



Review article

Serological diagnosis of allergic bronchopulmonary mycosis: Progress and challenges



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ABPM, allergic bronchopulmonary mycosis;

Abs, antibodies; ABPA, allergic

bronchopulmonary aspergillosis;

MnSOD, manganese superoxide dismutase;

AD, atopic dermatitis; MA, molecular-based

allergy; WHO/IUIS, World Health

Organization and International Union of

Immunological Societies; ABPC, allergic

bronchopulmonary candidiasis

ABSTRACT

Prompt diagnosis of allergic bronchopulmonary mycosis (ABPM) is an important clinical issue in preventing irreversible lung damage. Therefore, a good serological marker for the diagnosis of ABPM is desired in clinical practice.

The measurement of IgE antibody to crude *Aspergillus fumigatus* allergen is considered the first step in screening asthmatic patients for allergic bronchopulmonary aspergillosis (ABPA). However, presence of IgE to *A. fumigatus* does not always indicate genuine sensitization to *A. fumigatus* because of cross-reactivity between crude extracts from different fungal sources. The application of molecular-based allergy diagnosis can solve this problem. The specificity of testing can be greatly improved by measuring the IgE antibody to Asp f 1 and f 2, specific allergen components for genuine *A. fumigatus* allergy.

The problem of cross-reactivity between crude fungal extracts is also true for the identification of genuine causal fungi in each ABPM patient. Some patients with ABPM induced by fungi other than *Aspergillus* may be consistent with ABPA diagnostic criteria because current criteria depend on IgE/IgG reactivity to crude extracts. Accurate identification of genuine causal fungi for ABPM is of clinical importance, considering that clinical presentation, anti-fungal treatment strategies and disease prognosis can be influenced by different causal fungi. The diagnosis of causal fungi can be robustly validated by the confirmation of genuine sensitization to fungi after measuring IgE to specific allergen components, as well as repeated microbiological isolation of the fungi from their airway.

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Introduction

Allergic bronchopulmonary mycosis (ABPM) is a pulmonary hypersensitivity disease characterized by sensitization to fungi, recurrent transient radiographic infiltrate, peripheral and pulmonary eosinophilia, and bronchiectasis.^{1–5} The diagnosis of ABPM is an important clinical issue because ABPM may lead to ongoing decline in lung function, lung fibrosis, and early treatment is essential to prevent long-term tissue damage.⁶ The diagnosis of ABPM is not difficult if patients exhibit all diagnostic criteria of ABPM including central bronchiectasis and lung infiltration. However, ABPM should ideally be diagnosed before bronchiectasis

occurs, to prevent irreversible lung damage. Therefore, good serological markers for the early diagnosis of ABPM are desired. This review discusses the recent progress and challenges in serological diagnosis of ABPM.

Pathogenesis and causal fungal species of ABPM

The pathogenesis of ABPM is characterized by colonization of fungi in the airways and a strong humoral and cellular response to the fungi and its secreted proteolytic enzymes,^{7,8} resulting in increased levels of serum IgE and IgG antibodies (Abs) to these fungal allergens.⁹ A common feature of fungi which can induce ABPM includes thermotolerance,¹⁰ which enables these microbes to grow at human body temperature. *Aspergillus fumigatus* is the most common causal pathogen for ABPM, whereas *Aspergillus flavus*, *Aspergillus niger*,^{11–13} *Candida albicans*,^{14–16} *Bipolaris* spp.,¹⁷ and *Schizophyllum commune*^{18–23} are also reported to induce similar clinical conditions.

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The diagnostic criteria for ABPA and ABPM

There are no universally accepted set of criteria for the diagnosis of ABPM. However, it is generally accepted that the diagnosis of ABPM can be performed using the diagnostic criteria of allergic bronchopulmonary aspergillosis (ABPA) by replacing *A. fumigatus* with another fungus.^{4,5} ABPA is a subset of ABPM induced by *Aspergillus* species, first described in 1952 by Hinson *et al.*²⁴ As *A. fumigatus* is the most common fungal agent to cause ABPA, the diagnostic criteria for ABPA have been based on the patient's immunological reactivity to crude extracts of *A. fumigatus* in combination with radiological or clinical findings. Although its clinical presentations have been well described in the literature,^{3,25–28} there are no universally accepted diagnostic criteria for ABPA as yet.²⁹ The diagnostic criteria most widely accepted are those proposed by Rosenberg and Patterson,²⁵ which include 7 primary criteria. These include 1) asthma, 2) peripheral blood eosinophilia, 3) immediate skin reactivity to *Aspergillus* antigen, 4) precipitating Ab against *Aspergillus* antigen, 5) elevated total IgE, 6) history of pulmonary infiltrate, and 7) central bronchiectasis. Criteria 1), 3), 5) and “elevated serum IgE Ab to *A. fumigatus*” are also minimal essential criteria for seropositive ABPA, proposed by Greenberger.² The International Society for Human and Animal Mycology (ISHAM) working group has recently proposed new diagnostic criteria for ABPA,³ in which each criterion is divided into “obligatory” and “other” status. These newly proposed criteria also regard positive immediate cutaneous hypersensitivity to *Aspergillus* antigen or elevated IgE levels against *A. fumigatus* as “obligatory” criteria and the presence of precipitating or IgG Abs against *A. fumigatus* in serum as “other” criteria.

It should be stressed that both the Rosenberg–Patterson (modified by Greenberger) and ISHAM working group criteria uses the elevation of IgE Abs to crude extracts of *A. fumigatus* as an essential requirement for ABPA. Furthermore, both criteria do not include the isolation of *A. fumigatus* from sputum as an essential

requirement, which relates to the major challenges in the diagnosis of ABPM discussed in this paper. Since it is known that there is cross-reactivity between allergens from crude extracts of different fungi,³⁰ identification of IgE and/or IgG responses to *A. fumigatus* do not always indicate genuine sensitization to *A. fumigatus*. Therefore, those patients meeting current diagnostic criteria include patients with fungal allergy induced by fungi other than *A. fumigatus* (Fig. 1). In other words, current diagnostic criteria do not guarantee *A. fumigatus* colonization in the airway nor genuine antigen-specific IgE reactivity to *A. fumigatus*. These problems also apply for the diagnosis of ABPM induced by fungi other than *A. fumigatus*.

Isolation of fungi for the diagnosis and identification of causal pathogens of ABPM

Culture of *A. fumigatus* from sputum supports the diagnosis of ABPA, which is included as secondary criteria in the Rosenberg–Patterson criteria.²⁵ However the results of sputum culture have not been included in the primary criteria for the diagnosis of ABPA²⁵ because of its low sensitivity and specificity. The frequency of isolation of *A. fumigatus* from sputum of ABPA patients is not particularly high (63% in one report³¹) and can be influenced by the number of specimens examined and processing procedures.^{3,32,33} In addition, the pathogen is frequently isolated from the respiratory tract of asthmatic patients without ABPA,^{34,35} and also from patients with cystic fibrosis,³⁶ chronic obstructive pulmonary disease,³⁷ tuberculosis-related fibrocavity disease,^{38,39} and healthy individuals.³⁵ Identification of more than two fungal species from one specimen is relatively common.^{22,34,40} In addition, isolated fungi from a patient may vary by time of sampling, thus transient findings in sputum culture may not reflect the long-term history of fungal colonization. In contrast, the production of IgE Abs usually reflects long-term historical exposure to the fungal allergens thus serological findings may be superior to laboratory culture for the identification of genuine causal fungi. More recently, a study

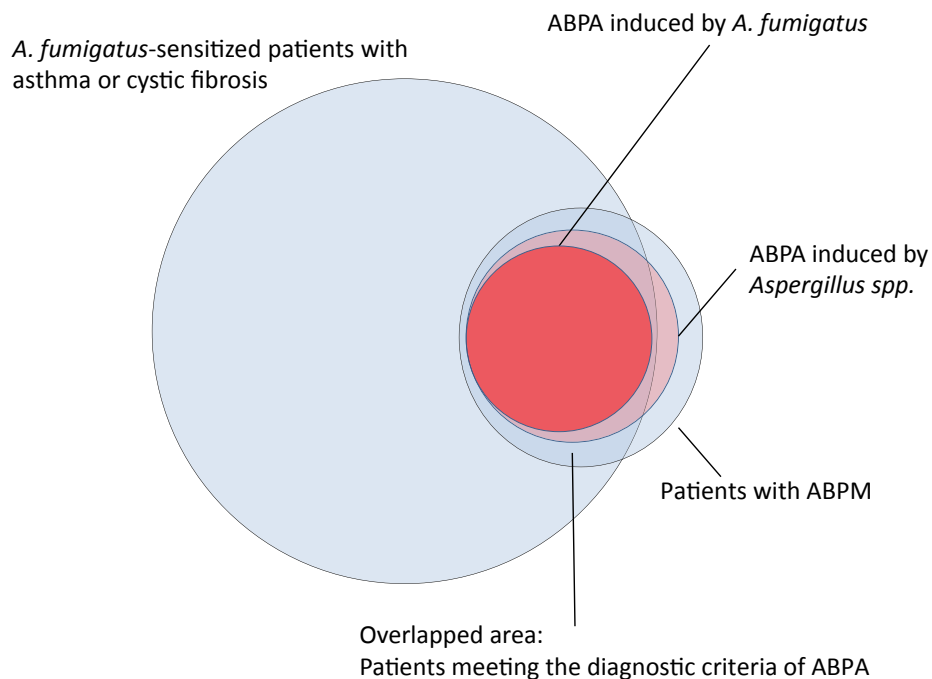


Fig. 1. The Venn diagram showing the relationship between patients meeting diagnostic criteria of ABPA and patients with ABPA genuinely induced by *Aspergillus fumigatus* or other *Aspergillus*. The patients meeting the current diagnostic criteria of ABPA (Rosenberg–Patterson or ISHAM working group) can theoretically include patients with ABPM induced by fungi other than the genus *Aspergillus* because of cross-reactivity to crude fungal allergen extracts.

showed that detection of *A. fumigatus* DNA in sputum using a very sensitive real-time polymerase chain reaction assay even for culture-negative ABPA.⁴¹ This technique is promising in that it can improve diagnostic sensitivity of fungi in the respiratory tract, but the problem of low specificity still remains.

The diagnosis of ABPM is well validated if a patient shows repeated isolation of the same fungal species from their airway and genuine antigen-specific IgE reactivity to the species isolated.⁴² However, the main problem is that the procedure of sputum culture for fungi other than *A. fumigatus* has not yet been standardized.^{21,32} In addition, assays for IgE/IgG Abs are not commercially available for all fungi which possibly induce ABPM.

Cross-reactivity of fungal allergens and the concept of molecular-based allergy diagnostics

The reason for cross-reactivity between crude fungal extracts is that they contain cross-reactive allergenic proteins (pan-allergens), which are highly conserved molecules with similar functions present in widely different species that belong to the same protein family.^{30,43} Numerous allergenic proteins have been identified as cross-reactive allergenic proteins and a list of these proteins are summarized in Table 1. Polysaccharides can also be the cause of fungal allergen cross-reactivity.⁴⁴ Since crude *A. fumigatus* extracts contains such cross-reactive allergenic proteins, apparent sensitization to crude *A. fumigatus* extract does not always indicate genuine *A. fumigatus* sensitization. For example, asthmatic patients with clinical *Alternaria* allergy can have elevated IgE Abs to *A. fumigatus*, even when the patients have not exposed/sensitized to allergenic proteins from *A. fumigatus*, due to cross-reactivity between pan-allergens such as manganese superoxide dismutase (MnSODs; Asp f 6 and Alt a 14), enolases (Asp f 22 and Alt a 6), or heat shock proteins (Asp f 12 and Alt a 3). Another example of such mimicry of *A. fumigatus* sensitization is co-morbid atopic dermatitis (AD) in asthmatic patients. AD is strongly associated with transdermal exposure and sensitization to *Malassezia* spp.,⁴⁵ and some patients with AD display marked elevation in the levels of IgE Abs to *Malassezia* spp., which results in positivity in IgE to *A. fumigatus* owing to the cross-reactivity between their shared pan-allergens.^{46,47} This phenomenon has been a major limitation in the serological diagnosis of fungal allergy using crude extracts.⁴⁸

More recently, measurement of IgE Abs to allergens (allergen components), that are either purified from their native sources or produced as recombinant proteins, have become available for the diagnosis of allergic diseases.^{40,48–51} Molecular-based allergy (MA) diagnostics,⁴³ formally known as component-resolved diagnostics,^{52,53} is an approach to allergy diagnosis using purified natural or recombinant allergenic molecules instead of crude allergen extracts. One of the most important advantages of MA diagnostics is its ability to distinguish genuine sensitization from

sensitization due to cross-reactivity, after evaluation of the sensitization profile to specific allergen components and cross-reactive allergen components. A specific allergen component is an allergenic protein which is specific to its allergenic source, a marker for genuine allergy. According to the concept of MA diagnostics,⁴³ a patient with sensitization to specific allergen components indicates that the patient have been genuinely exposed and sensitized to its corresponding allergenic source or a source of taxonomically closely related species. Most of the specific allergen components used in MA diagnosis are major allergens of their allergenic sources. A cross-reactive allergen is an allergenic protein which contributes to IgE or IgG cross-reactivity between similar allergenic molecules present in sources of different species, a marker for cross-reactivity (see Table 1). Sensitization only to cross-reactive components but without sensitization to specific components indicates that positivity in IgE Ab to crude allergen extract results from cross-reactivity from sensitization to other allergenic sources.

Specific allergen components of *A. fumigatus* as markers for genuine sensitization

In the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature database,⁵⁴ 23 allergenic proteins from *A. fumigatus* have been registered, whereas, as previously discussed, most of these allergenic proteins are cross-reactive allergens. Among these, it is possible to measure the levels of IgE and IgG Abs to recombinant Asp f 1, f 2, f 3, f 4, and f 6 using the commercially available ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden).

Asp f 1 is an 18-kD species-specific major allergen for *A. fumigatus*,⁵⁵ which is a member of the mitogillin family and almost identical to a restrictocin cloned from *Aspergillus restrictus*.⁵⁶ *In silico* genomic analysis of fungal allergenic proteins reported by Bowyer *et al.* showed that Asp f 1 has no homology with any known fungal gene (not including *A. restrictus* or *Aspergillus giganteus*), indicating that Asp f 1 is a highly specific allergen of *A. fumigatus*.⁵⁵ The Asp f 1 allergen is not present in spores, but produced after germination and growth of the fungi.⁵⁷ Since it is almost undetectable in house dust extracts,⁵⁸ respiratory sensitization to airborne Asp f 1 in the indoor environment seems to be uncommon, and sensitization to Asp f 1 indicates that the patients have been genuinely exposed and sensitized to *A. fumigatus*, which has germinated in their respiratory tract.⁵⁸ Asp f 2 is also a species-specific major allergen of *A. fumigatus*, with a 96% frequency of sensitization among those affected by ABPA.^{59–61} Asp f 4 is a 30-kD protein of unknown function and is another well-described highly specific allergen, with a 92% frequency of sensitization among those affected by ABPA.⁶² According to the concept of MA diagnostics,⁴³ Asp f 1, f 2, and f 4 can be considered specific allergen components, whereas Asp f 3 and f 6 are considered cross-reactive allergen components (see Table 1).

Table 1

Cross-reactive allergen components which contribute to cross-reactivity between crude extracts from different fungal sources.

Protein family	<i>Aspergillus fumigatus</i>	<i>Penicillium</i>	<i>Alternaria</i>	<i>Cladosporium</i>	<i>Candida</i>	<i>Malassezia</i>
Peroxisomal proteins	Asp f 3	Pen c 3			Cand b 2	Mala f 3, 4
MnSOD	Asp f 6		Alt a 14			Mala s 11
Cyclophilins	Asp f 27					Mala s 6
Thioredoxins	Asp f 28, 29					Mala s 13
Enolases	Asp f 22	Pen c 22	Alt a 6	Cl a h 6		
Heat shock proteins	Asp f 12		Alt a 3			Mala s 10
P1 ribosomal proteins		Pen b 26	Alt a 12	Cl a h 12		
P2 ribosomal proteins	Asp f 8		Alt a 5	Cl a h 5		

Only allergens from common fungal sources are shown according to WHO–IUIS Allergen Nomenclature database (<http://www.allergen.org/>).

Application of molecular-based allergy diagnostics on ABPA

Application of MA diagnostics can contribute to more accurate diagnosis of ABPA. Theoretically, considering the cross-reactive nature of crude fungal allergen extracts, the IgE Abs to crude *A. fumigatus* in the ABPA diagnostic criteria should be replaced by appropriate panels of *A. fumigatus* specific allergen components. However, clinical studies using MA diagnostics for ABPA have been controversial.^{62–65} We recently studied 306 consecutive *A. fumigatus*-sensitized Japanese asthmatic patients, comparing the levels of IgE Abs to Asp f 1, f 2, f 3, f 4, and f 6 in 53 ABPA patients with 253 control patients without ABPA.⁴⁰ ABPA can be effectively distinguished by the levels of IgE Abs to two major specific allergen components, Asp f 1 and f 2. Using a cut-off value of 0.7 kU_A/L, the combination of IgE positivity to Asp f 1 and/or f 2 showed good diagnostic performance, with a sensitivity and specificity of 77% and 85%, respectively. In contrast, IgE to Asp f 6 was a marker for cross-reactive (not genuine) sensitization to *A. fumigatus* courtesy of transdermal exposure and sensitization to *Malassezia* allergens in patients with co-morbid AD.

Most experts recommend the measurement of IgE Abs or skin prick testing to crude *A. fumigatus* as a first step in screening asthmatic patients for ABPA.^{3,66–68} If IgE Abs to *A. fumigatus* are positive and total IgE is elevated, other examinations for ABPA including precipitating or IgG Abs to *A. fumigatus* and chest CT scans are recommended.³ However, considering that the frequency of ABPA among the *A. fumigatus*-sensitized asthma population is not always high, coupled with limited medical resources and potential health risks relating to exposure to radiation, a more specific serological test for identification of patients with a high risk of

ABPA is desired. Measurement of the levels of IgE Abs to Asp f 1, f 2, and f 6 can be used for this purpose (see Fig. 2). Patients with positivity to IgE Abs for Asp f 1/f 2 can indicate patients with high risk of ABPA. Indeed, in our study,⁴⁰ 52.6% (41/78) of patients with positive IgE Abs to Asp f 1/f 2 (specific components) met the diagnostic criteria of ABPA, whereas 5.2% (12/228) of patients with negative IgE Abs to both Asp f 1 and f 2 met the diagnostic criteria of ABPA. According to this finding, we recommend that all patients with positive IgE to Asp f 1/f 2 should undergo other diagnostic examinations for ABPA, including precipitating Ab and chest CT scan, whereas patients who are seronegative for IgE to both Asp f 1 and f 2 should undergo other diagnostic examinations for ABPA only when the patients have other clinical features suggestive of ABPA. Regarding this study population, an Asp f 6-mono-sensitized pattern indicates co-morbid AD and the lowest risk of ABPA. The majority (95.7%, 45/47) of patients with an Asp f 6-mono-sensitized pattern had co-morbid AD. Generally, co-morbid AD in asthmatic patients hampers the diagnosis of ABPA because some patients with severe AD exhibit a cross-reactive IgE response to *A. fumigatus*, elevation of blood eosinophils, and marked elevation in total IgE. Sensitization from co-morbid AD can effectively be distinguished from sensitization related to ABPA using MA diagnostics.

All patients meeting ABPA diagnostic criteria do not always exhibit IgE seropositivity to Asp f 1 and f 2 (specific components). Indeed, in our study,⁴⁰ of 53 ABPA patients who met the diagnostic criteria of ABPA, 11 patients were seronegative (<0.35 kU_A/L) for IgE to both Asp f 1 and f 2. According to the concept of MA diagnostics, IgE responses to Asp f 1 and f 2 are thought to result from exposure to *A. fumigatus* allergens secreted in the airways. Conversely, negative findings for IgE to Asp f 1 and f 2 in patients meeting the

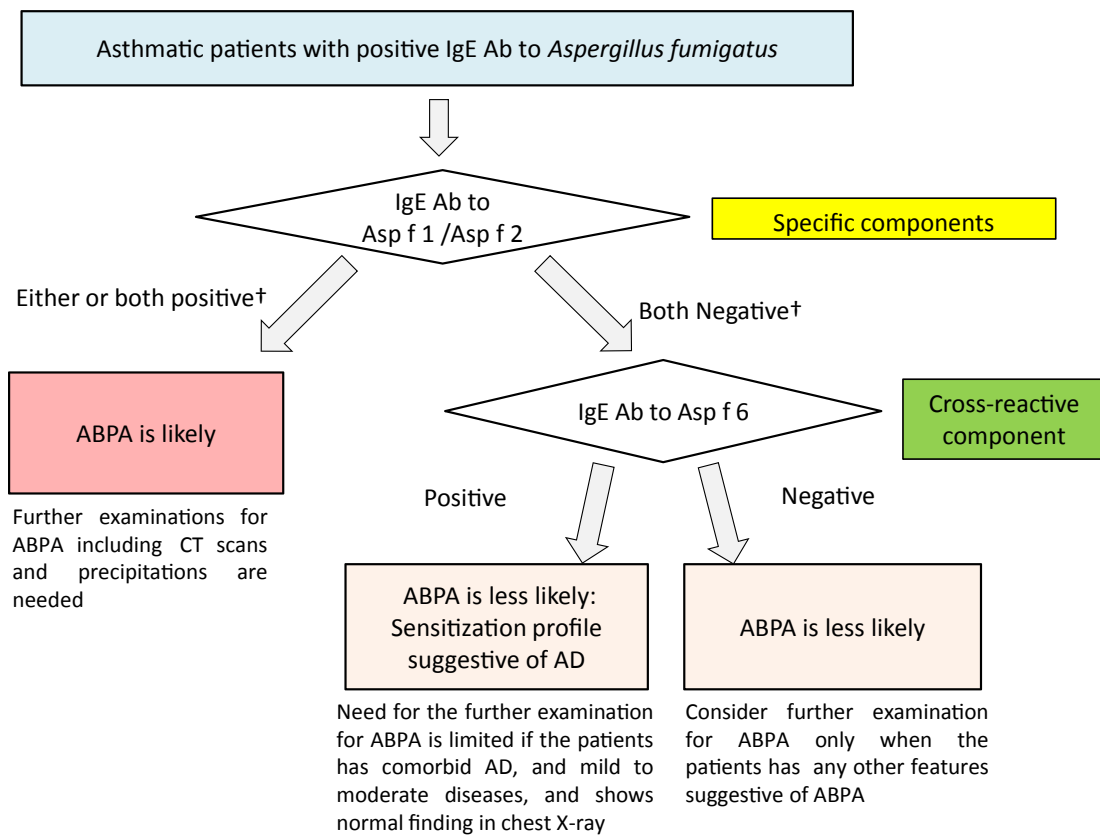


Fig. 2. Flowchart for the interpretation of results of serum IgE Abs to allergen components among *Aspergillus fumigatus*-sensitized asthma according to the finding of Ref. 40. † Cutoff of 0.7 kU_A/L is recommended if the ImmunoCAP system is used for IgE Ab testing. ABPA, allergic bronchopulmonary aspergillosis; Ab, antibody; AD, atopic dermatitis.

ABPA criteria may suggest the possibility that IgE seropositivity to crude *A. fumigatus* for such patients result from cross-reactivity from sensitization to other fungal allergens, and the genuine causal fungi may be fungi other than *A. fumigatus*. Therefore, repeated culture sputum samples, including mucus plug aspirated by bronchoscopy,^{33,69} may be informative especially for such patients, because the results of culture may suggest these are genuine causal fungi. However, the possibility remains that measurement of IgE Abs to additional allergen components other than Asp f 1 and f 2 are required for the identification of genuine *A. fumigatus* allergy. Asp f 9⁷⁰ and Asp f 34⁷¹ have been reported to have a relatively high sequence specificity for *A. fumigatus* and a high prevalence of sensitization among the ABPA population, indicating a possibility that these allergens have to be included in panels of specific allergen components, in addition to Asp f 1 and f 2.

Meanwhile, recent studies have shown IgE-sensitization to crude *A. fumigatus* is associated with reduced lung function even in patients with asthma who do not fulfill diagnostic criteria of ABPA.^{35,72} We speculate that the association between IgE-sensitization and reduced lung function can also become more apparent when we use IgE to Asp f 1 and f 2 instead of IgE to crude *A. fumigatus*.

Possible application of molecular-based allergy diagnostics on ABPM due to fungi other than *A. fumigatus*

Although ABPA and ABPM due to fungi other than *Aspergillus* share similar pathological backgrounds, the clinical presentations, treatment strategies and prognosis may be different between disease entities of different causal fungi.⁵ For example, approximately 70% of patients with ABPM due to fungi other than *Aspergillus* are reported not to have co-morbid asthma.⁵ Although there is strong evidence which supports the effect of anti-fungal therapy for ABPA,^{29,73–77} robust evidence for ABPM is limited.^{20,78} Therefore, identification of genuine causal fungi is needed for better management of ABPM. The results of sputum culture are typically used for this purpose, but MA diagnostics can also be useful.

Serological discrimination of ABPM induced by *S. commune* from ABPA is not always easy because of cross-reactivity between crude extracts from *A. fumigatus* and *S. commune*. Indeed, some of the ABPM reported cases induced by *S. commune* also display elevated levels of IgE Ab to *A. fumigatus*^{20,23} as well as *S. commune*. More recently, Toyotome *et al.* identified Sch c 1, a glucoamylase from *S. commune*, as a major allergen of *S. commune* allergy using sera from ABPM induced by *S. commune*.¹⁹ Homology between Sch c 1 and the glucoamylase from *Aspergillus* spp. is about 50%, which is average; thus the resultant IgE cross-reactivity is weak to moderate. IgE to Sch c 1 may be a promising candidate for allergen component which can discriminate ABPM induced by *S. commune* from ABPA.¹⁹

C. albicans is reported to be the most prevalent causal fungi of ABPM induced by fungi other than *Aspergillus*.⁵ However, we consider that caution should be exercised when we diagnose patients as ABPM induced by *C. albicans* (allergic bronchopulmonary candidiasis, ABPC), given that *C. albicans* is a common human commensal of the oral cavity. Until now, allergens from *C. albicans* have not been fully characterized at the molecular level,⁷⁹ and, to the best of our knowledge, there have been no studies showing IgE-sensitization to individual allergenic proteins of *C. albicans* using sera from ABPM supposedly induced by *C. albicans*. This indicates that allergen components associated with pathogenesis of ABPC are still unknown. Therefore, our clinical recommendation is that the diagnosis of ABPC should include all of the following items: 1) evidence of mono-IgE-sensitization or isolated strong IgE reactivity to *C. albicans* (crude extracts) after evaluating IgE Abs to panels of fungal allergens including *A. fumigatus*, 2) positive precipitating or

IgG Ab to *C. albicans*, and 3), repeated isolation of *C. albicans* from bronchi (mucus aspirated by bronchoscopy is ideal^{33,69}),^{14–16}

Aspergillus spp. other than *A. fumigatus* are frequently isolated from the airway of patients with ABPA. Studies from Japan have shown that *A. niger* is frequently isolated from the airway of patients with ABPA.^{22,40} However, because a specific allergen component as a marker for genuine *A. niger* sensitization among the ABPM population has not been identified, serological discrimination of ABPA induced by *A. niger* is currently difficult. Some case reports have shown that ABPA induced by *A. niger* has been observed among patients without asthma.^{12,13} This implies that the effect of *A. niger* colonization on the pathogenesis of ABPM may differ from that of *A. fumigatus*.

Precipitation antibody testing or specific IgG antibody

Positivity of precipitating Ab or elevation of serum IgG Ab levels to the causal fungi are a hallmark of ABPM and have been included as one of the items of the diagnostic criteria for ABPM.^{3,25,28} Traditionally, serum precipitating Abs have been commonly evaluated using the Ouchterlony double immunodiffusion technique using crude antigen extracts. However, an important limitation of this technique is the low sensitivity and lack of quantitativity. Approximately 15% of ABPA patients are reported to show negative results,²⁷ which is more frequently observed among patients receiving systemic corticosteroid therapy or during remission of the disease. *Aspergillus* IgG enzyme immunoassays can be used for the evaluation of IgG responses to the fungus.^{80,81} One study shows higher sensitivity of Phadia ImmunoCAP *Aspergillus* IgG (cut-off, >40 mg/L) and Bio-Rad Platelia *Aspergillus* IgG (cut-off, ≥10 AU/mL) for detection of *Aspergillus* IgG Ab when compared to counter-immunoelectrophoresis.⁸⁰

Positivity in precipitating or IgG Abs is not specific for ABPM. It is also positive in chronic pulmonary aspergillosis.^{80,82} As these techniques typically use crude fungal extracts, the problems of cross-reactivity with fungal allergens discussed for IgE Abs is also applicable to precipitating or IgG Abs. Although there is a possibility that precipitating Ab to *Aspergillus* components can improve the diagnostic specificity of ABPA, we are currently unable to measure these because recombinant or purified *Aspergillus* components are not commercially available. IgG Abs to *Aspergillus* components can be measured by ImmunoCAP systems. Although IgG to some *Aspergillus* components shows good diagnostic performance for ABPA, clinical utility of such measurements beyond that of the IgE Abs to *Aspergillus* components have not been characterized.⁴⁰

Conclusion

We have discussed the challenges in IgE and IgG Ab testing using crude fungal extracts for the diagnosis of ABPM and identification of causal fungi. Although sputum culture has advantages in that it can identify specific fungi, limitations include low sensitivity and reproducibility, as well as difficulty in discriminating between the genuine causal pathogen and commensals or contaminants. Measuring IgE Abs to allergen components from fungi can contribute to a more specific diagnosis of ABPM and identification of genuine fungal sensitization. We consider that IgE seropositivity to Asp f 1 and f 2, specific allergen components of *A. fumigatus*, can be used as a hallmark for historical exposure to *A. fumigatus* in their airways and genuine sensitization to fungi. However, regarding ABPM induced by *A. niger*, *C. albicans*, and *S. commune*, further studies for the identification of allergenic proteins which are specific and characteristic for these disease entities are needed, in order to apply the concept of MA diagnostics to diagnosis in clinical practice.

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Conflict of interest

The authors have no conflict of interests to declare.

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