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Diagnosis of Allergic Bronchopulmonary Aspergillosis in patients with persistent allergic asthma using three different diagnostic algorithms

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AUTHORS CONTRIBUTION

V.M. and S. S. contributed to acquisition of data, draft the article and agrees to be accountable for all aspects of the work related to its accuracy or integrity. S.A.M, M. S. M., M. P., M. H., P. M. and Z. P. contributed to acquisition of clinical data, agrees to be accountable for all aspects of the work related to its accuracy or integrity and reviewed the manuscript critically for important intellectual content. J. Y. C and N. B. contributed to analysis and interpretation of data and agrees to be accountable for all aspects of the work related to its accuracy or integrity. M.T.H. and S. S. made substantial contributions to conception and design of the study, given final approval of the version to be published and agrees to be accountable for all aspects of the work related to its accuracy or integrity.

ETHICAL STATEMENT

The study was approved by the Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran (Code: IR.MAZUMS.REC.94-2060) and informed consent were filled out by all study population.

CONFLICT OF INTEREST

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Abstract

Background: Allergic bronchopulmonary aspergillosis (ABPA) has been reported in various degrees among patients with persistent allergic asthma (PAA). Currently there is no gold standard approach for diagnosis of ABPA.

Objectives: In the current study, we aimed the evaluation of three different mainly used algorithms as Rosenberg & Patterson (A), ISHAM Working Group (B) and Greenberger (C) for diagnosis of ABPA in two hundred patients with underlying PAA.

Methods: All patients were evaluated using *Aspergillus* skin prick test (SPTAf), *Aspergillus*-specific IgE (sIgEAf) and IgG (sIgGAf), total IgE (tIgE), pulmonary function tests, radiological findings and peripheral blood eosinophil count. The prevalence rate of ABPA in PAA patients was estimated by three diagnostic criteria. We used Latent Class Analysis for the evaluation of different diagnostic parameters in different applied ABPA diagnostic algorithms.

Results: *Aspergillus* sensitization was observed in 30 (15.0%) patients. According to algorithms A, B and C, 9 (4.5%), 6 (3.0%) and 11 (5.5%) of patients were diagnosed with ABPA, respectively. The sensitivity and specificity of criteria B and C were (55.6% and 99.5%) and (100.0% and 98.9%) respectively. sIgEAf and sIgGAf showed the high significant sensitivity. The performance of algorithm A, in terms of sensitivity and specificity was somewhat better than algorithm B.

Conclusion: Our study demonstrated that the sensitivity of different diagnostic algorithms could change the prevalence rate of ABPA. We also found that all of three criteria resulted an adequate specificity for ABPA diagnosis. A consensus patterns combining elements of all three criteria may warrant a better diagnostic algorithm.

Keywords

Persistent allergic asthma; Allergic bronchopulmonary aspergillosis (ABPA); *Aspergillus* sensitization; Diagnostic algorithm; prevalence rate

1. Introduction

Aspergillus species are one of the most frequent fungi in both indoor and outdoor environments. ¹ Following inhalation of aspergilli conidia by susceptible hosts, a wide variety of clinical presentations can be seen ², chronic pulmonary aspergillosis, invasive pulmonary aspergillosis and invasive bronchial aspergillosis. ² Various type of allergic aspergillosis may also be observed in atopic hosts, including severe asthma with fungal sensitization, ABPA, *Aspergillus*-induced asthma, and allergic *Aspergillus* rhinosinusitis. ³

ABPA is the most common form of allergic aspergillosis among patients with persistent asthma and cystic fibrosis (CF) with an estimated prevalence rate of 1% to 3.5% of all

asthmatics patients, 7% to 14% of poorly controlled asthma and 7% to 9% of CF patients worldwide.⁴

ABPA is clinically diagnosed by wheezing and reduced lung function, worsening asthma, lung infiltration, mucosal airway plugging and bronchiectasis.⁵ Type of ABPA can also be determined by radiological findings. Patients who fulfill the criteria for serological tests only, are labeled as ABPA-Seropositive (ABPA-S), and those with positive central bronchiectasis are considered as ABPA-central bronchiectasis (ABPA-CB).⁶ An undiagnosed and untreated ABPA can cause progressive pneumonia, bronchiectasis, chronic production of sputum, loss of lung function, honeycomb lung fibrosis or cavitary lung disease and inappropriate control of asthma and eventual respiratory failure.⁵ Therefore, timely diagnosis and treatment of ABPA is important to prevent progressive and irreversible damage of the lung due to bronchiectasis.⁵ Although several attempts have been made to establish a standardized method, there has not been a uniform criteria for diagnosis of ABPA yet.⁶ The criteria proposed by Rosenberg et al.^{7,8}, the International Society for Human and Animal Mycology (ISHAM) Working Group⁹ and Greenberger et al.^{10,11} are three main employed algorithms used.

Due to lack of a gold standard methodology, the prevalence rate of ABPA in patients with asthma is not rectified properly in Iran. Therefore, in this present study, we evaluated the diagnosis of ABPA in patients with persistent allergic asthma (PAA) using three different diagnostic algorithms.

2. MATERIALS AND METHODS

2.1. Patients

During January 2016 to February 2018, all patients with a diagnosis of moderate to severe PAA (according to the Global Initiative for Asthma (GINA 2015) guidelines)¹², who admitted to the Hospital of National Reference Center for Tuberculosis and Pulmonary Diseases of Iran, Tehran, were included in this study. A detailed demographic information, history of used medication and symptoms were collected. Patients with aged ≤ 18 years old, mild asthma, pregnancy, smoking, non-persistent allergic asthma, other widespread lung diseases such as tuberculosis (TB), cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), antifungal therapy in last two months and malignancy were excluded from the study. The study was approved by the Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran (Code: IR.MAZUMS.REC.94–2060) and informed consent were filled out by all study population.

2.2. Assessment of atopy and asthma severity

The severity of asthma was evaluated retrospectively by the level of treatment required to control asthma symptoms and exacerbation. Based on GINA 2015 guidelines, moderate and severe asthma are two phenotype of asthma that is well controlled by Step 3 treatment (e.g. low dose inhaled corticosteroids (ICS)/ long-acting beta-agonists (LABA)) and Step 4 or 5 treatment (e.g. high-dose ICS/LABA), respectively.¹² The atopy was determined by skin

prick testing with various types of allergens including pollens (grass, tree and weed), food allergens and different fungi, and a positive history of atopy in family.

2.3. Clinical and paraclinical testing

All study subjects were evaluated by high resolution computed tomography (HRCT) and/or chest X-Ray for any obvious evidence of bronchiectasis, centrilobular nodules/ mucoid impaction/ hyper-dense mucus by focusing on bronchiectasis. The patients also were screened for serum specific IgE against *Aspergillus fumigatus* (sIgE_{Af}) using HYTEC 288 (HYCOR Biomedical, Hannover, Germany), serum specific IgG against *A. fumigatus* (sIgG_{Af}) (IBL ELISA Kit, Hamburg, Germany), total IgE (tIgE) levels (Genesis, Omega Diagnostic Group, UK) and SPT with commercial *Aspergillus fumigatus* extracts (SPT_{Af}) (Alk-Abello, Lincoln Diagnostics, Dallas, Tx, USA). sIgE_{Af} level greater than 0.35 KUA/L and sIgG_{Af} values > 12 U/ml were considered as positive result. Total IgE levels were separately represented in > 1000 IU/ml and > 417 IU/ml.

The peripheral blood eosinophil count was also reported for all patients. Eosinophilia was considered with a count of >500 eosinophils/ μ l.⁹

2.4. Mycological examinations of sputum

At least two spontaneous sputum samples were taken from all patients who were included in the study. Each collected sputum samples were homogenized with 0.5% pancreatin and then centrifuged at 3000 rpm for 10 min. The obtained sediment was vortexed for 30 s and then divided into two samples; one for fungal culture and the other for direct microscopic examination mounted with 20% potassium hydroxide. The sample for fungal culture was inoculated into Malt extract agar (MEA, QUELAB, Canada). The cultured plates were kept at 27–30°C for 5 – 7 days. The grown colonies were independently sub-cultured onto MEA and were then identified at species level by macroscopic and microscopic characteristics followed by PCR sequencing of *Aspergillus* β -tubulin.

2.5. ABPA diagnostic criteria

In order to determine the prevalence rate of ABPA, the algorithms proposed by Rosenberg et al (A)^{7,8}, the International Society for Human and Animal Mycology (ISHAM) Working Group (B)⁹ and Greenberger et al (C)^{10,11} were employed for ABPA diagnosis (table 1). The algorithms A was hypothetically considered as gold standard for ABPA diagnosis.

2.6. Statistical analysis

Data were analysed using the software SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Kappa weighted test was used to find concordance between criteria A, criteria B and criteria C. The quantitative variables were presented as the mean $\pm$ standard deviation ($\pm$ SD) or the mean (range) and number of study population in percentage (%). The sensitivity, specificity and accuracy of three criteria were calculated. The agreement between three applied algorithms was evaluated by Kappa test.¹³ We used the R version (R-4.0.2) and TAGS software for performing Latent Class Analysis (LCA) to estimate the accuracy of two created model of ABPA diagnostic algorithms.¹⁴ To perform LCA, we created two models of ABPA diagnostic algorithms (Table 5). The relationship between observed variables (in the current

study, the various diagnostic parameters) evaluate by LCA, in which an invisible or hidden variable inaccurately estimates the actual disease status (ABPA). Next, the maximum likelihood estimate for different Models is calculated by the LCA to obtain their sensitivity and specificity with 95% CI (bootstrap derived from 10000 samples). Model 1 is similar to Algorithm A and somewhat to Algorithm C and Model 2 is designed similar to Algorithm B.

3. Result

Overall, 111 (55.5%) out of 200 patients with PAA were females. The mean age was 45.8 ± 13.03 years with an asthma duration (geometric mean) of 10.04 ± 9.9 years. The history of cough with sputum, shortness in breathing, wheeze and atopy were also recorded for all patients. The rhinosinusitis and nasal polypsis were observed in 108 (54.0%) and 66 (33%) of the cases, respectively. The demographic, clinical and paraclinical information of the study population summarised in Table 2.

Elevated tIgE values > 1000 IU/ml and > 417 IU/ml were observed in 6 (3.0%) and 65 (32.5%) of patients, respectively. Nevertheless, 11 out of 65 patients with tIgE levels > 417 IU/ml, were also positive for SPTA*f*, raised sIgEA*f* ≥ 0.35 KUA/L and sIgGA*f*. The geometric mean of sIgEA*f* and sIgGA*f* were 1.5 ± 6.0 (0.1 – 61.3) IU/ml and 35.2 ± 42.6 (0 – 184) IU/ml, respectively. The radiological findings revealed the evidence of bronchiectasis, opacity, transient infiltration and mucus impaction in 28 (14.0%), 11 (5.5%), 6 (3.0%) and 4 (2.0%) of asthmatic patients, respectively. Overall, 8 (4.0%) patients with asthma showed bronchiectasis suggestive of ABPA-CB. The bronchiectasis observed in 20 patients with asthma without ABPA.

In addition, 17 (8.5%) and 58 (29.0%) of study subjects showed evidence of peripheral eosinophil count > 1000 cell/ μ l and above 500 cell/ μ l, respectively (Table 2).

3.1. Assessment of three different diagnostic algorithms for ABPA

Taken together *Aspergillus* sensitization (patients with positive sIgEA*f* and/ or SPTA*f*) was observed in 30 (15.0%) patients with PAA of which 27 (90.0%) were positive for SPTA*f*. According to algorithms A, B and C, 9 (4.5%), 6 (3.0%) and 11 (5.5%) of patients met the diagnosis of ABPA, respectively. Using algorithm B, 3 patients (1.5%) were also classified as probable ABPA. These patients were positive for SPTA*f*, raised sIgEA*f* and sIgGA*f* levels, peripheral eosinophil count greater than 500 cell/ μ l and central bronchiectasis with a tIgE lower than 1000 IU/ml. Out of 11 patients with ABPA, 8 cases showed the evidence of bronchiectasis in HRCT findings. According to employed algorithms, ABPA patients showed different rate of ABPA-CB or ABPA-S (table 3).

There was a high positivity concordance among all patients diagnosed with ABPA relying on SPTA*f* and sIgEA*f* results (Table 3). The sensitivity and specificity by algorithm B, were 55.6% (21.2% to 86.3%, 95% CI) and 99.5% (97.1% to 99.9%, 95% CI), respectively with an accuracy of 97.5% (94.3% to 99.2%, 95% CI). The sensitivity and specificity of algorithm C were 100.0% (66.4% to 100.0%, 95% CI) and 99.0% (96.3% to 99.9%, 95% CI), respectively with accuracy of 99.0% (96.4% to 99.9%, 95% CI) (Table 4). The measures of agreement between algorithms A and B, A and C, and B and C in terms of their ABPA

prevalence rate was moderate (kappa value = 0.654), good (kappa value = 0.895) and moderate (kappa value = 0.694), respectively.

Additionally, out of 200 sputum collected samples from patients with PAA, 110 (55.5%) patients were positive for fungal growth. 113 *Aspergillus* relevant colonies were isolated from 30 patients (15.0%). The identified *Aspergillus* species were *A. fumigatus* (4, 44.4%), *A. tubingensis* (3, 33.3%), *A. terreus* (1, 11.1%) and *A. flavus* (1, 11.1%). The complete list of *Aspergillus* species isolated from ABPA patients are summarized in Table 3.

3.2. Performance of various criteria using LCA

The evaluated sensitivity and specificity of various parameters for ABPA diagnosis using LCA are presented in Table 5 using two different form. The goodness-of-fit test for contingent independence was passable ($p = 0.99$) and the remaining correlations between the parameters were indeliberately distributed at about zero. In both of the algorithms, sIgE_{Af} (>0.35 kUA/L) showed the high significant sensitivity value (mean = 99.0%). The other parameter that showed high sensitivity value was positive sIgG_{Af} (>26.9 IU/mL) with good specificity. The performance of algorithm A, in terms of sensitivity and specificity was somewhat better than algorithm B.

4. Discussion

In this present study, the prevalence rate of ABPA was 4.5%, 3.0% and 5.5% in patients with PAA based on algorithms A, B and C, respectively. In comparison, several previous studies have also reported a prevalence rate of 16%¹⁵ (using criteria A), 7.5%¹⁶ (using algorithm A) and 27.2%¹⁷ (using the algorithm proposed by Agarwal et al) of ABPA in asthmatic patients. A meta-analysis by Agarwal et al.¹⁸, the reported rate of ABPA prevalence in patients with asthma ranged from 2% to 32%, with a pooled prevalence of 12.9%. The prevalence rate of ABPA in industrialized and developing countries were also reported as 25.6% and 12.7%, respectively. However our reported rates of ABPA using different algorithms were in range of previous several studies, this present study showed that using different algorithms for ABPA diagnosis may result various prevalence rate of ABPA.

In contrary to other applied algorithms, in algorithm B, a higher cut-off value for tIgE (≥ 1000 IU/ml) was considered as one of the main parameter for ABPA diagnosis, which resulted a lower sensitivity (55.6%) compared to algorithm A (100.0%) as well as criteria C (100.0%). This may causes a decreased prevalence rate of ABPA (3.0%) using algorithm B in comparison with two others employed algorithms, in this present study. Using algorithms B, three patients with a lower tIgE (≥ 417 IU/mL - <1000 IU/ml) were also classified as probable ABPA. With adding these probable ABPA cases, the measures of agreement between algorithms B and A, algorithms B and C as well as the sensitivity, specificity and accuracy of algorithm B increased in comparison to algorithm A as the gold standard (Table 4). Of our 11 confirmed ABPA patients, algorithms A and C agreed to identify nine ABPA patients however two other ABPA patients (P8 and P10) diagnosed by algorithm C, met only 5 major parameters of algorithm A. These two cases were negative for bronchiectasis and presence of transient pulmonary infiltrates (fleeting shadows) or fixed pulmonary opacities as well as with eosinophil counts below 1000 cell/ μ l. Therefore, by using algorithm A,

ABPA was not approved in these cases (Table 3). On the other hand, out of 9 ABPA patients, the agreement between algorithms A and B (patients with probable and proven ABPA) in ABPA diagnosis was in 8 cases. Our results are in agreement with Agarwal et al.¹⁹ study, revealed that the algorithm A (employing at least six main parameters) with a high sensitivity, specificity, accuracy and validity can be considered as an appropriate diagnostic criteria for ABPA. Several studies^{17, 19} have applied only five parameters of algorithm A for the diagnosis of ABPA which resulted different rates of ABPA. It is also suggested that the specificity of tIgE for ABPA detection is poor therefore, employing of parameter tIgE ≥ 1000 IU/ml was recommended in ABPA diagnostic criteria.¹⁹

In our study, 32.5% of patients with PAA showed tIgE ≥ 417 and six patients (3.0%) all with ABPA had tIgE greater than 1000 IU/mL with a mean of 1187.5 IU/mL. A higher rate of tIgE greater than 1000 IU/mL (10.43%) in comparison with our study was reported by Lou et al.²⁰ in patients with asthma. Similarly, some previous studies have also shown a higher level of tIgE in ABPA patients.^{17, 21}

In this present study, 22 (11.0%) patients with PAA showed sIgE Af values > 0.35 kUA/L of which 50.0% cases with ABPA diagnosis. A report by Lou et al.²⁰ showed a higher rate of sIgE Af (22.2%) in asthmatic patients. However our finding was in line with Sarkar et al.²² and Prasad et al.²³ The sIgE Af value as one of the most sensitive and specific parameter in ABPA diagnostic criteria along with *Aspergillus* skin test make algorithm A as the most widely used criteria for ABPA diagnosis.¹⁹

Typically, the first choice in screening of patients for ABPA diagnosis is SPT Af . In our study, the positivity of SPT Af was 13.5% in patients with PAA. The positivity rate of SPT Af was reported from 16.0% to 43.0% in asthma patients. Our positivity rate of SPT Af was lower than reported rate by Hendrick et al. (16.0%)²⁴, Eaton et al (18.4%)²⁵, Maurya et al (28.5%)¹⁶ and Agarwal et al¹⁷ (39.5%) studies. However in our study we observed a higher prevalence of SPT Af positivity than those of reported by Ma et al²⁶ (5.5%) and Mathur and Mathur²⁷ (9.0%) studies. Several factors may affect the positivity ratio of SPT Af prevalence, including the use of various fungal allergens or exclusive use of *A. fumigatus* antigens, using different diagnostic criteria for interpreting of a positive skin test and difference in the type of skin test (SPT or intradermal skin test).²⁸ In contrast to Agarwal et al.¹⁷, our finding showed a concordance between positive results of SPT Af and sIgE Af in all eleven diagnosed ABPA patients. Our finding in line with previous studies have also shown that using sIgE Af along with SPT Af increase the accuracy of Rosenberg & Patterson criteria (algorithm A) for ABPA diagnosis compared with other applied criteria.^{7,8,19, 29}

Due to lack of an approved cut-off value of sIgG Af for Iranian population and high exposure with *Aspergillus* species, we also observed a higher positivity rate of sIgG Af (57.0%) in PAA patients. In our study, 9 of 11 (81.8%) patients with ABPA showed *Aspergillus* species growth in sputum samples. Several studies reported a lower rate of positivity of sputum cultures for *Aspergillus* as 40 to 60%.^{30, 31} However the isolation of *Aspergillus* in sputum culture was not considered as the major criteria for diagnosis of ABPA, it is suggested that the identification of these organisms can be helpful in antifungals susceptibility tests.⁵ Radin et al.³¹ showed a positive relationship between the level of *Aspergillus* exposure and

exacerbation in ABPA. In our study, among 30 patients with *Aspergillus*-positive sputum culture, 13.3% had $sIgE_{Af} > 0.35$ kUA/L but no ABPA. In the present study, all patients with ABPA showed the positive results in SPT_{Af} and $sIgE_{Af}$ however, the sputum culture of patients yielded different species of *Aspergillus*. This result may due to shared antigen component and cross reactivity among different species of *Aspergillus*. Recently, Kuwabara et al.³² demonstrated a high cross-reactivity of specific IgE and IgG among different species of *Aspergillus*.

According to analysis by LCA statistical modeling method, in this present study, we found that none of the diagnostic parameters have 100% sensitivity and specificity in identifying patients with ABPA, alone. Therefore, using a comprehensive diagnostic criteria consisting of several parameters can help to receive an accurate diagnosis of ABPA. Our findings, in line with Agarwal et al.¹⁹ study and in contrast to the Saxena et al.³³ study, showed that algorithm A have a better diagnostic performance compared to algorithm B. It should be noted, however, in Saxena et al.³³ study, Algorithm B had a slightly better diagnostic performance than Algorithm A in terms of sensitivity and specificity. In concordance with Agarwal et al.¹⁹ and Saxena et al findings³³, this present study revealed that $sIgE_{Af}$ was the most sensitive parameter in diagnosis of ABPA. In our study, $sIgG_{Af}$ as a functional arm of diagnostic parameter showed a moderate specificity and high sensitivity which can play a useful role in identifying patients with ABPA. SPT_{Af} was considered as one of the most important screening tools in asthma patients for ABPA, however, it has been suggested that using only a skin test to evaluate the ABPA will result in a loss of 6 to 12% of the disease.¹⁹ On the other hand, $sIgE_{Af}$ parameter was potentially most sensitive test in our study. Therefore, it could be suggested that simultaneous performance of $sIgE_{Af}$ and SPT_{Af} tests can lead to better results for identifying more patients with ABPA. Several studies have reported a rising level of tIgE in many individuals with asthma without ABPA,^{34,35} however our findings indicate despite the high specificity of tIgE > 1000 IU/mL (99.5%), the sensitivity of this parameter is poor (55.6%) and observed only in a limited number of patients with asthma regardless of ABPA. Therefore, these finding may predict a low false-positive rate of ABPA in patients with asthma, however the diagnosis of ABPA in many patients may be missed because of its poor sensitivity. In contrast, our finding also showed a high sensitivity and an appropriate specificity for tIgE ≥ 417 IU/mL which can serve as a useful tool for ABPA screening and follow up of ABPA in asthma patients.

5. Conclusion

Our study demonstrated that the sensitivity of different diagnostic algorithms could change the prevalence rate of ABPA. We also found that all of three criteria resulted an adequate specificity for ABPA diagnosis. A consensus patterns combining elements of all three criteria may warrant a better diagnostic algorithm. Our findings have also shown the algorithm A by using six parameters, as the most accurate and sensitive diagnostic criteria for ABPA. According to our results we can also recommend a periodic screening of PAA patients by SPT_{Af} along with $sIgE_{Af}$ for the diagnosis of ABPA.

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Table 1.

Various diagnostic algorithms for allergic bronchopulmonary aspergillosis

Rosenberg et al. ^{7,8} (algorithm A)	ISHAM Working Group ⁹ (algorithm B)	Greenberger et al. ^{10, 11} (algorithm C)
Major criteria	Predisposing conditions	Minimum essential criteria
1. Asthma	Bronchial asthma or Cystic fibrosis	1. Asthma or Cystic Fibrosis
2. Immediate cutaneous hyper reactivity on <i>Aspergillus</i> skin test (type I reaction)	Obligatory criteria (both should be present)	2. Immediate cutaneous reactivity on skin-prick testing
3. Elevated serum <i>A. fumigatus</i> specific IgE (>0.35 kUA/L)	1. Type I <i>Aspergillus</i> skin test positive (immediate cutaneous hypersensitivity to <i>Aspergillus</i> antigen) Or 2. elevated IgE levels against <i>A. fumigatus</i> >0.35 KUA/L	3. Total serum IgE >1000 ng/mL (417 IU/mL)
4. Precipitating antibodies in serum against <i>A. fumigatus</i> or Positive specific IgG against <i>A. fumigatus</i>	3. Elevated total IgE levels (>1,000 IU/mL)*	Plus one or both of the following
5. Elevated serum total IgE (≥417 IU/mL)	Other criteria (at least two of three)	1. Elevated serum IgE and IgG levels to <i>A. fumigatus</i> (at least twice asthma controls)
6. Eosinophilia (>1000 cells/μL)	1. Presence of precipitating or IgG antibodies against <i>A. fumigatus</i> in serum	2. Proximal (central) bronchiectasis on radiograph (inner two-thirds of lung on CT)
7. Central bronchiectasis	2. Radiographic pulmonary opacities consistent with ABPA	
8. Presence of transient pulmonary infiltrates (fleeting shadows) or fixed pulmonary opacities	3. Total eosinophil count >500 cells/μL in steroid naïve patients (may be historical)	*1 IU/mL = 1 kU/L = 2.4 ng/mL
Minor criteria	The chest radiographic features consistent with ABPA may be transient (i.e. consolidation, nodules, tram-track opacities, toothpaste/finger-in-glove opacities, fleeting opacities) or permanent (i.e. parallel line and ring shadows, bronchiectasis and pleuropulmonary fibrosis).	
1. Positive sputum culture for <i>Aspergillus</i> species	*If the patient meets all other criteria, an IgE value < 1000 IU/mL may be acceptable	
2. Expectoration of golden brownish sputum plugs		
3. Arthus reactivity (late skin reactivity) to <i>Aspergillus</i> antigen		
*ABPA was confirmed if patient met 6 out of 8 major criteria and one or two minor criteria	**ABPA was confirmed if patient met all Obligatory criteria and at least 2 out of 3 other criteria	

Table 2:

Demographic and laboratory findings of patients with persistent allergic asthma

		Patients (n=200)
Age (year) Mean($\pm$ SD)		45.8 $\pm$ 13.03
Gender		
Men n (%)		89 (44.5)
Women n (%)		111 (55.5)
Severity of asthma		
Moderate n (%)		103 (51.5)
Severe n (%)		97 (48.5)
Duration of asthma (In years) (mean $\pm$ SD)		10.04 $\pm$ 9.9
Rhinosinusitis n(%)		108 (54)
Nasal polyps n(%)		66 (33)
Spirometry		
FEV1 < 80% n (%)		136 (68)
FVC < 80% n (%)		120 (60)
SPTAf(positive %)		27 (13.5)
Immunological test		
sIgEAf(KUA/L) $\geq$ 0.35 (KUA/L)	Mean($\pm$ SD)	1.5 ($\pm$ 6.0)
	n (%)	22 (11)
sIgGAf(IU/ml)	Mean($\pm$ SD)	35.2 ($\pm$ 42.6)
> 12 (IU/ml)	n (%)	114 (57)
tIgE (IU/ml)	Mean($\pm$ SD)	316.2 ($\pm$ 305.4)
$\geq$ 417 IU/ml	n (%)	65 (32.5)
$\geq$ 1000 IU/ml	n (%)	6 (3.0)
Radiological test		
Bronchiectasis n (%)		28 (14.0)
Transient infiltration n (%)		6 (3.0)
Mucus impaction n (%)		4 (2.0)
Haematological test		
Eosinophil count cell/ μ l n (%)	> 500 cell/ μ l	58 (29.0)
	> 1000 cell/ μ l	17 (8.5)

FVC; forced vital capacity, FEV1; forced expiratory volume in one second, SPTAf; skin prick test with *A. fumigatus*, sIgEAf; serum immunoglobulin E against *A. fumigatus*, sIgGAf; serum immunoglobulin G against *A. fumigatus*, tIgE; total immunoglobulin E

Table 3:

Characteristics of 11 patients with ABPA

Variables	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Gender	F	F	F	M	M	F	M	M	M	M	M
Age(year)	39	40	45	23	35	42	34	36	78	50	46
Moderate asthma	-	-	+	-	-	+	-	-	-	+	-
Severe asthma	+	+	-	+	+	-	+	+	+	-	+
Asthma duration	3	5	5	4	34	14	1	30	11	8	42
FVC%	54	87	81	78	81	90	56	59	49	89	55
FEV1%	52	86	78	65	46	87	71	53	30	75	29
Normal CT	-	-	-	-	-	+	-	+	-	+	-
Bronchiectasis	+	+	+	+	+	-	+	-	+	-	+
Transient infiltration	-	-	-	-	-	-	-	-	-	-	-
Mucus impaction	+	-	-	+	+	-	-	-	-	-	-
SDM	+	+	+	+	+	+	+	+	+	-	+
SFC	+	+	+	+	+	-	+	+	+	-	+
Sputum appearance	With brown plaque	Normal	With blackish plaque	With yellowish plaque	Normal	With golden brown plaque	With yellowish plaque	Normal	With black plaque	Normal	With brown plaque
<i>Aspergillus</i> species (Accession number)	<i>A. tubingensis</i> (MK012368)	<i>A. fumigatus</i> (MK012369)	<i>A. tubingensis</i> (MK012373)	<i>A. flavus</i> (MK012371)	<i>A. fumigatus</i> (MK012367)	Neg	<i>A. terreus</i> (MK012370)	<i>A. fumigatus</i> (MK012374)	<i>A. tubingensis</i> (MK012375)	Neg	<i>A. fumigatus</i> (MK012372)
SPTA _f	+	+	+	+	+	+	+	+	+	+	+
slgEa _f KU/L	0.36	31.8	14.8	1	61.3	19.9	0.82	3.82	3.29	0.42	27.19
slgGa _f IU/ml	47	54	114	109	165	37	150	129	72	34	100
tIgE IU/ml	1123	1155	575	919	1300	597	1197	1250	765	609	1100
Blood Eosinophil count cells/mL	1456	536	1487	1344	4565	1570	1150	684	514	140	608
ABPA by algorithm A	+	+	+	+	+	+	+	-	+	-	+

Variables	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
ABPA by algorithm B	+	+	+/-	+/-	+	-	+	+	+/-	-	+
ABPA by algorithm C	+	+	+	+	+	+	+	+	+	+	+
Total ABPA prevalence			algorithm A (4.5%)			algorithm B Proven (3.0%) Probable (1.5%)			algorithm C (5.5%)		
ABPA-CB prevalence			(4.0%)			Proven (2.5%) Probable (1.5%)			(4.0%)		
ABPA-S prevalence			(0.5%)			(0.5%)			(1.5%)		

P; patient, F; female, M; male, FVC; forced vital capacity, FEV1; forced expiratory volume in one second, SDM; sputum direct microscopy, SFC; sputum fungal culture, SPT/AF; skin prick test with *A. fumigatus*, sIgE/AF; serum immunoglobulin E against *A. fumigatus*, sIgG/AF; serum immunoglobulin G against *A. fumigatus*, tIgE; total immunoglobulin E, +/-; Probable ABPA

Table 4:

The sensitivity, specificity, accuracy and measure of agreement of different diagnostic algorithms compared with algorithm A

95% CI	algorithm B (proven ABPA)	algorithm B (probable and proven ABPA)	algorithm C
Sensitivity	55.6% (21.2 – 86.3)	88.9% (51.7 – 99.7)	100.0% (66.4 – 100.0)
Specificity	99.5% (97.1 – 99.9)	99.5% (97.1 – 99.9)	98.9% (96.3 – 99.9)
Accuracy	97.5% (94.3 – 99.2)	99.0% (96.4 – 99.9)	99.0% (96.4 – 99.9)
Measure of agreement (kappa value)	0.654	0.884	0.895

Table 5:

Sensitivity and specificity of various parameters for ABPA diagnosis using Latent Class Analysis

Model 1	Sensitivity	Specificity
Positive SPT <i>Af</i>	91.5 (87.8 – 96.5)	91.1 (88.9 – 95.7)
tIgE (≥ 417 IU/mL)	97.1 (96.2 – 98.8)	70.7 (64.6 – 79.3)
sIgE <i>Af</i> (>0.35 kUA/L)	98.7 (97.9 – 99.8)	93.2 (90.1 – 97.7)
sIgG <i>Af</i> >26.9 (IU/mL)	99.1 (98.9 – 99.6)	61.3 (54.6 – 72.5)
Total eosinophil count >1000 cells/mL	66.7 (61.4 – 74.5)	94.2 (90.9 – 98.3)
HRCT Central bronchiectasis	88.9 (84.5 – 93.7)	89.5 (87.3 – 93.4)
Model 2		
Positive <i>A. fumigatus</i> skin-prick testing	97.3 (96.4 – 98.9)	92.2 (89.7 – 96.6)
tIgE (>1000 IU/mL)	55.6 (48.3 – 65.8)	99.5 (99.3 – 99.9)
sIgE <i>Af</i> (>0.35 kUA/L)	99.2 (89.9 – 99.6)	73.8 (68.5 – 79.1)
sIgG <i>Af</i> >26.9 (IU/mL)	96.5 (95.9 – 97.4)	68.3 (64.5 – 73.9)
Total eosinophil count >1000 cells/mL	73.2 (68.7 – 79.6)	93.6 (90.7 – 97.4)
HRCT Central bronchiectasis	87.8 (83.2 – 92.4)	90.2 (88.3 – 95.4)

tIgE: total immunoglobulin E; SPT*Af*: skin prick test with *A. fumigatus*, sIgE*Af*: serum immunoglobulin E against *A. fumigatus*, sIgG*Af*: serum immunoglobulin G against *A. fumigatus*, tIgE; HRCT: high resolution computed tomography