduct of the study. T. Oguma reports grants from Japan Agency for Medical Research and Development during the conduct of the study. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication May 26, 2020; revised August 9, 2020; accepted for publication August 17, 2020.

Available online September 10, 2020.

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0091-6749

https://doi.org/10.1016/j.jaci.2020.08.029

Abbreviations used
ABPA: Allergic bronchopulmonary aspergillosis
ABPM: Allergic bronchopulmonary mycosis
AUC: Area under the curve
CT: Computed tomography
ISHAM: International Society for Human and Animal Mycology
ROC: Receiver-operating characteristic

Allergic bronchopulmonary aspergillosis (ABPA) was first reported by Hinson et al[1] as bronchopulmonary aspergillosis characterized by bronchitis, eosinophilia, bronchiectasis and/or mucus plugs, and the presence of *Aspergillus fumigatus* in the lungs. Subsequent studies showed that ABPA pathogenesis is associated with types I and III hypersensitivity reactions to *A fumigatus* inhaled and colonized in the bronchi and is based on high serum IgE levels; immediate cutaneous hypersensitivity reaction with or without an Arthus reaction; and presence of IgE, IgG, and/or precipitating *A fumigatus*–specific antibodies.[2-5] In addition to *A fumigatus*, other *Aspergillus* species such as *A flavus* and *A niger* and other filamentous fungi such as *Penicillium* and *Schizophyllum commune* can cause similar pathologies called allergic bronchopulmonary mycosis (ABPM).[6,7]

Rosenberg et al[5] proposed the first diagnostic criteria for ABPA in 1977, which included 7 primary components (asthma, peripheral blood eosinophilia, positive immediate cutaneous hypersensitivity to *Aspergillus* antigen, presence of precipitating antibody to *Aspergillus* antigen, increased total serum IgE levels, transient or fixed pulmonary opacities, and central bronchiectasis) and 3 secondary components (isolation of *A fumigatus* in sputum, history of expectoration of brownish plug or flecks, and Arthus-type reactivity to *Aspergillus* antigen). Later, Greenberger and Patterson[4] added *A fumigatus*–specific IgE and IgG to these criteria. Criteria for patients with cystic fibrosis as a predisposing condition were proposed in 2003,[8] consisting of high total serum IgE levels; immediate skin reactivity to *Aspergillus* antigen or specific IgE; precipitating antibody or IgG to *Aspergillus* antigen; and any typical radiographic findings such as pulmonary infiltrates, mucus plugs, or central bronchiectasis. Based on these preceding studies, the International Society for Human and Animal Mycology (ISHAM) proposed new diagnostic criteria in 2013,[2] which define asthma or cystic fibrosis as predisposing conditions and included 2 obligatory criteria: (1) immediate cutaneous hypersensitivity to *Aspergillus* antigen or elevated IgE levels against *A fumigatus*, and (2) elevated total IgE levels. They also included 3 minor criteria, at least 2 of which should be satisfied for ABPA diagnosis, namely: (1) presence of precipitating or IgG antibodies to *A fumigatus*, (2) radiographic features in the lungs consistent with ABPA, and (3) peripheral blood eosinophilia.

However, pulmonary/allergy specialists consider a substantial proportion of cases with clinical ABPA do not fulfill the diagnostic criteria. Agarwal et al[9] examined the sensitivity of an 8-component criteria, consisting of presence of *A fumigatus*–specific IgE and 7 primary components in Rosenberg-Patterson criteria. They found that only 39% of the cases with clinical ABPA checked at least 7 of the 8 components. In a nationwide Japanese survey,[10] 22% of 148 patients with physician-diagnosed ABPA accompanied by high attenuation mucus in the central bronchi, a highly compatible feature with ABPA/ABPM,[11] lacked either the asthma or cystic fibrosis necessary for ABPA diagnosis with existing criteria. Owing to the difficulty of differential diagnosis, some researchers have proposed allergic fungal airway disease as an inclusive disease concept encompassing severe asthma with fungal sensitization and ABPA.[12]

Diagnosis of non-*Aspergillus* ABPM is more challenging as traditional criteria[2,4,5] are specific to ABPA caused by *A fumigatus*, with no diagnostic criteria available for ABPM. Laboratory tests for specific IgE/IgG are not necessarily present for the ABPM-causing fungi, and clinical ABPM characteristics are often atypical. Ishiguro et al[13] reported 17 cases with biopsy-confirmed ABPM and finding that 7 cases lacked asthma or cystic fibrosis, 7 cases lacked peripheral blood eosinophilia, and 3 cases had <1000 IU/mL total serum IgE.

The Japan ABPM Research Program, supported by the Japan Medical Research and Development Organization, developed new 10-component diagnostic criteria for ABPA/ABPM in patients who do not have cystic fibrosis (Table I). We compared the sensitivity and specificity of the new and conventional criteria to discriminate pathological and physician-diagnosed ABPA/ABPM from related diseases, including fungus-negative mucoid impaction in bronchi, chronic eosinophilic pneumonia, fungus-sensitized severe asthma, and chronic pulmonary aspergillosis.

METHODS

Clinical diagnostic criteria for ABPM

The new diagnostic criteria for ABPM are presented in Table I. Current or previous physician-diagnosed asthma or asthma-like symptoms, including wheezing, are required for component 1. Peripheral blood eosinophil counts and serum IgE levels at the diagnosis or recent peak values can be used for components 2 and 3. The fungal type for component 5 (precipitating antibody or IgG) must be matched at the genus level with component 4 (immediate cutaneous hypersensitivity or IgE). Component 6 (positive fungal culture) can be counted when the same fungal genus for serum tests (component 4 and/or 5) was identified in culture. Component 6 can be counted if there were no positive serum tests, but there was a positive fungal culture. Components 4 to 6 are applicable for filamentous fungi, but not for yeast-like fungi such as *Candida* and *Saccharomyces*. Mucus plugs obtained by expectoration or by bronchoscopy can be used for pathological examinations (component 7). Components 8 to 10 are radiological findings, but bronchoscopy identification or expectoration history is applicable for mucus plugs in component 9. High attenuation mucus (component 10) is defined as mucus plugs with visually higher densities than paravertebral muscles on high-resolution computed tomography (CT).[11] Patients who checked 6 or more of these components were diagnosed with definite ABPM, and those who checked 5 components were diagnosed with probable ABPM.

The criteria proposed by Rosenberg et al[5] and ISHAM,[2] which were originally specific for ABPA, were modified for cases with non-*Aspergillus* ABPM (see Tables E1 and E2 in this article's Online Repository at www.jacionline.org). For Rosenberg-Patterson criteria, cases satisfying all 7 primary components are considered as definite ABPM, and cases fulfilling 6 primary components, except for central bronchiectasis, were designated as probable ABPM. Cases satisfying ISHAM criteria in the absence of predisposing conditions (asthma or cystic fibrosis) are considered as probable ABPM.

Subjects with pathological ABPM and control diseases

Mucus plugs expectorated in sputum or obtained by aspiration with bronchoscopy were routinely examined microscopically by pathologists experienced with diagnosis of allergic fungus-related lung diseases, in 2 institutes (National Hospital Organization Tokyo National Hospital and Saitama Cardiovascular and Respiratory Center). Pathological ABPM was

TABLE I. Clinical diagnostic criteria for ABPM in patients without cystic fibrosis

1. Current or previous history of asthma or asthmatic symptoms
2. Peripheral blood eosinophilia (≥500 cells/mm^3)
3. Elevated total serum IgE levels (≥417 IU/mL)
4. Immediate cutaneous hypersensitivity or specific IgE for filamentous fungi
5. Presence of precipitins or specific IgG for filamentous fungi
6. Filamentous fungal growth in sputum cultures or bronchial lavage fluid
7. Presence of fungal hyphae in bronchial mucus plugs
8. Central bronchiectasis on CT
9. Presence of mucus plugs in central bronchi, based on CT/bronchoscopy or mucus plug expectoration history
10. High attenuation mucus in the bronchi on CT

Filamentous fungi in criteria 4 to 6 should be identical.
Patients that meet 6 or more of these criteria are diagnosed with ABPM.

diagnosed based on the presence of fungal hyphae in the eosinophilic mucus plugs (allergic mucin).[14] Fungal hyphae were determined using a fluorescent dye (Fungiflora Y; Alfresa Pharma, Osaka, Japan), or Grocott staining.

Cases with intrabronchial allergic mucin lacking fungal hyphae, even after an extensive survey by the pathologists, were used as a control group. The second control group included patients with chronic eosinophilic pneumonia without culture-positive fungi in either sputum or bronchoalveolar lavage fluid. The third control group included subjects with severe asthma and fungal sensitization[15] specifically sensitized to *A fumigatus* for this analysis. Severe asthma with *A fumigatus* sensitization was defined as severe asthma: (1) requiring Global Initiative for Asthma[16] step 4 or 5 treatment, under the management by certified pulmonologists; (2) positive for specific IgE to *A fumigatus* antigen; and (3) without fulfilling ABPA criteria proposed by Rosenberg et al.[5] The fourth control group included patients with chronic pulmonary aspergillosis who presented with (1) typical radiological features and (2) positive sputum culture of *Aspergillus* species and/or precipitating antibodies to *A fumigatus*.

Clinical and radiographic data were retrospectively reviewed in the medical charts. This study was approved by the institutional review boards of Tokai University School of Medicine (no. 16R-239), National Hospital Organization Tokyo National Hospital (no. 190033), Saitama Cardiovascular and Respiratory Center (no. 2019028), and National Hospital Organization Sagamihara Hospital (no. 2019-030) and was implemented in compliance with the Declaration of Helsinki. The need for informed patient consent was waived by the institutional review boards for this study, given the anonymity of the data and the retrospective observational nature of the study.

Subjects with physician-diagnosed ABPM

A retrospective, cross-sectional survey of ABPM in Japan was performed in 2013.[10] This study was approved by the Institutional Review Board of Tokai University School of Medicine (no. 13R-105), and it was implemented in compliance with the Declaration of Helsinki. A total of 499 cases of physician-diagnosed ABPM were reported from 132 clinical centers certificated by the Japanese Respiratory Society and/or the Japanese Society of Allergology. Clinical, laboratory, and radiological parameters at ABPM diagnosis were evaluated in the physician questionnaire. Cases without appropriate data for peripheral blood eosinophil counts, total serum IgE levels, fungus-specific IgE or immediate cutaneous reactions, fungus-specific IgG or precipitating antibody, and thoracic CT scan data were excluded from the analysis.

Statistical analysis

Numerical data were expressed as mean and SD or median and interquartile range, while categorical data were presented as numbers with respective percentages. Numerical data were analyzed using the Mann-Whitney *U* test or Kruskal-Wallis test, while categorical data were analyzed using Fisher exact test. Receiver-operating characteristics (ROC) curve analysis and area under the curve (AUC) were used to compare the accuracy of the different diagnostic criteria.

Data of cases with pathological ABPM and physician-diagnosed disease were merged for the subgroup analyses to examine the sensitivity of the diagnostic criteria in patients without asthma and the cases with non-*Aspergillus* ABPM. Because data for pathological examination of mucus plugs were not available for cases with physician-diagnosed ABPM, component 7 (positive fungal hyphae in mucus plugs) was excluded from the new diagnostic criteria for these analyses.

The statistical analyses were performed using GraphPad Prism (version 5.0; GraphPad Software Inc, La Jolla, Calif). *P* values <0.05 were considered statistically significant.

RESULTS

Pathological ABPM

One hundred sixteen cases with eosinophilic mucus plugs in the lower airways were enrolled from 2 institutes. A total of 79 cases (68%) presenting with fungal hyphae in the mucus plugs were diagnosed as pathological ABPM. Demographic and laboratory data of the cases with pathological ABPM are presented in Table II. Mucus plugs were obtained under bronchoscopy in 97% of the cases, while sputum-derived samples were examined in 2 cases. Filamentous fungi, including *Aspergillus* spp, *S commune*, and *Penicillium* spp, were culture-positive in 40 (49%), 6 (7%), and 4 cases (5%), respectively. Demographic and laboratory data were relatively consistent, irrespective of the institute, as demonstrated in Table E3 (in this article's Online Repository at www.jacionline.org), but differences were present between the institutes in the prevalence of non-*Aspergillus* fungi isolation, fungus-specific IgE or immediate cutaneouos reactions, and some radiographic findings (central bronchiectasis and mucus plugs in the bronchi).

Eleven cases with pathological ABPM checked all 7 components of modified Rosenberg-Patterson criteria. Nine other cases fulfilled 6 criteria, except for central bronchiectasis. Therefore, 25.3% of the cases with pathological ABPM could be diagnosed as ABPM, according to the criteria (Fig 1, *A*). ISHAM criteria were fulfilled in 61 cases (77.2%), but 24 cases (30.4%) did not present with predisposing conditions and were defined as probable cases. Using the new diagnostic criteria, 71 cases (89.9%) were diagnosed as definite ABPM and 5 cases (6.3%) as probable ABPM (Fig 1, *A*).

Physician-diagnosed ABPM

Appropriate clinical, laboratory, and radiologic data were available in 179 of the cases with possible ABPA/ABPM reported in the nationwide survey. Data for fungal culture and radiographic findings on mucus plugs were missing in 70 (39%) and 35 cases (19%), respectively. Pathological examination of mucus plugs was not performed in any physician-diagnosed ABPM cases. Demographic data are presented in Table II. There were no differences in age, sex, total serum IgE levels, positive rate of fungus-specific type I hypersensitivity, and fungus culture between pathological and physician-diagnosed ABPM. Asthma prevalence, peripheral blood eosinophil counts, and positive rate of fungus-specific type III hypersensitivity were higher in patients with physician-diagnosed ABPM.

Diagnosis of definite ABPM was made with the new diagnostic criteria in 143 cases (79.9%). An additional 26 cases (14.5%) were

TABLE II. Demographic and laboratory data of patients with ABPM

	Pathological ABPM	Physician-diagnosed ABPM	*P* value
No. of subjects	79	179	
Age (y)	59 ± 15	61 ± 14	NS
Female	51 (65)	98 (55)	NS
Asthma	46 (58)	156 (87)	<.001
Peripheral blood eosinophil counts (/μL)	900 (574-1700)	1280 (720-2214)	<.001
Serum IgE levels (IU/mL)	1983 (515-5600)	2240 (1033-5950)	NS
Fungus-specific hypersensitivity			
Fungus-specific IgE or immediate cutaneous reactions	74 (94)	171 (96)	NS
Fungus-specific precipitin, IgG, or Arthus-type cutaneous reactions	43 (57)*	137 (77)	.003
Fungal culture in sputum or bronchial samples	50 (64)†	81 (74)‡	NS
Aspergillus spp	40 (51)†	68 (62)‡	NS
A fumigatus	35 (44)	38 (35)	
Aspergillus spp other than *A fumigatus*	5 (6)	13 (12)	
Aspergillus spp, unclassifiable	0 (0)	17 (16)	
Other filamentous fungi	10 (13)†	13 (12)‡	NS
Schizophyllum commune	6 (8)	4 (4)	
Penicillium spp	2 (3)	2 (2)	
Others or unclassifiable	2 (3)	7 (6)	
Fungal hyphae in mucus plugs	79 (100)	N/A	—
Thoracic CT			
Lung opacities	78 (99)	159 (89)	<.001
Central bronchiectasis	46 (58)	148 (83)	<.001
Mucus plugs in bronchi	58 (73)	118 (82)§	NS
High attenuation mucus	41 (52)	67 (47)§	NS

N/A, Not analyzed; *NS*, not significant.
Values are mean ± SD, n (%), or median (interquartile range).
*n = 76.
†n = 78.
‡n = 109.
§n = 144.

diagnosed as probable ABPM. Therefore, the sensitivity of the new criteria for definite/probable ABPM was 94.4%, and the sensitivity for definite/probable ABPM with Rosenberg-Patterson criteria and ISHAM criteria were 49.2% and 82.7%, respectively (Fig 1, *B*).

Control population

Among 116 cases with eosinophilic mucus plugs, 37 cases (32%) were negative for fungal hyphae, even with extensive examination by experienced pathologists. Demographic data are presented in Table III. All but one case presented with asthma. Among these cases, 2 (5.4%), 4 (10.8%), and 4 (10.8%) cases were diagnosed as ABPM based on modified Rosenberg-Patterson criteria, ISHAM criteria, and the new criteria, respectively (Table IV). Another 3 cases (8.1%) were diagnosed as probable ABPM using the new criteria.

Approximately one-half of the cases with chronic eosinophilic pneumonia (n = 64) exhibited asthma. A substantial proportion (23%) of the patients with this condition showed mucus plugs in the bronchi. No case presented with high attenuation mucus. No case in this population fulfilled Rosenberg-Patterson criteria, but 3 cases (4.7%) were compatible with probable ABPM based on ISHAM criteria and the new diagnostic criteria (Table IV).

Among the cases with severe asthma sensitized with *A fumigatus* that did not fulfill Rosenberg-Patterson criteria of ABPA (n = 26), 14 cases (53.8%) presented with serum IgE levels >1000 IU/mL, and 6 patients (23.1%) fulfilled ISHAM criteria. One case (3.8%) with high attenuation mucus on thoracic CT was compatible with definite ABPA, according to the new criteria, and another 3 cases (11.5%) were diagnosed with probable ABPM (Table IV).

Seven cases (29.2%) fulfilled ISHAM criteria among the patients with chronic pulmonary aspergillosis (n = 24), but only 1 case also exhibited asthma, which is a predisposing condition for the criteria. One case (4.2%) was compatible with probable ABPM in the new criteria, and no case fulfilled the Rosenberg-Patterson criteria (Table IV). Mucus plugs were not microscopically examined in any of the severe asthma or chronic pulmonary aspergillosis cases.

ROC curve analysis

Sensitivity and specificity for ABPM diagnosis were examined using data from cases with pathological ABPM (n = 79), fungus-negative mucus plugs (n = 37), chronic eosinophilic pneumonia (n = 64), severe asthma with *Aspergillus* sensitization (n = 26), and chronic pulmonary aspergillosis (n = 24).

The AUC of ROC curve was 0.85, 0.90, and 0.98 for modified Rosenberg-Patterson criteria (with or without central bronchiectasis), ISHAM criteria, and the new criteria (Fig 2, *A*), respectively. The Youden index was largest for the new diagnostic criteria (0.84 or 0.86) when the cutoff value was 5 or 6, which is compatible with the definitions of probable and definite ABPM. The sensitivities for probable/definite and definite ABPM were 96.2% and 89.9%, respectively. The specificities for probable/definite and definite ABPM were 87.4% and 96.0%, respectively.

We also validated the new diagnostic criteria excluding component 7 (positive fungal hyphae in mucus plugs), which requires pathological examination. AUC for the new diagnostic criteria was 0.95 in this analysis. The Youden index was largest when the cutoff value was 5, which was compatible with the definition of probable ABPM with the sensitivity and specificity of 90.0% and 91.4%, respectively.

Subgroup analyses

Data of 79 cases with pathological ABPM and 179 patients with physician-diagnosed disease were merged for subgroup analyses. A total of 56 ABPM cases had no asthma history (see Table E4 in this article's Online Repository at www.jacionline.org). These patients could not be diagnosed as ABPM using Rosenberg-Patterson criteria because the presence of asthma is mandatory for the criteria. Sensitivity for the diagnosis of ABPM without asthma was relatively high for ISHAM criteria

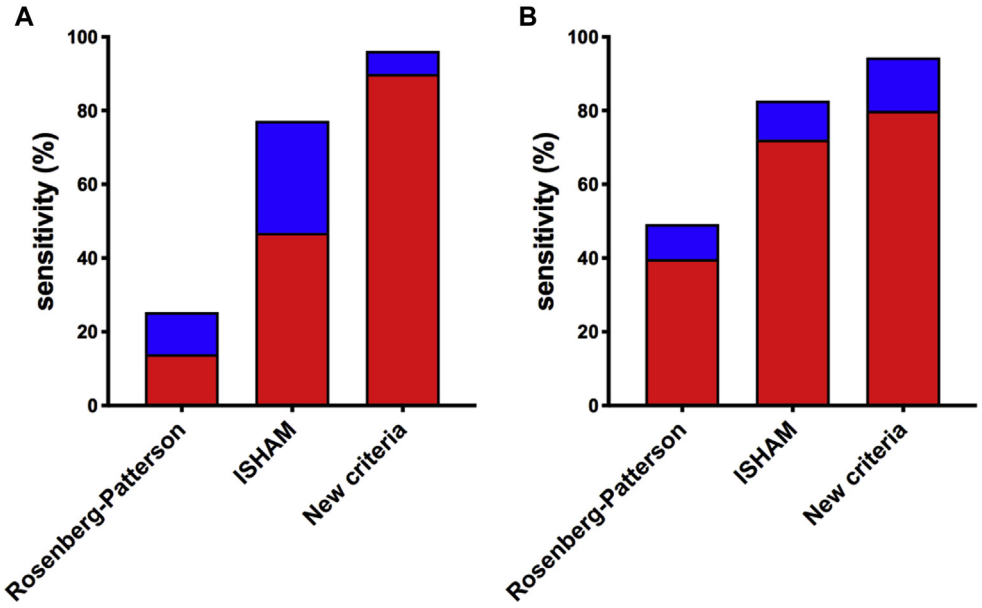

FIG 1. Sensitivity of diagnostic criteria for pathological and physician-diagnosed ABPM. Sensitivity for pathological ABPM (n = 79) **(A)** and physician-diagnosed ABPM (n = 179) **(B)** were compared among the Rosenberg-Patterson criteria, ISHAM criteria, and the new diagnostic criteria. *Red bars* represent definite ABPM. *Blue bars* show probable cases.

TABLE III. Demographic and laboratory data of the control population

	Mucus plugs without fungal hyphae	Chronic eosinophilic pneumonia	Severe asthma with *Aspergillus* sensitization	Chronic pulmonary aspergillosis
No. of subjects	37	64	26	24
Age (y)	54 ± 14	55 ± 12	64 ± 14	65 ± 13
Female	18 (49)	44 (69)	11 (42)	4 (17)
Asthma	36 (97)	31 (48)	26 (100)	3 (13)
Peripheral blood eosinophil counts (/mL)	1104 (559-2104)	1400 (600-2700)	317 (167-530)	201 (74-713)
Serum IgE levels (IU/mL)	326 (170-1389)	371 (182-943)	1155 (231-3035)	107 (36-1048)
Fungus-specific hypersensitivity				
Fungus-specific IgE or immediate cutaneous reactions	8 (22)	11 (17)	26 (100)	13 (54)
Fungus-specific precipitin, IgG, or Arthus-type cutaneous reactions	5 (14)	0 (0)	5 (19)	19 (79)
Fungal culture in sputum or bronchial samples	0 (0)	0 (0)	5 (33)*	12 (52)†
Aspergillus spp	0 (0)	0 (0)	5 (33)	10 (43)
Other filamentous fungi	0 (0)	0 (0)	0 (0)	2 (5)
Fungal hyphae in mucus plugs	0 (0)	N/A	N/A	N/A
Thoracic CT				
Lung opacities	25 (68)	67 (100)	11 (42)	18 (75)
Central bronchiectasis	2 (6)	2 (3)	3 (12)	6 (25)
Mucus plugs in bronchi	7 (19)	15 (23)	1 (4)	2 (8)
High attenuation mucus	6 (16)	0 (0)	1 (4)	0 (0)

Values are mean ± SD, n (%), or median (interquartile range).
*n = 15.
†n = 23.

(76.8%) and the new criteria (85.7%, definite/probable ABPM) (Fig 3, *A*).

Fungal culture was positive in 131 cases. A total of 108 cases were positive for *Aspergillus* spp, and 23 cases were positive for non-*Aspergillus* fungi only (see Table E5 in this article's Online Repository at www.jacionline.org). Sensitivity was modest in cases culture-positive for *Aspergillus* spp with Rosenberg-Patterson criteria (42.2%), but was higher with ISHAM criteria (89.9%), and highest with the new criteria (100%) (Fig 3, *B*). In contrast, sensitivity for the cases culture-positive for non-*Aspergillus* fungi alone decreased to 13.0% with Rosenberg-Patterson criteria and 47.8% with ISHAM criteria but remained high for the new criteria (91.3%) (Fig 3, *B*).

DISCUSSION

We validated traditional and new ABPA/ABPM diagnostic criteria by examining cases with pathological ABPM and

TABLE IV. Pseudo-positive cases in control diseases

		Rosenberg-Patterson		ISHAM		New criteria	
	No.	Definite	Definite/probable	Definite	Definite/probable	Definite	Definite/probable
Mucus plugs without fungal hyphae	37	1 (3)	2 (5)	4 (11)	4 (11)	4 (11)	7 (19)
Chronic eosinophilic pneumonia	64	0 (0)	0 (0)	3 (5)	3 (5)	0 (0)	3 (5)
Severe asthma with *Aspergillus* sensitization	26	N/A	N/A	6 (23)	6 (23)	1 (4)	4 (15)
Chronic pulmonary aspergillosis	24	0 (0)	0 (0)	1 (4)	7 (29)	0 (0)	1 (4)

Values are n (%).

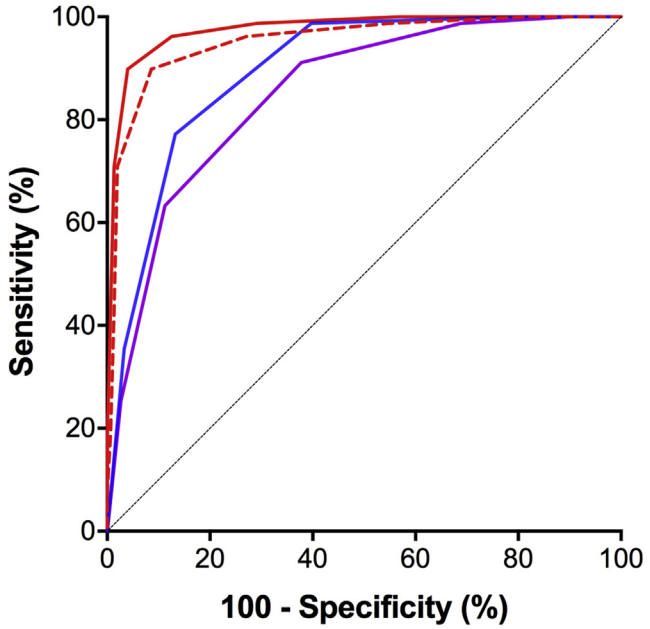

FIG 2. ROC curve analysis for the diagnosis of pathological ABPM. ROC curve analysis for differential diagnosis between pathological ABPM (n = 79) and control diseases (fungus-negative mucus plugs, chronic eosinophilic pneumonia, severe asthma with *Aspergillus* sensitization, and chronic pulmonary aspergillosis; n = 151) with Rosenberg-Patterson criteria (*purple line*), ISHAM criteria (*blue line*), and new criteria (*red line*). The *red dotted line* represents the result with new diagnostic criteria excluding component 7 (positive fungal hyphae in mucus plugs), which requires pathological examination.

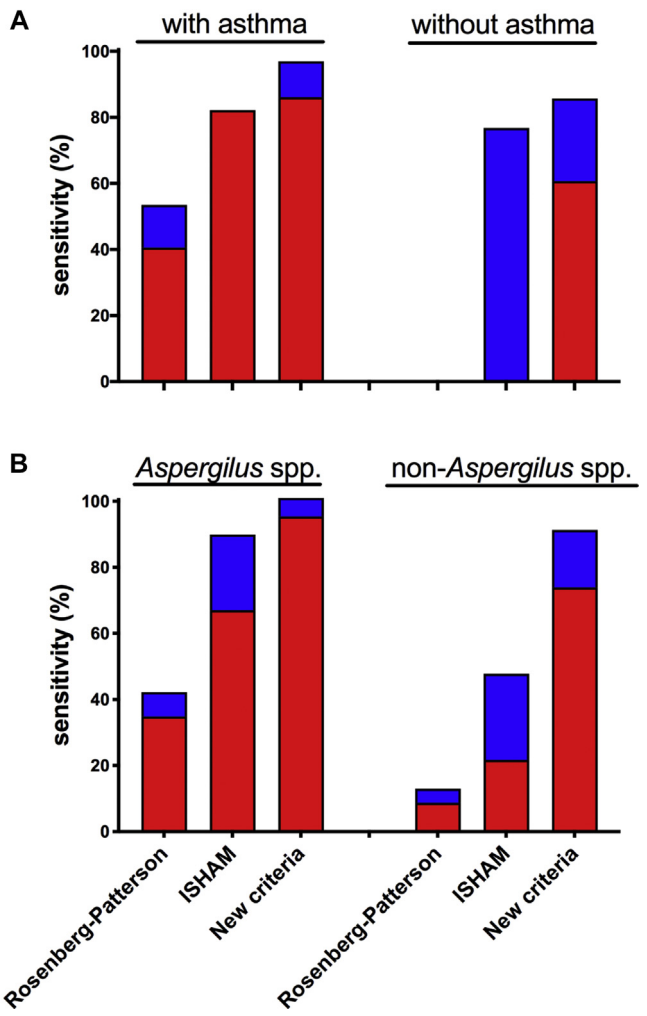

FIG 3. Sensitivity of diagnostic criteria for ABPM with/without asthma and *Aspergillus*/non-*Aspergillus* ABPM. Sensitivity for ABPM with asthma (n = 202) and without asthma (n = 56) **(A)** and culture-positive ABPA (n = 108) and non-Aspergillus ABPM (n = 21) **(B)** were compared using Rosenberg-Patterson criteria, ISHAM criteria, and the new diagnostic criteria. *Red bars* represent definite ABPM, and *blue bars* indicate probable cases.

physician-diagnosed ABPM. The classic diagnostic criteria for ABPA proposed by Rosenberg et al.[5] were highly specific with low sensitivity, as previously reported.[9] We are the first to validate the criteria proposed by ISHAM,[2] showing that the criteria presented substantially improved sensitivity, with a modest decrease in specificity. However, the sensitivity was poor for cases with non-*Aspergillus* ABPM, due to the absence of appropriate serum tests for pathogens excluding *Aspergillus* spp. Therefore, we proposed and validated new diagnostic criteria that showed improved sensitivity and specificity compared to the previous criteria, even in atypical cases without asthma or non-*Aspergillus* ABPM.

A major strength of this study is the number and quality of cases with pathologically diagnosed ABPM identified through collaboration among experienced physicians, bronchoscopists, and pathologists. Although resected lungs with ABPA demonstrate various pathological features including mucoid impaction of bronchi, bronchocentric granulomatosis, exudative bronchiolitis, eosinophilic pneumonia, and noninvasive fungal hyphae,[14] it is not practical to perform surgical biopsies for the diagnosis of ABPM. Therefore, pathologists proposed that "the finding of mucoid impaction of bronchi containing allergic mucin should suggest the diagnosis of ABPA; if fungal hyphae are present, it is diagnostic."[14] The pathological diagnosis based on the presence of fungal hyphae in allergic mucin, which is an eosinophil-rich mucin in the airways, has also been widely accepted for the diagnosis of allergic fungal rhinosinusitis, an allergic disease associated with fungal colonization in the upper

airways.[17] In contrast to allergic fungal rhinosinusitis criteria, pathological criteria for ABPM diagnosis have not been popular in clinical practice, due to the requirement of bronchoscopy examinations to obtain appropriate lower airway samples. Instead, immunological tests such as *Aspergillus*-specific IgE, IgG, and precipitin have been widely used to diagnose ABPA. Therefore, the limited availability of appropriate serum tests for non-*Aspergillus* fungi has complicated non-*Aspergillus* ABPM diagnosis.

Rosenberg-Patterson criteria were highly specific for ABPM but with low sensitivity, especially for cases with pathological ABPM, partly due to the absence of asthma in 42% of these cases. Even in cases with asthma, the sensitivities were 40% and 56% with Rosenberg-Patterson criteria for pathological and physician-diagnosed ABPM, respectively. These results are consistent with the previous report by Agarwal et al[9] who examined the sensitivity of these criteria in 372 asthmatic patients without previous ABPA diagnosis. Using modified Rosenberg-Patterson criteria with an addition of elevated serum *A fumigatus*-specific IgE, only 12.5% of the 56 cases with possible ABPA checked all 8 components.[9]

This is the first study to validate ISHAM criteria for ABPA/ABPM diagnosis. Sensitivities of 77.2% for pathological ABPM and 82.7% for physician-diagnosed disease were significantly better than Rosenberg-Patterson criteria, but with slightly lower specificity, especially for cases with severe asthma with *Aspergillus* sensitization. ISHAM criteria emphasize that high total IgE serum levels (≥1000 IU/mL) are essential for differentiating ABPM from asthma with fungal sensitization. However, 23% of the patients with severe asthma with *Aspergillus* sensitization showed IgE levels of 1000 IU/mL or higher in the present study, as did 36% of the cases with severe asthma with fungal sensitization in our previous study.[18] Furthermore, a substantial proportion of patients with ABPA present with IgE levels <1000 IU/mL in our nationwide survey in Japan[10] and in other studies from East Asia.[19,20] High levels of total serum IgE alone are not specific enough for differential diagnosis between ABPM and severe asthma with fungal sensitization. Another pitfall for diagnosis with ISHAM criteria is the handling of predisposing conditions such as asthma and cystic fibrosis. If the predisposing conditions are mandatory for ABPM diagnosis, a substantial proportion of patients with pathological and physician-diagnosed ABPM could not have been accurately diagnosed. On the other hand, the ability to differentiate ABPM from chronic pulmonary aspergillosis with ISHAM criteria could be compromised if the presence of asthma is excluded. The third problem for ISHAM criteria is the reduced sensitivity in cases with non-*Aspergillus* ABPM.

We developed new diagnostic criteria that emphasize the presence of fungi in the mucus (components 6 and 7), and the importance of mucus plugs in the bronchi (components 9 and 10). Although there is controversy regarding the necessity of separating ABPA/ABPM from severe asthma with fungal sensitization,[12] ABPA with intrabronchial mucus plugs that cause central bronchiectasis by expanding inflamed bronchial wall is significantly different pathophysiologically and should be distinguished from fungus-sensitized asthma. Our new criteria showed better sensitivity than previous criteria for diagnosing pathological and physician-diagnosed ABPA/ABPM, with reasonable specificity. Even in the absence of pathological examinations of mucus plugs (component 7), the new criteria, compared with previous criteria, still showed improved AUC for ROC curve analysis. These results were further confirmed in cases with physician-diagnosed ABPM where pathological examination of mucus plugs was not performed. Therefore, it is practical to apply components 1 to 6 and 8 to 10 of the new diagnostic criteria for ABPM screening. A total score of 6 or more establishes a definite diagnosis without further examination. A bronchoscopy examination to harvest mucus plugs, with a detailed microscopic analysis, would be suggested to confirm the diagnosis for a score of 5.

Our study has some limitations. First, our study included only Japanese cases. There are some differences in the ABPA/ABPM disease phenotypes between East Asian and South Asian populations.[3] Furthermore, there are few patients with cystic fibrosis in Japan and the new criteria have not been validated for ABPM predisposed with this condition. Therefore, an international study to validate the new diagnostic criteria is necessary. Second, we employed tentative cutoff values for laboratory tests, such as peripheral blood eosinophil counts and total and fungus-specific IgE, IgG, and precipitins, based on the previous diagnostic criteria. However, there are substantial discrepancies between the measurement and interpretation of these parameters, such as *Aspergillus*-specific IgG and precipitins, as previously demonstrated.[21] Further studies are required to determine appropriate cutoff values, especially for patients with different regional/ethnic backgrounds. Third, serum tests for this study were mostly performed using crude fungal extracts, and the presence of cross-reactivity among different fungi could have compromised ABPM diagnosis. Molecular allergy diagnostics based on allergenic component-specific serum tests may be a promising way to improve the accuracy of ABPA/ABPM diagnosis.[20]

In conclusion, the new 10-component diagnostic criteria would be useful in diagnosing ABPA/ABPM with improved sensitivity and specificity.

Key messages

- **New diagnostic criteria, consisting of 10 components, for ABPM in patients without cystic fibrosis are proposed and validated.**
- **The new criteria showed high sensitivity and specificity for ABPM, which improved on the previous criteria proposed by Rosenberg and Patterson and by ISHAM.**
- **The new criteria are useful both for *Aspergillus* and non-*Aspergillus* ABPM.**

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TABLE E1. Modified Rosenberg-Patterson diagnostic criteria

1. Episodic bronchial obstruction (asthma)
2. Peripheral blood eosinophilia (≥500/mm^3)
3. Immediate skin reactivity or specific IgE antibody to filamentous fungal antigen
4. Precipitating antibodies or IgG antibodies against filamentous fungal antigen
5. Elevated serum IgE concentrations (≥417 IU/mL)
6. History of pulmonary infiltrates (transient or fixed)
7. Central bronchiectasis

Cases that checked all 7 components were considered definite ABPM. Cases fulfilling 6 components, except for central bronchiectasis (no. 7), were designated as probable cases.

TABLE E2. Modified ISHAM diagnostic criteria

Predisposing conditions
Asthma, cystic fibrosis
Obligatory criteria (both should be present)
Immediate cutaneous hypersensitivity or elevated IgE levels against filamentous fungi
Elevated serum IgE concentrations (≥1000 IU/mL)
Other criteria (at least 2)
Precipitating antibodies or IgG antibodies against filamentous fungal antigen
Radiographic pulmonary opacities consistent with ABPA*
Peripheral blood eosinophilia (≥500/mm^3)

An IgE value <1000 IU/mL may be acceptable if the patient met all other criteria. A case without any predisposing conditions was considered probable ABPM.
*The chest radiographic features consistent with ABPA may be transient (ie, consolidation, nodules, tram-track opacities, toothpaste/finger-in-glove opacities, or fleeting opacities) or permanent (ie, parallel line and ring shadows, bronchiectasis, or pleuropulmonary fibrosis).

TABLE E3. Comparison of demographic data for pathological ABPM between institutes

	Institute A (n = 54)	Institute B (n = 25)	*P* value
Age (y)	59 ± 15	59 ± 15	NS
Female	35 (65)	16 (64)	NS
History of asthma	35 (64)	11 (44)	NS
Peripheral blood eosinophil counts	967 (602-1764)	900 (450-1600)	NS
Total serum IgE levels	1707 (447-4961)	2007 (855-6476)	NS
Fungus-specific hypersensitivity			
Fungus-specific IgE or immediate cutaneous reactions	53 (98)	21 (84)	.03
Fungus-specific precipitin, IgG, or Arthus-type cutaneous reactions	31 (57)	12 (48) [n = 22]	NS
Fungal culture	30 (57) [n = 53]	20 (80)	NS
Aspergillus spp	28 (53)	12 (48)	NS
Other filamentous fungi	2 (4)	8 (32)	.001
Thoracic CT			
Lung opacities	53 (98)	25 (100)	NS
Central bronchiectasis	21 (39)	25 (100)	<.001
Mucus plugs in bronchi	34 (63)	24 (96)	.001
High attenuation mucus	28 (52)	13 (52)	NS

Values are mean ± SD, n (%), or median (interquartile range).

TABLE E4. Comparisons of demographic data for ABPM with or without asthma

	With asthma (n = 202)	Without asthma (n = 56)	*P* value
Age (y)	59 ± 14	65 ± 13	.005
Female	114 (57)	35 (63)	NS
Peripheral blood eosinophil counts	1210 (700-2204)	1086 (594-1508)	NS
Total serum IgE levels	2439 (1034-6111)	1499 (520-3933)	NS
Fungus-specific hypersensitivity			
Fungus-specific IgE or immediate cutaneous reactions	191 (95)	54 (96)	NS
Fungus-specific precipitin, IgG, or Arthus-type cutaneous reactions	146 (72)	34 (64) [n = 53]	NS
Fungal culture	93 (67) [n = 139]	38 (80) [n = 48]	NS
Aspergillus spp	83 (59)	25 (48)	.002
Other filamentous fungi	10 (7)	13 (32)	
Thoracic CT			
Lung opacities	182 (90)	55 (98)	NS
Central bronchiectasis	157 (78)	37 (66)	<.001
Mucus plugs in bronchi	130 (78) [n = 167]	46 (82)	.001
High attenuation mucus	83 (50) [n = 166]	25 (45)	NS

Values are mean ± SD, n (%), or median (interquartile range).

TABLE E5. Comparisons of demographic data between culture-positive ABPA and non-*Aspergillus* ABPM

	Culture-positive ABPA (n = 108)	Non-*Aspergillus* ABPM (n = 23)	*P* value
Age (y)	62 ± 15	60 ± 9	NS
Female	66 (62)	16 (70)	NS
History of asthma	83 (77)	10 (44)	.002
Peripheral blood eosinophil counts	1344 (710-2206)	780 (470-1300)	.004
Total serum IgE levels	2305 (1095-6538)	1391 (460-4293)	NS
Fungus-specific hypersensitivity			
Fungus-specific IgE or immediate cutaneous reactions	106 (98)	22 (96)	NS
Fungus-specific precipitin, IgG, or Arthus-type cutaneous reactions	87 (81)	7 (35) [n = 20]	<.001
Thoracic CT			
Lung opacities	101 (94)	20 (87)	NS
Central bronchiectasis	83 (77)	17 (74)	NS
Mucus plugs in bronchi	72 (78) [n = 92]	21 (96) [n = 22]	.004
High attenuation mucus	46 (50) [n = 92]	16 (73) [n = 22]	NS

Values are mean ± SD, n (%), or median (interquartile range).