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# Which are the optimal criteria for the diagnosis of allergic bronchopulmonary aspergillosis? A latent class analysis

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**What is already known about this topic?**

Several criteria are available for the diagnosis of ABPA. However, in the absence of a reference standard, the diagnostic performance of the various criteria remains unknown.

**What does this article add to our knowledge?**

The ISHAM-ABPA working group diagnostic criteria were found to perform slightly better than the Rosenberg-Patterson criteria on latent class analysis. The diagnostic performance improved by modifying the existing ISHAM criteria.

**How does this study impact current management guidelines?**

We evaluated various benchmarks for identifying ABPA among asthmatics and provide an updated optimal criterion. Our data support modifying the original ISHAM criteria for improved sensitivity. The new criteria will help standardize the diagnosis of ABPA for future research and practice.

## ABSTRACT

**Background:** The ideal criteria for diagnosing allergic bronchopulmonary aspergillosis (ABPA) remains unknown because of the lack of a gold standard. Latent class analysis (LCA) using a probabilistic modeling technique can circumvent the need for a reference standard.

**Objective:** To compare the diagnostic performance of various criteria used for evaluating ABPA.

**Methods:** We prospectively enrolled consecutive cases of bronchial asthma and performed a series of investigations used for the diagnosis of ABPA. We used LCA to analyze the performance of various existing and novel diagnostic criteria.

**Results:** Of the 543 subjects (mean age, 37 years; 319 women), 338 (62.2%), and 205 (37.8%) were labeled as 'mild-to-moderate' and 'severe' asthma, respectively. The subjects with severe asthma had a longer duration of asthma and a higher number of exacerbations in the previous year. The prevalence of *Aspergillus fumigatus* sensitization was 41% and 30%, using the *A.fumigatus*-specific IgE and skin test, respectively. The prevalence of ABPA was 16%, using both the Rosenberg-Patterson and the ISHAM-ABPA working group criteria. The ISHAM criteria were slightly more sensitive (99% vs. 98%) and specific (89% versus 81%) than the Patterson criteria. We obtained optimal diagnostic performance by altering the existing ISHAM criteria (serum total IgE >500 IU/mL, excluding the skin test, and using CT thorax instead of chest radiograph).

**Conclusions:** The ISHAM-ABPA working group criterion was only marginally better than the Patterson criteria in diagnosing ABPA among asthmatics <66 years of age. The diagnostic performance however improved by modifying the prevailing ISHAM criteria, but with increased cost.

**Key-words:** allergic bronchopulmonary mycosis; *Aspergillus*; ABPA; ABPM; fungal sensitization; allergy; asthma; latent class analysis

**Abbreviations used**

|    |                    |                                                     |
|----|--------------------|-----------------------------------------------------|
| 81 |                    |                                                     |
| 82 |                    |                                                     |
| 83 | <i>A.fumigatus</i> | <i>Aspergillus fumigatus</i>                        |
| 84 | Ig                 | Immunoglobulin                                      |
| 85 | CT                 | Computed Tomography                                 |
| 86 | ABPA               | Allergic Bronchopulmonary Aspergillosis             |
| 87 | AST                | <i>Aspergillus</i> skin test                        |
| 88 | ISHAM              | International Society for Human and Animal Mycology |
| 89 | FEV <sub>1</sub>   | Forced expiratory volume in the first second        |
| 90 | FVC                | Forced vital capacity                               |
| 91 | LCA                | Latent Class Analysis                               |
| 92 | FEIA               | Fluorescent enzyme immunoassay                      |
| 93 | kUA/L              | Kilounit of antigen-specific antibodies per liter   |
| 94 | mgA/L              | Milligrams of antigen-specific antibodies per liter |
| 95 | IU/mL              | International units per milliliter                  |

## INTRODUCTION

Allergic bronchopulmonary aspergillosis (ABPA) is a complex disease affecting patients with asthma and cystic fibrosis.<sup>1,2</sup> The prevalence of ABPA in asthma varies from 3-13%, depending on the echelon of medical care.<sup>3</sup> The most common presentation of ABPA is with poorly controlled asthma. However, if ABPA is not recognized or treated, it may cause irreversible airway damage and bronchiectasis,<sup>4</sup> emphasizing the need for early diagnosis.

In 1977, Rosenberg et al. proposed a standard for the diagnosis of ABPA based on the analysis of 20 patients seen over nine years at one center (Table 1).<sup>5</sup> The Rosenberg-Patterson criteria have been widely used despite several limitations. The shortcomings include the lack of consensus on the number of components in the criteria to make a diagnosis, disagreement on the cut-off values for various immunological tests, assigning equal weight to all the individual components, and the failure to use a standardized methodology for immunological tests.<sup>2</sup> These inadequacies led to ambiguity as clinicians and researchers employed the Patterson criteria variably. To overcome the limitations, the ABPA working group of the International Society for Human and Animal Mycology (ISHAM) suggested a new model for the diagnosis of ABPA based on the existing evidence and consensus (Table 1).<sup>6</sup> The group further called for evaluating new criteria to improve the accuracy of identifying ABPA.<sup>6</sup> Regrettably, without a reference standard, it becomes impossible to accurately assess the performance of the diagnostic criteria.

Latent class analysis (LCA) has been used to derive estimates of diagnostic performance when no gold standard is available.<sup>7-10</sup> In a previous study, we had evaluated the performance of various individual tests used in the diagnosis of ABPA using LCA.<sup>11</sup> While we had also assessed the performance of a few criteria, the ISHAM-ABPA working group criteria were not evaluated, as the study was performed before the publication of these criteria. Over the last five years, several new pieces of evidence have emerged, which can potentially improve the detection of ABPA.<sup>11-13</sup> In this

120 study, we evaluate the diagnostic performance of various criteria for the identification of ABPA in  
121 a sequential cohort of asthmatics using LCA.

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**METHODS**

**Study design and setting:** We conducted a prospective observational study between July 2017 and September 2018 at our institute's chest clinic. The study protocol was approved by the Institute Ethics Committee. We obtained written informed consent from all the participants.

**Study population:** All consecutive cases of bronchial asthma attending the chest clinic during the study period were eligible for inclusion. We defined bronchial asthma by the presence of any three of the following: (1) history of recurrent episodes of breathlessness, chest tightness, or cough; (2) wheeze on auscultation of the chest; (3) evidence of obstruction on spirometry defined as a ratio of forced expiratory volume in the first second (FEV<sub>1</sub>) to forced vital capacity (FVC) less than the lower limit of normal, with significant bronchodilator reversibility (increment of FEV<sub>1</sub> or FVC >12% and 200 mL, after 400 µg of inhaled salbutamol); and, (4) clinical response to asthma therapy or improvement in FEV<sub>1</sub> by at least 12% and 200 mL documented on spirometry after 4-8 weeks of treatment. An assessment of asthma severity was made as per the 2004 Global Initiative for Asthma (GINA) recommendations, which includes the effect of treatment on disease severity.<sup>14</sup>

We excluded subjects with any of the following: (1) age >65 years; (2) smokers with a smoking index >300 (smoking index was defined as the number of bidis or cigarettes smoked per day multiplied by the number of years smoked); (3) subjects on oral steroids for >3 weeks within the preceding three months; (4) subjects with a prior diagnosis of ABPA; (5) chronic obstructive pulmonary disease; (6) immunosuppressive states (chronic liver disease, chronic renal failure, cytotoxic therapy, uncontrolled diabetes mellitus, and others); (7) pregnancy; and, (8) failure to provide informed consent.

**Outcomes:** The study's primary outcome was to compare the diagnostic performance of the Rosenberg-Patterson criteria with the ISHAM-ABPA working group criteria. We also investigated the diagnostic performance of three newer standards for the detection of ABPA.

**Measurements:** A detailed history and clinical examination were undertaken. We performed the following investigations in all the subjects: spirometry, *Aspergillus* skin test, *A.fumigatus*-specific IgE, *A.fumigatus*-specific IgG, serum total IgE, peripheral blood eosinophil count, chest radiograph, and computed tomography (CT) of the chest.

**Spirometry:** All subjects underwent spirometry on an ultrasonic flow-sensing spirometer (model Easy on-PC; Version V02, NDD, Medizintechnik AG, Zurich, Switzerland). We recorded the FEV<sub>1</sub>, FVC, FEV<sub>1</sub>/FVC, and the changes after bronchodilator therapy. We interpreted the spirometric values using the protocol described previously.<sup>15, 16</sup>

***Aspergillus* Skin Test (AST):** We performed AST using the intradermal technique by injecting 0.2 mL of 100 protein nitrogen units/mL of crude *A.fumigatus* antigen (Alcit India, New Delhi, India) into the forearm. Phosphate buffer saline (0.2 mL) in the other forearm served as the negative control. The injection site was inspected every 15 minutes for one hour. A type 1 reaction was present if a “wheal and erythema” formed in one minute, maximized after 10-20 minutes and subsided in one hour. Also, the size of the reaction on the test arm had to be >8 mm compared to the control arm.<sup>3, 11, 17</sup>

***Serum A.fumigatus*-specific IgE:** We assayed *A.fumigatus*-specific IgE in serum using the fluorescent enzyme immunoassay technology (Phadia 100, Thermofisher Scientific, Uppsala, Sweden). The antigen (m3) used was a purified crude extract obtained from the mycelia and conidia of *A.fumigatus*. The samples were diluted at 1:100, and the results were expressed as kilounits of antigen-specific antibodies (kUA)/L. We interpreted a value >0.35 kUA/L as positive.

***Serum A.fumigatus*-specific IgG:** We measured serum *A.fumigatus*-specific IgG using the automated fluorescent enzyme immunoassay method (Phadia 100, Thermofisher Scientific, Uppsala, Sweden) with the m3 Immucap. The samples were diluted at 1:100, and the results were interpreted as milligrams of antigen-specific antibodies (mgA)/L. We considered a value >27 mgA/L as positive.<sup>12</sup>

Serum total IgE: we quantified serum total IgE using the fluorescent enzyme immunoassay technology (Phadia 100, Thermofisher Scientific, Uppsala, Sweden). We used various cut-offs of 417 IU/mL, 500 IU/mL, and 1000 IU/mL, to define serum total IgE as elevated.

Total eosinophil count (TEC): We first recorded the white blood cell count using a hematology auto-analyzer. The total eosinophil count was calculated as a product of white blood cell count, and the percentage of eosinophils (100 white blood cells were counted on a peripheral blood smear to determine the differential leukocyte count).

Chest radiograph: A chest radiograph was performed for all subjects, and previously available radiographs were compared. We labeled the radiological findings as transient (fleeting opacities, tram-track shadows, consolidation, toothpaste shadows, nodules, finger-in-glove opacities) or permanent (ring shadows, parallel lines, and pleuropulmonary fibrosis).<sup>2</sup>

CT thorax: A 16-slice multidetector CT scanner was used to obtain CT of the chest. The acquisition of images was contiguous. The images were reconstructed into a soft tissue mediastinal window (20-30 kernel) and a lung window in the sharp algorithm (60-80 kernel) at 1.5 mm section thickness. We defined bronchiectasis, mucus impaction, and high-attenuation mucus (HAM) according to previously described criteria.<sup>18-23</sup>

Sputum culture for *Aspergillus*: Sputum samples were concentrated as per the standard protocol. We then inoculated the sputum specimen on two tubes of Sabouraud dextrose agar, one at 25°C and the other at 37°C. A part of the sample was also inoculated and incubated at 25°C on brain heart infusion agar with antibiotics. We labeled the result as negative for subjects unable to expectorate sputum.

**Statistical analysis**: Data analysis was done using the commercial statistical package SPSS version 22.0 (IBM SPSS Inc., Armonk NY, US). Data are presented as number (percentage), mean (95% confidence intervals [CI]), or median [interquartile range (IQR)]. We analyzed the differences between continuous variables using the t-test (for normally distributed data) or the Mann-

whitney U test (for skewed data). The differences between categorical variables were analyzed using the chi-square test. We used Cohen's weighted kappa to assess the agreement between two tests.<sup>24</sup> The kappa results were interpreted as follows: 0 as no agreement, 0.01-0.2 none to slight, 0.21-0.4 fair, 0.41-0.6 moderate, 0.61-0.8 substantial, and 0.81-1.0 as almost perfect agreement.

We performed LCA using the R version of the TAGS software.<sup>7</sup> For performing LCA, we categorized the study population into two groups, namely mild-to-moderate asthma (consisting of mild intermittent, mild persistent, and moderate persistent) and severe asthma (comprising of severe persistent), according to the 2004 GINA guidelines.<sup>14</sup> We made this division as the treatment and outcome of severe asthma is different from that of mild-to-moderate asthma,<sup>25</sup> and subjects with severe asthma are more likely to have ABPA.<sup>26</sup> The diagnostic performance of various criteria used for evaluating ABPA was tested in the above-mentioned two groups. The LCA examines the relationship between the observed variables (in the current study, the different diagnostic criteria) that imperfectly estimate a non-observable or latent variable, the actual disease status (ABPA).<sup>27-32</sup> Each of the individual tests ( $n=7$ ) or criteria ( $n=5$ ) was applied in every subject. The presence or absence of the test or criteria was reported in a binary fashion (0 or 1). Depending on the number of tests or criteria to be evaluated, different permutations were possible (number of permutations being  $2^n$ , where  $n$  is the number of individual tests or criteria under evaluation). For instance, we can have 32 permutations when evaluating five criteria (Table E1). The frequency with which each permutation occurred in the data was noted and entered in the R-TAGS program. The LCA then calculates the maximum likelihood (ML) estimates for the various investigations and criteria to provide the sensitivity and specificity with 95% CI (corresponding to 2.5-97.5% bootstrap CIs obtained by bootstrapping 5000 samples). Thus, the sensitivity and specificity are derived without creating a 2 x 2 table, as would be routinely performed in the presence of a reference standard. Two iterative methods, namely the Newton-Raphson algorithm and the expectation-maximization algorithm, were used for the ML estimates.<sup>7</sup>

we tested the assumption of conditional independence by the goodness-of-fit test, and after that, residual correlations between tests were deduced. A p-value  $>0.05$  and the random distribution of the residuals near-zero indicated a good fit of the model.

A multidisciplinary team (MDT) analyzed the cases classified as ABPA by Patterson, the ISHAM, and the best performing newer criteria in the classification of ABPA. We used the DAG\_stat application for calculating the correct classification rate (efficiency) of the various criteria.<sup>33</sup>

**RESULTS**

During the study period, we screened 605 consecutive subjects with asthma, and 589 met the criteria for inclusion in the study. We excluded three subjects from the analysis because of uncertainty in the diagnosis, while 43 were excluded due to incomplete data. Finally, we included 543 asthmatics for data analysis.

**Baseline characteristics**

The mean (95% CI) age of the study population was 36.8 (35.6-38.0) years and were non-smokers. The frequencies of mild intermittent, mild persistent, moderate persistent and severe persistent asthma were 23 (4.2%), 58 (10.7%), 257 (47.3%), and 205 (37.8%), respectively, using the 2004 GINA classification. There were 338 (62.2%) subjects in the 'mild-to-moderate' asthma group and 205 (37.8%) subjects in the 'severe' asthma group. The severe asthma group had a higher mean age (39.9 vs. 34.9 years), a higher proportion of women, longer duration of asthma, and a higher number of exacerbations in the previous year (Table 2). A history of expectoration of brownish-black mucus plugs was also more frequent in the severe asthma group. More subjects in the severe asthma group had obstruction on spirometry (72.7% vs. 59%;  $p=0.002$ ), and this group also had a lower mean FEV1. The other characteristics, including a history of atopy, the microbiological (sputum culture), immunological, and imaging findings, were not statistically different between the two groups (Table 2). Sputum culture grew *Aspergillus* species in 4.1% (22/543) subjects. Of these, 17, 2, and 1 subject grew *A.flavus*, *A.fumigatus*, and *A.terreus* respectively, while two grew both *A.fumigatus* and *A.flavus*.

The *A.fumigatus*-specific IgE value was greater than 0.35 kUA/L in 40.5% (220/543) subjects, whereas immediate cutaneous reactivity to *A.fumigatus* antigen was seen in 30.2% (164/543) subjects. There was a moderate level of agreement between the two tests (Cohen's weighted  $\kappa$  value of 0.45,  $p < 0.001$ ). Eighty-six (15.8%) of the 543 subjects satisfied the Rosenberg-

Patterson criteria, while 16.4% (89/543) met the ISHAM working group criteria for ABPA. There was substantial agreement between the two criteria (Cohen's weighted  $\kappa$  value of 0.78,  $p < 0.001$ ).

#### **Performance of various individual tests using latent class analysis**

We initially tested the performance of the individual diagnostic tests to be included in the formulation of the composite diagnostic criteria using three models (Table 3). In the first model, we included AST, *A.fumigatus*-specific IgE  $>0.35$  kUA/L, *A.fumigatus*-specific IgG  $>27$  mgA/L, TEC  $>500$  cells/ $\mu$ L, IgE  $>417$  IU/mL (1000 ng/mL), bronchiectasis, and HAM. In the second model, the IgE cut-off was increased to 1000 IU/mL, while the other parameters remained the same. The third model was like the first except for an IgE cut-off of 500 IU/mL, and mucoid impaction replaced high-attenuation mucus. The goodness-of-fit test for conditional independence was acceptable for all the models (the  $p$  values for goodness-of-fit for models 1, 2, and 3 were 0.74, 0.46, and 0.22, respectively, indicating a good fit), and the residual correlations between the tests were randomly distributed around zero. *A.fumigatus*-specific IgE displayed the highest sensitivity while HAM on CT thorax showed the greatest specificity. The serum total IgE value  $>500$  IU/mL showed better sensitivity than a value  $>1000$  IU/mL; however, the specificity was much worse (49.3% vs. 73.4%, respectively).

#### **Performance of various criteria using latent class analysis**

We then tested the performance of five different diagnostic criteria using LCA (Table 4). The first two criteria were like the original Patterson criteria and the ISHAM criteria, respectively, with certain modifications. In the Patterson criteria, we used *A.fumigatus*-specific IgG levels  $>27$  mgA/L instead of *A.fumigatus* precipitins, and *Aspergillus* sensitization was defined by either a positive AST or elevated *A.fumigatus*-specific IgE. In the ISHAM criteria (Table 1), *A.fumigatus*-specific IgG replaced precipitins.

The newly evaluated criteria were all modifications of the existing ISHAM criteria. In the first modification (Table 4, criteria 1), we used a lower cut-off of serum total IgE (500 IU/mL),

removed ASI, and replaced chest radiograph with CT chest. The second criterion (Table 4, criterion 2) was like criterion 1 except that we included mucoid impaction on CT thorax in the 'other criteria', and at least three of the four 'other criteria' had to be satisfied for diagnosing ABPA. The IgE cut-off was also kept at 1000 IU/mL in criterion 2. The third criterion resembled criterion 2 except that at least two of the four 'other criteria' had to be present for diagnosing ABPA (Table 4, criterion 3).

On LCA, the p-value for the goodness-of-fit test for conditional independence was 0.03, indicating that the model does not fit. The ISHAM criteria performed slightly better than the Patterson criteria in terms of both sensitivity and specificity. Amongst the newly evaluated criteria, criterion 1 had the best diagnostic performance, with sensitivity and specificity approaching 100% (Table 4).

A total of 108 subjects (Figure 1) were diagnosed as ABPA by any of the three criteria (Patterson [n=86], the ISHAM [n=89], and the modified ISHAM [criterion 1, Table 4; n=102]). An MDT further evaluated these cases. Of the 108, 106 cases were adjudged to have ABPA (Table 5), and 71 of the 106 were correctly classified as ABPA by all the three criteria (Figure 1). The efficiency (Table 5) of the classification was best for the modified ISHAM criteria (Criteria 1, Table 4).

## DISCUSSION

Of the two major diagnostic criteria currently available for identifying ABPA among asthmatics, we found the ISHAM criteria<sup>6</sup> to fare slightly better than the Rosenberg-Patterson criteria.<sup>5</sup> Further, the sensitivity of the ISHAM criteria improved with certain modifications (modified ISHAM criteria).

We initially evaluated the performance of the seven most widely used diagnostic tests for ABPA using three models. In the first, the IgE level cut-off was kept at 417 IU/mL (as in the Patterson criteria), while the cut-off was 1000 IU/mL in the second model (as in the ISHAM criteria). In the third model, we replaced HAM with mucoid impaction and used 500 IU/mL as the cut-off for serum total IgE. In all the models, we used *A.fumigatus*-specific IgG instead of *Aspergillus* precipitins, because of the superior sensitivity of the former test.<sup>12</sup> The serum total IgE cut-off at 500 IU/mL demonstrated better sensitivity, albeit a lower specificity, compared to a level of 1000 IU/mL. *A.fumigatus*-specific IgE showed a higher sensitivity than AST in all the models, similar to a previous study.<sup>11</sup> However, we should bear in mind that the antigen used for skin testing is different from the antigen used for measuring *A.fumigatus*-specific IgE. Also, if a more reactive skin test antigen becomes available, the results might be different. Additionally, the skin test is cheaper compared to *A.fumigatus*-specific IgE. Skin tests may be used in populations with high prevalence or economic constraints, where a negative test may exclude ABPA.<sup>34</sup> While most investigations are directed towards the fungi, the eosinophil count despite being nonspecific remains an important component of the diagnostic criteria and should be routinely performed.

Subsequently, we evaluated five different criteria for the diagnosis of ABPA (Table 4). Apart from the existing Patterson and ISHAM criteria, we evaluated three new criteria. We chose *A.fumigatus*-specific IgE (instead of AST), CT evidence of bronchiectasis (instead of HAM), and IgE level cut-off of 500 IU/mL, given the better sensitivity of these tests (Table 3). In criteria 2 and 3, we retained the IgE cut-off at 1000 IU/mL, as the lower cut-off may reduce the specificity of the

model (Table 4). we also added mucoid impaction to the above two criteria as it is believed to be a characteristic feature,<sup>20-22, 35</sup> and has also been previously used as a diagnostic criterion.<sup>36</sup>

We found that the ISHAM criteria performed only marginally better than the Patterson criteria, both in sensitivity and specificity. However, the modifications to the existing ISHAM criteria (Criteria 1, Table 4) offered the best diagnostic performance. The newly proposed criterion resembles the minimal version of the original Patterson criteria,<sup>37</sup> albeit with some changes. The addition of mucoid impaction did not improve the diagnostic performance, possibly because mucoid impaction and bronchiectasis on CT chest are often found in concert.

What is the clinical relevance of our study? The previous criteria for ABPA, namely the Patterson and the ISHAM criteria, are consensus-based and have never been compared prospectively, for diagnostic performance. We provide an evidence-based estimate of the diagnostic performance of various criteria used in the diagnosis of ABPA. The modified version of the ISHAM criteria (criterion 1, Table 4) has a better correct classification rate than the existing criteria (Table 5). However, the inclusion of CT and *A.fumigatus*-specific IgE (instead of AST) increases the cost substantially. Once confirmed with studies from other centers, the newer (Table 4, criteria 1) criteria could serve as a standard for patient care and research.

Finally, our study is not without limitations. One major flaw is that the sample population represents only asthmatic patients visiting a single tertiary care referral center. Hence, our study had a larger population of moderate and severe persistent patients than mild intermittent and persistent variants. For the same reason, the prevalence of *Aspergillus* sensitization (40%) and bronchiectasis (25%) was also considerably high. The study results may not be applicable in other populations, especially cystic fibrosis. Also, we included non-smoking participants less than 66 years of age. The other significant drawback relates to the statistical technique, which may be associated with incorrect assumptions, unknown dependence between variables, and erroneous interpretations of the LCA results.<sup>29, 30</sup> The assumption is that these five criteria are independent.

345 However, since the evaluated criteria, in general, contain many of the same individual  
346 components, they may not be completely independent. Also, the goodness-of-fit test for the  
347 model indicated that the model did not fit. Thus, the assumption of conditional independence was  
348 not met, and the estimates of sensitivity and specificity may be inflated. We partially overcame  
349 this drawback by performing an MDT review to examine the accuracy of various criteria. The MDT  
350 vs. the LCA modeling provides additional evidence that the estimates of sensitivity and specificity  
351 may be exaggerated. The sputum fungal culture positivity was low overall as we used routine  
352 sputum cultures. The use of high volume culture,<sup>38</sup> and nucleic acid amplification assay may  
353 improve the yield of fungus detection in sputum.<sup>39</sup> Growing evidence suggests that the use of  
354 recombinant *A.fumigatus* antigens may simplify the diagnosis of ABPA.<sup>40</sup> Unfortunately, we have  
355 not evaluated the performance of recombinant antigens in the current study. Also, our study  
356 results are not applicable to patients receiving oral glucocorticoids, as steroids rapidly cause a  
357 decline in serum total IgE. The strengths of our study are the large size of the study population  
358 and the fact that all immunological tests were conducted using standardized techniques.

359 In conclusion, the ISHAM ABPA working group criteria was only a tad better than the  
360 Patterson criteria. The diagnostic performance was however improved by modifying the existing  
361 ISHAM criteria. Future studies are suggested to validate our findings.

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465 and Clinical Utility of Recombinant *Aspergillus fumigatus* Antigens in the Diagnosis of  
466 Allergic Bronchopulmonary Aspergillosis. J Allergy Clin Immunol Pract 2020; 8:579-87.

467 **Table 1:** Diagnosis of allergic bronchopulmonary aspergillosis (ABPA)*Rosenberg-Patterson criteria*

|           |                                                                                                                                                                                                                                                                                                                                                                                                                       |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Primary   | <ol style="list-style-type: none"> <li>1. Presence of asthma</li> <li>2. Transient or fixed radiographic infiltrates</li> <li>3. Positive type I skin reaction to <i>A.fumigatus</i></li> <li>4. Serum total IgE &gt;1000 ng/mL (&gt;417 IU/mL)</li> <li>5. Positive <i>A.fumigatus</i> precipitins</li> <li>6. Total eosinophil count &gt;1000 cells/<math>\mu</math>L</li> <li>7. Central bronchiectasis</li> </ol> |
| Secondary | <ol style="list-style-type: none"> <li>1. Expectoration of mucus plugs</li> <li>2. Isolation of <i>Aspergillus</i> in the culture of sputum</li> <li>3. Positive type III skin reaction to <i>A.fumigatus</i></li> </ol>                                                                                                                                                                                              |

The authors labeled the entity as ABPA-CB (central bronchiectasis) if proximal bronchiectasis was present and ABPA-S (serological), if not.

The diagnosis of ABPA is highly probable if the first six of the seven primary criteria are present.

The presence of all seven criteria makes the diagnosis almost certain.

*ISHAM diagnostic criteria for ABPA*

|                                               |                                                                                                                                                                                                                                                              |
|-----------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Predisposing conditions                       | Presence of asthma or cystic fibrosis                                                                                                                                                                                                                        |
| Obligatory criteria<br>(both must be present) | <ol style="list-style-type: none"> <li>1. Positive type I skin reaction to <i>A.fumigatus</i> or <i>A.fumigatus</i>-specific IgE &gt;0.35 KUA/L</li> <li>2. Serum total IgE levels &gt;1000 IU/mL*</li> </ol>                                                |
| Other criteria ( $\geq 2$ )                   | <ol style="list-style-type: none"> <li>1. Positive <i>A.fumigatus</i> precipitins or <i>A.fumigatus</i>-specific IgG &gt;27 mgA/L</li> <li>2. Chest radiograph favoring ABPA**</li> <li>3. Total eosinophil count &gt;500 cells/<math>\mu</math>L</li> </ol> |

\* IgE level <1000 IU/mL can be accepted if all other conditions are satisfied.

\*\*Transient opacities like fleeting opacities, tram-track shadows, consolidation, toothpaste shadows, nodules or finger-in-glove opacities or permanent opacities like ring shadows, parallel lines, and pleuropulmonary fibrosis.

**Table 2:** Baseline characteristics of the study population

|                                                     | <sup>a</sup> Mild-to-moderate asthma (n=338) | <sup>b</sup> Severe asthma (n=205) | Total (n=543)    | P value |
|-----------------------------------------------------|----------------------------------------------|------------------------------------|------------------|---------|
| Age (years)                                         | 34.9 (33.5-36.4)                             | 39.9 (38.0-41.8)                   | 36.8 (35.6-38.0) | <0.001  |
| Female sex                                          | 186 (55.0)                                   | 133 (64.9)                         | 319 (58.8)       | 0.024   |
| Duration of asthma (years)                          | 9.02 (8.1-10.0)                              | 12.0 (10.6-13.4)                   | 10.2 (9.4-11.0)  | <0.001  |
| Number of exacerbations in the previous year        | 0.35 (0.3-0.4)                               | 1.23 (0.9-1.6)                     | 0.68 (0.5-0.8)   | <0.001  |
| History of atopy                                    | 102 (30.2)                                   | 71 (34.6)                          | 173 (31.9)       | 0.28    |
| History of atopy in family                          | 131 (38.8)                                   | 93 (45.4)                          | 224 (41.3)       | 0.13    |
| Allergic rhinitis                                   | 220 (65.1)                                   | 143 (69.8)                         | 363 (66.9)       | 0.26    |
| History of hemoptysis                               | 31 (9.2)                                     | 26 (12.7)                          | 57 (10.5)        | 0.20    |
| History of expectorating brownish-black mucus plugs | 38 (11.2)                                    | 38 (18.5)                          | 76 (14.0)        | 0.018   |
| History of anti-tuberculous treatment               | 18 (5.3)                                     | 17 (8.3)                           | 35 (6.5)         | 0.17    |
| Obstruction in spirometry                           | 202 (59.8)                                   | 149 (72.7)                         | 351 (64.6)       | 0.002   |
| FEV1 (% predicted)                                  | 77.7 (75.2-80.2)                             | 66.6 (62.9-70.3)                   | 73.5 (71.4-75.6) | <0.001  |
| Growth of <i>Aspergillus</i> in sputum culture      | 13 (3.9)                                     | 9 (4.4)                            | 22 (4.1)         | 0.76    |
| <b>Immunologic investigations</b>                   |                                              |                                    |                  |         |
| Type 1 <i>Af</i> skin test positive                 | 103 (30.5)                                   | 61 (29.8)                          | 164 (30.2)       | 0.86    |
| <i>A.fumigatus</i> -specific IgE level, kUA/L       | 0.1 (0.03-2.3)                               | 0.1 (0.03-2.5)                     | 0.1 (0.03-2.4)   | 0.57    |
| Serum total IgE level, IU/mL                        | 614 (202-2208)                               | 687 (189-2440)                     | 624 (200-2293)   | 0.91    |
| <i>A.fumigatus</i> -specific IgG level, mgA/L       | 17.60 (7.9-46.5)                             | 18.0 (8.7-48.5)                    | 17.7 (8.1-48)    | 0.99    |
| Total eosinophil count, cells/ $\mu$ L              | 395 (200-670)                                | 420 (220-710)                      | 400 (200-700)    | 0.12    |
| Total eosinophil count >500 cells/ $\mu$ L          | 132 (39.1)                                   | 91 (44.4)                          | 223 (41.1)       | 0.22    |
| Total eosinophil count >1000 cells/ $\mu$ L         | 40 (11.8)                                    | 27 (13.2)                          | 67 (12.3)        | 0.65    |
| IgE level >417 IU/mL (1000 ng/mL)                   | 199 (58.9)                                   | 122 (59.5)                         | 321 (59.1)       | 0.88    |
| IgE level >1000 IU/mL                               | 128 (37.9)                                   | 84 (40.9)                          | 212 (39)         | 0.47    |
| IgE level >500 IU/mL                                | 186 (55)                                     | 114 (55.6)                         | 300 (55.3)       | 0.90    |
| <b>Chest radiograph</b>                             |                                              |                                    |                  |         |
| Parallel opacities                                  | 14 (4.1)                                     | 14 (6.8)                           | 28 (5.2)         | 0.17    |
| Ring opacities                                      | 27 (7.9)                                     | 22 (10.7)                          | 49 (9)           | 0.28    |
| Finger-in-glove opacities                           | 18 (5.3)                                     | 8 (3.9)                            | 26 (4.8)         | 0.45    |
| Tram-track opacities                                | 16 (4.7)                                     | 13 (6.3)                           | 29 (5.3)         | 0.42    |
| Fleeting opacities                                  | 15 (4.4)                                     | 6 (2.9)                            | 21 (3.9)         | 0.38    |
| Any of the above                                    | 47 (13.9)                                    | 35 (17.1)                          | 82 (15.1)        | 0.32    |
| <b>CT chest findings</b>                            |                                              |                                    |                  |         |
| Bronchiectasis                                      | 73 (21.6)                                    | 59 (28.8)                          | 132 (24.3)       | 0.059   |
| Centrilobular nodules                               | 49 (14.5)                                    | 36 (17.6)                          | 85 (15.7)        | 0.34    |
| High-attenuation mucus                              | 24 (7.1)                                     | 15 (7.3)                           | 39 (7.2)         | 0.93    |
| Mucus impaction                                     | 43 (12.7)                                    | 27 (13.2)                          | 70 (12.9)        | 0.88    |

All values are represented as mean (95% confidence intervals) or number (percentage). FEV1- forced expiratory volume in the first second. <sup>a</sup>Mild-to-moderate asthma consisted of mild intermittent, mild persistent, and moderate persistent while the <sup>b</sup>severe asthma comprised of severe persistent category as per the 2004 GINA guidelines

**Table 3:** Sensitivity and specificity of individual diagnostic tests with three different models using  
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|                                              | Sensitivity      | Specificity      |
|----------------------------------------------|------------------|------------------|
| <b>Model 1</b>                               |                  |                  |
| <i>A.fumigatus</i> skin test positivity      | 82.2 (74.1-90.7) | 81.5 (77.5-85.6) |
| <i>A.fumigatus</i> -specific IgE >0.35 kUA/L | 99.2 (96.2-100)  | 72.4 (67.5-77.2) |
| <i>A.fumigatus</i> -specific IgG >27 mgA/L   | 91.6 (83.8-100)  | 81.5 (77.4-85.5) |
| Total eosinophil count >500 cells/ $\mu$ L   | 71.5 (62.0-81.3) | 64.7 (60.0-69.5) |
| Serum total IgE >417 IU/mL (1000 ng/mL)      | 96.3 (91.8-100)  | 47.5 (42.7-52.7) |
| Bronchiectasis                               | 95.3 (86.9-100)  | 93.4 (90.4-96.0) |
| High-attenuation mucus                       | 36.0 (26.0-46.4) | 100.0 (99.5-100) |
| <b>Model 2</b>                               |                  |                  |
| <i>A.fumigatus</i> skin test positivity      | 82.7 (72.8-89.5) | 82.5 (78.5-85.8) |
| <i>A.fumigatus</i> -specific IgE >0.35 kUA/L | 99.0 (90.4-99.1) | 73.6 (69.0-77.7) |
| <i>A.fumigatus</i> -specific IgG >27 mgA/L   | 91.9 (80.9-96.9) | 77.8 (73.6-81.6) |
| Total eosinophil count >500 cells/ $\mu$ L   | 73.3 (63.4-81.4) | 66.7 (62.1-71.0) |
| Serum total IgE >1000 IU/mL                  | 90.9 (82.1-95.6) | 73.4 (69.0-77.5) |
| Bronchiectasis                               | 94.3 (82.9-98.2) | 92.5 (89.3-94.9) |
| High-attenuation mucus                       | 35.1 (26.3-45.0) | 99.5 (97.3-100)  |
| <b>Model 3</b>                               |                  |                  |
| <i>A.fumigatus</i> skin test positivity      | 82.9 (74.0-90.9) | 81.4 (77.7-85.3) |
| <i>A.fumigatus</i> -specific IgE >0.35 kUA/L | 98.2 (94.1-100)  | 72.1 (67.9-76.8) |
| <i>A.fumigatus</i> -specific IgG >27 mgA/L   | 94.6 (86.9-100)  | 77.2 (73.2-81.4) |
| Total eosinophil count >500 cells/ $\mu$ L   | 73.3 (63.5-82.4) | 66.0 (61.7-70.6) |
| Serum total IgE >500 IU/mL                   | 97.7 (92.3-100)  | 49.3 (44.8-54.3) |
| Bronchiectasis                               | 100 (93.3-100)   | 92.5 (89.7-95.5) |
| Mucoid impaction on CT chest                 | 67.7 (55.2-77.5) | 98.9 (97.5-100)  |

The figures in parenthesis correspond to 2.5-97.5% bootstrap confidence intervals obtained by bootstrapping 5000 samples

The p values for goodness-of-fit for models 1, 2, and 3 are 0.74, 0.46, and 0.22, respectively, indicating a good fit

**Table 4:** Performance of different criteria for the diagnosis of allergic bronchopulmonary

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|                                                                                                                                                                                                                                                                                                                                                                                            | Sensitivity      | Specificity      |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|------------------|
| <b>Existing criteria</b>                                                                                                                                                                                                                                                                                                                                                                   |                  |                  |
| <sup>a</sup> <b>Patterson criteria:</b> asthma, chest radiographic infiltrates, type I AST positivity, serum total IgE >417 IU/mL, <i>A.fumigatus</i> -specific IgG >27 mgA/L, eosinophil count >1000 cells/μL, bronchiectasis, <i>A.fumigatus</i> -specific IgE >0.35 KUA/L (presence of any six of the above)                                                                            | 81.3 (73.3-88.6) | 98.2 (96.9-99.6) |
| <sup>b</sup> <b>ISHAM criteria:</b> Presence of all of the following: (1) asthma; (2) positive type I skin reaction to <i>A.fumigatus</i> or <i>A.fumigatus</i> -specific IgE >0.35 KUA/L; (3) serum total IgE levels >1000 IU/mL; and, two of the other components: (1) <i>A.fumigatus</i> -specific IgG >27mgA/L; (2) chest radiograph favoring ABPA; (3) eosinophil count >500 cells/μL | 88.5 (80.2-94.6) | 99.1 (98.0-99.8) |
| <b>Newly evaluated criteria (with certain modifications of the existing ISHAM criteria)</b>                                                                                                                                                                                                                                                                                                |                  |                  |
| <b>Criteria 1:</b> Presence of all of the following: (1) asthma; (2) <i>A.fumigatus</i> -specific IgE >0.35 KUA/L; (3) serum total IgE levels >500 IU/mL; and, two of the following: (1) <i>A.fumigatus</i> -specific IgG >27mgA/L; (2) bronchiectasis on CT chest; (3) eosinophil count >500 cells/μL                                                                                     | 100 (96.6-100)   | 100 (100-100)    |
| <b>Criteria 2:</b> Presence of all of the following: (1) asthma; (2) <i>A.fumigatus</i> -specific IgE >0.35 KUA/L; (3) serum total IgE levels >1000 IU/mL; and, three of the following: (1) <i>A.fumigatus</i> -specific IgG >27mgA/L; (2) bronchiectasis on CT chest; (3) eosinophil count >500 cells/μL; (4) mucus impaction on CT chest                                                 | 80.2 (71.3-88.1) | 100 (100-100)    |
| <b>Criteria 3:</b> Presence of all of the following: (1) asthma; (2) <i>A.fumigatus</i> -specific IgE >0.35 KUA/L; (3) serum total IgE levels >1000 IU/mL; and, two of the following: (1) <i>A.fumigatus</i> -specific IgG >27mgA/L; (2) bronchiectasis on CT chest; (3) eosinophil count >500 cells/μL; (4) mucus impaction on CT chest                                                   | 100 (100-100)    | 98.9 (97.8-100)  |

Confidence intervals correspond to 2.5–97.5% bootstrap confidence intervals obtained by bootstrapping 5000 samples

CT: computed tomography; ISHAM: international society for human and animal mycology

**a:** This model was similar to Rosenberg-Patterson criteria, except that *A.fumigatus*-specific IgG levels >27 mgA/L replaced precipitating antibodies against *A.fumigatus* and *Aspergillus* sensitization was described by skin test or *A.fumigatus* specific IgE

**b:** Same as ISHAM criteria (Table 1), except that precipitins were not used

**Criteria 1:** Similar to ISHAM criteria except that IgE cut-off was reduced to 500 IU/mL, AST was removed, and CT evidence of bronchiectasis replaced chest radiograph. Precipitins were not used

**Criteria 2:** Similar to criteria 1, except IgE cut-off was retained at 1000 IU/mL, and the presence of mucus impaction on CT thorax was added to “other criteria” and at least three out of four of the “other criteria” had to be present for diagnosing ABPA

**Criteria 3:** Similar to criteria 2, except that at least two out of four of the “other criteria” had to be present for diagnosing ABPA

The p value for goodness-of-fit for the models is 0.03, respectively, indicating that the model does not fit

**Table 5:** Summary of the 108 subjects classified as ABPA by any of the three ABPA diagnostic

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| Criteria                  | MDT, Not ABPA (n=2) |        | MDT, ABPA (n=106) |        | Correct classification rate (Efficiency), 95% CI |
|---------------------------|---------------------|--------|-------------------|--------|--------------------------------------------------|
|                           | Present             | Absent | Present           | Absent |                                                  |
| Patterson criteria        | 0                   | 2      | 86                | 20     | 81.5, 72.9-88.3                                  |
| ISHAM criteria            | 2                   | 0      | 87                | 19     | 80.6, 71.8-87.5                                  |
| Modified ISHAM criteria 1 | 0                   | 2      | 102               | 4      | 96.3, 90.8-98.9                                  |

CI: confidence intervals; MDT: multidisciplinary team

**Legend to Figure 1:**

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Patterson (white circle), ISHAM (gray), and the modified ISHAM criteria 1 (blue)

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Table E1: Frequency of permutations ( $2^5=32$ ) of the 5 different criteria evaluated in the two populations used in our study

| Permutations | Criteria<br>1 | Criteria<br>2 | Criteria<br>3 | Criteria<br>4 | Criteria<br>5 | Frequency in<br>mild/moderate<br>asthma | Frequency in<br>severe asthma |
|--------------|---------------|---------------|---------------|---------------|---------------|-----------------------------------------|-------------------------------|
| 1            | 0             | 0             | 0             | 0             | 0             | 275                                     | 159                           |
| 2            | 1             | 0             | 0             | 0             | 0             | 2                                       | 2                             |
| 3            | 0             | 1             | 0             | 0             | 0             | 2                                       | 1                             |
| 4            | 1             | 1             | 0             | 0             | 0             | 1                                       | 0                             |
| 5            | 0             | 0             | 1             | 0             | 0             | 0                                       | 0                             |
| 6            | 1             | 0             | 1             | 0             | 0             | 0                                       | 0                             |
| 7            | 0             | 1             | 1             | 0             | 0             | 0                                       | 0                             |
| 8            | 1             | 1             | 1             | 0             | 0             | 0                                       | 0                             |
| 9            | 0             | 0             | 0             | 1             | 0             | 0                                       | 0                             |
| 10           | 1             | 0             | 0             | 1             | 0             | 0                                       | 0                             |
| 11           | 0             | 1             | 0             | 1             | 0             | 0                                       | 0                             |
| 12           | 1             | 1             | 0             | 1             | 0             | 0                                       | 0                             |
| 13           | 0             | 0             | 1             | 1             | 0             | 0                                       | 0                             |
| 14           | 1             | 0             | 1             | 1             | 0             | 0                                       | 0                             |
| 15           | 0             | 1             | 1             | 1             | 0             | 0                                       | 0                             |
| 16           | 1             | 1             | 1             | 1             | 0             | 0                                       | 0                             |
| 17           | 0             | 0             | 0             | 0             | 1             | 2                                       | 0                             |
| 18           | 1             | 0             | 0             | 0             | 1             | 0                                       | 3                             |
| 19           | 0             | 1             | 0             | 0             | 1             | 0                                       | 0                             |
| 20           | 1             | 1             | 0             | 0             | 1             | 0                                       | 0                             |
| 21           | 0             | 0             | 1             | 0             | 1             | 2                                       | 0                             |
| 22           | 1             | 0             | 1             | 0             | 1             | 2                                       | 1                             |
| 23           | 0             | 1             | 1             | 0             | 1             | 5                                       | 5                             |
| 24           | 1             | 1             | 1             | 0             | 1             | 3                                       | 1                             |
| 25           | 0             | 0             | 0             | 1             | 1             | 0                                       | 0                             |
| 26           | 1             | 0             | 0             | 1             | 1             | 0                                       | 0                             |
| 27           | 0             | 1             | 0             | 1             | 1             | 0                                       | 0                             |
| 28           | 1             | 1             | 0             | 1             | 1             | 0                                       | 0                             |
| 29           | 0             | 0             | 1             | 1             | 1             | 0                                       | 1                             |
| 30           | 1             | 0             | 1             | 1             | 1             | 2                                       | 3                             |
| 31           | 0             | 1             | 1             | 1             | 1             | 2                                       | 3                             |
| 32           | 1             | 1             | 1             | 1             | 1             | 40                                      | 26                            |

**Criteria 1** <sup>a</sup>**Patterson criteria:** asthma, chest radiographic infiltrates, type I AST positivity, serum total IgE >417 IU/mL, *A.fumigatus*-specific IgG >27 mgA/L, eosinophil count >1000 cells/ $\mu$ L, bronchiectasis, *A.fumigatus*-specific IgE >0.35 kUA/L (presence of any six of the above)

**Criteria 2** <sup>b</sup>**ISHAM criteria:** Presence of all of the following: (1) asthma; (2) positive type I skin reaction to *A.fumigatus* or *A.fumigatus*-specific IgE >0.35 KUA/L; (3) serum total IgE levels >1000 IU/mL; and, two of the other components: (1) *A.fumigatus*-specific IgG >27mgA/L; (2) chest radiograph favoring ABPA; (3) eosinophil count >500 cells/ $\mu$ L

**Criteria 3** **Modification of ISHAM criteria 1:** Presence of all of the following: (1) asthma; (2) *A.fumigatus*-specific IgE >0.35 KUA/L; (3) serum total IgE levels >500 IU/mL; and, two of the following: (1) *A.fumigatus*-specific IgG >27mgA/L; (2) bronchiectasis on CT chest; (3) eosinophil count >500

|                   |                                                                                                                                                                                                                                                                                                                                                                        |
|-------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                   | cells/ $\mu$ L                                                                                                                                                                                                                                                                                                                                                         |
| <b>Criteria 4</b> | <b>Modification of ISHAM criteria 2:</b> Presence of all of the following: (1) asthma; (2) <i>A.fumigatus</i> -specific IgE >0.35 KUA/L; (3) serum total IgE levels >1000 IU/mL; and, three of the following: (1) <i>A.fumigatus</i> -specific IgG >27mgA/L; (2) bronchiectasis on CT chest; (3) eosinophil count >500 cells/ $\mu$ L; (4) mucus impaction on CT chest |
| <b>Criteria 5</b> | <b>Modification of ISHAM criteria 3:</b> Presence of all of the following: (1) asthma; (2) <i>A.fumigatus</i> -specific IgE >0.35 KUA/L; (3) serum total IgE levels >1000 IU/mL; and, two of the following: (1) <i>A.fumigatus</i> -specific IgG >27mgA/L; (2) bronchiectasis on CT chest; (3) eosinophil count >500 cells/ $\mu$ L; (4) mucus impaction on CT chest   |

