

REVIEW

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Diagnosis of allergic bronchopulmonary aspergillosis: a case-based approach

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ABSTRACT Allergic bronchopulmonary aspergillosis is a pulmonary disease occurring in patients with asthma or cystic fibrosis, consequent to a dysregulated immune response to inhaled *Aspergillus* conidia. The usual presentation is with poorly controlled asthma. Patients may also present with expectoration of mucus plugs, hemoptysis, constitutional symptoms and radiological opacities. Patients may experience smoldering lung destruction despite well-controlled asthma. With emerging data, the diagnostic criteria transcribed by an International Expert Committee in 2013 are the latest evidence-based guidelines. Herein, we utilize a case-based approach to elaborate on the diagnosis of this disease. The review intends to provide a lucid understanding of the diagnostic process for the expert as well as the primary physician, involved in management of this enigmatic disorder.

Allergic bronchopulmonary aspergillosis (ABPA) is a respiratory disorder consequent to a perturbed immune response to inhaled *Aspergillus fumigatus* conidia, occurring in patients with bronchial asthma or cystic fibrosis (CF) [1]. The disease is associated with considerable morbidity resulting in worsening of the underlying disease and may result in smoldering lung destruction [2]. Diagnosis is easy in a patient presenting with classical clinical manifestations and characteristic laboratory test results [3]. In others, it may be perplexing. The diagnostic criteria for ABPA have been revised several times over the past six decades since the malady was first brought to attention [4–7]. But the diagnostic process is far from perfect, primarily because of the lack of a gold standard. The recommendations proposed by an International Working Group of ABPA complicating asthma (formed by the International Society for Human and Animal Mycology) in 2013 are the latest evidence-based guideline on the diagnosis, staging and management of this disease [7]. The article focuses on the diagnosis of ABPA complicating bronchial asthma using a case-based approach. Five case scenarios from our chest clinic are discussed in the article (**Box 1**), which are representative of the various entities related to ABPA. Using these representative cases, the major clinical manifestations and laboratory features of ABPA are explained in detail to highlight the peculiarities of this perplexing condition. The article then delves into a logical approach towards diagnosis of ABPA utilizing a combination of several laboratory investigations to arrive at the diagnosis.

KEYWORDS

- ABPA • *Aspergillus* • asthma
- azoles

How does ABPA manifest clinically?

Although many patients with ABPA are diagnosed in the third or fourth decade of life [8], there is no specific age or gender predilection for its occurrence [9]. Even children and the elderly may be afflicted [10,11]. Familial occurrence has been documented in up to 5% of cases [12]. Patients usually have previous diagnosis of asthma with the average duration of asthma prior to the diagnosis of ABPA being 6–15 years [8,9,11]. Asthma may be controlled (20%) or uncontrolled (80%)

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Box 1. Initial case information.

- Case 1: A 51-year-old patient with asthma diagnosed 9 years ago presents with fever, expectoration of mucus plugs and increasing breathlessness. She has been treated for 'pneumonia' thrice over the past 2 years
- Case 2: A 32-year-old asthmatic patient of 8 years duration maintains good asthma control with moderate doses of inhaled corticosteroids and has had two exacerbations over the past 3 years requiring oral steroids
- Case 3: A 26-year-old man presents to our chest clinic with history of well-controlled asthma for 6 years. He is being treated with low doses of inhaled steroids
- Case 4: A 44-year-old lady with history of asthma for 10 years requires high doses of inhaled corticosteroids for control of asthma and has had frequent exacerbations requiring short courses of oral steroids
- Case 5: A 43-year-old woman presents with history of asthma of 2 years duration. The asthma is well controlled with low doses of inhaled steroids. She has had one exacerbation requiring oral steroid therapy in the last 2 years

with the usual asthma medications [13]. Rarely, patients may present *de novo* without prior history of asthma [14]. The typical patient presents with fever, weight loss, cough, expectoration of mucus plugs, hemoptysis, breathlessness and wheezing [8,15–16], although the most common presentation is that of poorly controlled asthma. Expectoration of brownish mucus plugs, considered to be a characteristic symptom has been reported in 30–70% of patients [13,17–18]. Physical examination is usually noncontributory except for finding of polyphonic wheeze on auscultation. In approximately 15% of cases, crackles are present indicating the presence of bronchiectasis, and a similar proportion may reveal clubbing (especially in those with long-standing bronchiectasis) [13]. Many classical manifestations are less common in recent studies as compared with the early ones due to early diagnosis [9,13,17–18]. In fact, chest clinics may not encounter bronchiectasis in ABPA, and see only the serological stage of ABPA (ABPA-S) [19].

Should all asthmatics be screened for ABPA?

All asthmatic patients should be screened for ABPA as clinical symptoms and signs lack sensitivity and specificity (Box 2). Moreover, many patients with ABPA, including those with bronchiectasis, surprisingly do not manifest with worsening asthma control. There is also considerable burden of the disease with an estimated 4.8 million patients globally [20]. The prevalence of ABPA complicating asthma in special clinics has been estimated to approximately 13% [21]. Importantly, early diagnosis and treatment of ABPA can prevent the development of bronchiectasis or halt its progression once it has already

set in [22]. Also, the investigations used in screening have no adverse effects. These points underscore the need for routine screening of asthmatic patients for ABPA.

What investigations are used for screening of ABPA?

Aspergillus fumigatus-specific IgE levels and *Aspergillus* skin test (AST) are the investigations most commonly used for diagnosing sensitization to *A. fumigatus* (defined as asthma with immediate cutaneous hypersensitivity to *A. fumigatus* or elevated specific IgE levels). Let us examine the utility of each of them in the diagnosis of ABPA.

• *A. fumigatus*-specific IgE levels

In 1974, Patterson *et al.* identified the presence of IgE antibodies directed toward *A. fumigatus* in the sera of patients with ABPA, and proposed this investigation as the most specific test in diagnosis of ABPA [23]. We now know that presence of *A. fumigatus*-specific IgE antibodies is not specific (specificity: 69–78%) for ABPA as they are also seen in *Aspergillus*-sensitized asthma, albeit at a lower level. However, *A. fumigatus*-specific IgE is probably the most sensitive test in diagnosis of ABPA. In a latent class analysis, *A. fumigatus* specific IgE level >0.35 kilo units of antibodies per liter (kUA/l) showed 100% sensitivity in the diagnosis of ABPA in three different models [8]. Although a cutoff value more than that of the pooled serum samples from patients with *Aspergillus*-sensitized asthma was proposed [24,25], the clinical significance of this cutoff is not known. Clearly, more studies from different geographical regions are needed for defining a cutoff for *A. fumigatus*-specific IgE levels. At

Box 2. Case commentary.

- All our patients have asthma of several years duration. Cases 1 and 4 have poorly controlled asthma while cases 2, 3 and 5 have well-controlled asthma. Case 1 provides history of recurrent episodes of 'pneumonia' and now has fever and expectoration of mucus plugs which may be a pointer toward allergic bronchopulmonary aspergillosis. It is, however, difficult to predict allergic bronchopulmonary aspergillosis in the other cases

present, a value of 0.35 kUA/l, as agreed upon by the recently convened expert group meeting, might be accepted as the cutoff for a positive result [7].

- ***Aspergillus* skin test**

The presence and degree of cutaneous reactivity acts as a surrogate marker for sensitization within target organs, that is, lung in case of ABPA [26]. Skin testing for determining *Aspergillus* sensitization is performed by introducing a small quantity of *A. fumigatus* antigen (commercial or locally prepared) into the epidermis either by skin prick test (SPT), and if negative, followed by an intradermal injection [21]. The outcome of skin test is influenced by the relative concentration of the allergens in the extract and proper performance of the skin test [27]. A positive Type I reaction is typical of ABPA and represents the presence of *A. fumigatus*-specific IgE antibodies. Intradermal skin tests are more sensitive than SPT for the diagnosis of *Aspergillus* sensitization. In a meta-analysis, intradermal tests fared better than SPT for the detection of *Aspergillus* sensitization in bronchial asthma (28.7 vs 24.8%, $p = 0.002$); however, the prevalence did not vary with the type of antigen used (commercial or

indigenous) [21]. A positive skin test, similar to *A. fumigatus*-specific IgE, is not pathognomonic of ABPA as 28% of asthmatics have a positive AST, and of these only 40% are diagnosed with ABPA [21]. There is an association, albeit weak between *Aspergillus* sensitization (without ABPA) and severity of asthma [28]. In fact, patients who manifest severe asthma, fungal sensitization and in whom ABPA is excluded are classified as severe asthma with fungal sensitization [29–32].

AST has been recommended as the preferred test for screening asthmatic patients for ABPA [3,33]. However, a positive skin test is probably not the gold standard for *Aspergillus* sensitization in the context of ABPA. In a recent study, *Aspergillus* skin testing (intradermal method using an indigenous antigen) had sensitivity ranging between 88 and 94% for the diagnosis of ABPA in asthmatics (vs 100% for *A. fumigatus*-specific IgE) [8]. Thus, one in ten patients of ABPA can be potentially missed if AST is used as the screening test for ruling out ABPA (**Box 3**). Therefore, we recommend that all patients with persistent asthma be routinely investigated with an *A. fumigatus* specific IgE to rule out ABPA. An AST may be performed if specific IgE assay is not available; however, the clinician should

Box 3. Case update: initial workup.

- Case 1: The patient is further investigated. She is found to have positive *Aspergillus* skin test, and a serum *A. fumigatus*-specific IgE level of 3.15 kUA/l
- Case 2: The patient is screened with an *Aspergillus* skin test which turns out to be negative. He seeks advice of another physician and is found to have serum *A. fumigatus*-specific IgE level of 1.89 kUA/ml
- Case 3: On investigating, serum *A. fumigatus*-specific IgE level is 0.53 kUA/l and *Aspergillus* skin test is positive
- Case 4: Further workup reveals a positive *Aspergillus* skin test and serum *A. fumigatus*-specific IgE level of 1.21 kUA/l
- Case 5: She is screened for allergic bronchopulmonary aspergillosis with serum *A. fumigatus*-specific IgE level, which is 0.04 kUA/l

Case commentary

- Cases 1, 2, 3 and 4 have *A. fumigatus*-specific IgE levels above the presently accepted cutoff of 0.35 kUA/l. Thus, all of them are sensitized to *A. fumigatus*. Case 5 does not have sensitization to *A. fumigatus*, and further workup for allergic bronchopulmonary aspergillosis is not indicated in this patient. It is noteworthy that case 2 had a negative *Aspergillus* skin test, however was found to have a positive *A. fumigatus*-specific IgE. This highlights the point that allergic bronchopulmonary aspergillosis can be potentially overlooked if *Aspergillus* skin test is used as the screening test for ruling out allergic bronchopulmonary aspergillosis

understand that up to 6–12% of patients could be potentially missed if skin test is used as a screening modality [8].

What is the next investigation for an asthma patient found to be sensitized to *A. fumigatus*?

The next best step in the algorithmic workup of ABPA is total serum IgE (**Box 4**).

• Total serum IgE levels

The serum total IgE level is a valuable test both in the initial diagnosis, and during follow-up of ABPA. A patient's current symptoms cannot be attributed to ABPA in the face of a normal serum IgE (provided that the patient is not on systemic glucocorticoid therapy) [2]. As for specific IgE, the cutoff value of IgE level that should be used for diagnosis of ABPA remains uncertain. This is due to the fact that the levels of IgE overlap between normal individuals, atopic individuals (with or without fungal sensitization) and ABPA [34]. In 1970, Heiner *et al.* reported raised IgE values in patients with allergic aspergillosis [35]. The original paper published in 1977, describing the criteria proposed by the Patterson group did not provide a cutoff for total IgE [4]. The same group suggested a cutoff of 1500 ng/ml in 1978 [24], while it was stated as 2500 ng/ml (for the acute stage) in 1982 [36]. In 1986, they proposed that an IgE <1000 ng/ml along with an absence of *Aspergillus* precipitins probably excluded ABPA while in those with IgE >2000 ng/ml and presence of precipitins, determination of *A. fumigatus* specific IgE (and IgG levels) was required [37]. The Northwestern Allergy Immunology series of ABPA used an IgE cutoff of 1000 ng/ml [38]. Later series have

used a cutoff of 1000 IU/ml for diagnosing ABPA in asthma too [9,30,39–40]. In a latent-class analysis, a cutoff of 1000 IU/ml gave a sensitivity and specificity of 92 and 40%, respectively, whereas using a value of 417 IU/ml (1000 ng/ml) reduced the specificity to 24% with only a marginal increase in sensitivity to 96% [8]. The latest expert guideline therefore recommends the use of a 1000 IU/ml cutoff for the diagnosis of ABPA [7].

What is the next step in the protocol for the diagnosis of ABPA?

A peripheral blood eosinophil count, serum precipitins (or IgG) against *A. fumigatus* and a chest radiograph along with a high-resolution computed tomogram (HRCT) of the thorax should be obtained (**Box 5**).

• Eosinophil count

A peripheral blood eosinophil count >1000 cells/ μ l has been used as a threshold in the diagnosis of ABPA. However, as many as 60% of patients with ABPA present with an eosinophil count <1000 cells/ μ l at diagnosis, and a quarter of ABPA patients have a count lesser than 500 cells/ μ l [41]. Therefore, eosinophil count should not be used as a screening test for ABPA. In a recent study, the sensitivity and specificity of eosinophil count >1000 cells/ μ l were found to be approximately 30 and 93%, respectively, for the diagnosis of ABPA among asthmatics [8]. Due to the poor sensitivity of this cutoff, an eosinophil count >500 cells/ μ l is proposed as the limit in the recent diagnostic criteria [7]. One should remember that an elevation in eosinophil count may be observed in several systemic disorders including parasitic infestations,

Box 4. Case update: IgE levels.

- Case 1: Serum total IgE level of 10,900 IU/ml
- Case 2: Serum total IgE level of 8900 IU/ml
- Case 3: Serum total IgE level of 300 IU/ml
- Case 4: Serum total IgE level of 612 IU/ml

Case commentary

- Cases 1 and 2 have raised serum total IgE levels. Thus, they may potentially have active allergic bronchopulmonary aspergillosis (ABPA). Case 3 has well-controlled asthma on low doses of inhaled steroids, is sensitized to *A. fumigatus* but has a normal total IgE (below any cutoff, either 417 or 1000 IU/ml for ABPA). Thus, the patient has *Aspergillus*-sensitized asthma but no ABPA. Case 4 is also sensitized to *A. fumigatus* and has a normal total IgE, but her asthma is severe. Her diagnosis is severe asthma with fungal sensitization. Rarely, patients with untreated ABPA might have IgE levels between 417 and 1000 IU/ml, especially if they have received a course of oral corticosteroids for the control of their asthma. Therefore, we should go ahead with further workup of ABPA for case 4 as well

Box 5. Case update: other investigations.

- Case 1: The patient has positive precipitins against *A. fumigatus* and eosinophil count of 1789 cells/ μ l. High-resolution computed tomography (HRCT) of the chest shows mucoid impaction in bilateral upper lobes (with presence of high attenuation mucus in the right lower lobe) and bronchiectasis in both upper lobes and right lower lobe. A review of the chest radiographs during her 'pneumonia' episodes reveals that those were fleeting opacities in the same areas as the mucoid impaction in the present CT scan
- Case 2: The laboratory findings in this patient are positive precipitins against *A. fumigatus*, peripheral blood eosinophil count of 975 cells/ μ l and normal HRCT of the chest
- Case 3: Workup reveals positive precipitins against *A. fumigatus* and total eosinophil count of 432 cells/ μ l. HRCT of thorax reveals minimal bronchiectasis in the right upper lobe
- Case 4: She has positive precipitins against *A. fumigatus*, total eosinophil count of 389 cells/ μ l and normal HRCT of the chest

Churg–Strauss syndrome and other eosinophilic lung diseases. Further, patients receiving oral steroids (common in asthma) can have lower or normal eosinophil levels.

- **Serum precipitins or specific IgG against *A. fumigatus***

A positive precipitin reaction against *A. fumigatus* is found in nearly 80% of patients with ABPA [34,39]. Precipitins refer to the IgG antibodies mounted against the organism, usually detected by immunoprecipitation techniques. The double gel diffusion has been the preferred method for detection of precipitins over the years [4,37,39]. Automated commercial enzyme immunoassays are now available, which have been found to be sensitive and reproducible [42,43]. It should be borne in mind that IgG antibodies are not specific for ABPA and can be seen in semi-invasive forms of aspergillosis like chronic pulmonary aspergillosis. Chronic pulmonary aspergillosis can complicate the course of ABPA [20], and presence of constitutional symptoms, pulmonary cavities along with positive IgG (in high titres) may point toward its occurrence [44].

- **Chest radiograph**

A host of abnormalities can be seen on chest radiograph in ABPA but are generally nonspecific [45]. Chest radiograph may be normal in 50% of the cases [46]. Certain radiographic features occur during acute episodes and clear up during remission [47]. They include consolidation, lobar or segmental atelectasis, band-like or toothpaste shadows, tramline shadows and gloved-finger shadows [47,48]. Serial radiographs may reveal the transient nature of these migratory infiltrates, termed as 'fleeting shadows' [49]. Permanent changes include parallel line

shadows, ring shadows, generalized overinflation and localized emphysema [47,48]. Perihilar opacities representing infiltrates that surround the dilated, secretion-filled central bronchi may be seen, which can be mistaken for hilar adenopathy [50]. Toothpaste and gloved-finger shadows represent mucus impaction, tramline shadows represent bronchial wall thickening, while parallel line and ring shadows represent dilated bronchi and/or mucoid impaction. Consolidation, considered to be the most common chest radiograph finding, most often is found to represent mucus-filled bronchiectatic cavities, when imaged with an HRCT chest [46]. Chest radiographs need to be performed in all cases as they can be done during follow-up, and thus preclude the need for repeated CT scans.

- **Computed tomography**

Computed tomography is more sensitive than chest radiography in identification of bronchiectasis (**Figure 1**), and thus allows earlier detection of the onset and progression of lung damage [51,52]. HRCT of the chest is as sensitive and more acceptable than bronchography, the earlier gold standard for discernment of bronchiectasis [53]. Bronchiectasis is the most common (73–95%) abnormality seen in ABPA on CT chest along with bronchial wall thickening (95%) [39,54]. Bronchiectasis may be cylindrical (less severe), varicose or cystic (considered more severe forms) [54,55]. It may be central (medial half or two-thirds of the lung field, on an imaginary line drawn from the hilum to the chest wall) or may extend to the periphery (in approximately 40% of the cases) [46,56]. Because of the lack of specificity of central bronchiectasis in diagnosis of ABPA [54], and the fact that a significant proportion of patients with ABPA

Box 6. New diagnostic criteria for the diagnosis of allergic bronchopulmonary aspergillosis.

Predisposing conditions

- Bronchial asthma, cystic fibrosis

Obligatory criteria (both must be present)

- Elevated serum IgE levels against *A. fumigatus* (>0.35 kUA/l) or immediate cutaneous hypersensitivity to *A. fumigatus* antigen (positive Type I *Aspergillus* skin test)
- Raised serum total IgE levels (>1000 IU/ml)[†]

Other criteria (at least two of three)

- Presence of precipitating (or IgG) antibodies against *A. fumigatus* in serum
- Thoracic imaging findings consistent with allergic bronchopulmonary aspergillosis[‡]
- Peripheral blood eosinophil count >500 cells/ μ l (may be historical)

[†]An IgE value <1000 IU/ml may be acceptable, if all other criteria are met.

[‡]The features on thoracic imaging consistent with allergic bronchopulmonary aspergillosis may be transient (i.e., fleeting opacities, toothpaste/gloved finger opacities, tram-track opacities, consolidation, nodules) or permanent (i.e., bronchiectasis, parallel lines, ring shadows and pleuropulmonary fibrosis).

have ‘peripheral’ bronchiectasis, the term central has been removed by the recent expert group [7]. Not specific to ABPA, bronchiectasis is also seen in patients with *Aspergillus*-sensitized asthma, though less commonly than ABPA [57,58]. Bronchiectasis affecting three or more lobes with mucoid impaction is suggestive but not characteristic of ABPA [54]. An important HRCT finding, considered pathognomonic for ABPA, is airway plugging with high attenuation mucus (HAM) [59–63]. This term is used if the mucus plug is visually denser than the normal paraspinal skeletal muscle (Figure 2) [64]. The density may range from 108 to 168 Hounsfield units (HU) [13], and is attributed to the presence of calcium salts and ions of iron and manganese [65] or desiccated mucus [66]. Approximately 20% of ABPA patients demonstrate this finding on HRCT [13]. On latent-class analysis, the specificity of HAM was found to be 100% [8]. The

presence of bronchiectasis and/or HAM indicate an immunologically severe disease with predisposition to recurrent relapses [9,13,67].

Centrilobular nodules (32–90%), tree-in-bud and mucoid impaction (70%) are commonly seen especially in the acute stage [47]. Other parenchymal abnormalities include consolidation, segmental or lobar collapse, parenchymal scarring, cavities and bullae [56]. Pleural abnormalities include thickening/fibrosis and, rarely, pneumothorax and effusion [56–57,68–69]. Uncommon findings include pulmonary masses [70], miliary nodules [14], aspergilloma [71] and total lung collapse [72]. HRCT of the chest is found to be normal in a third of the patients; in that case they are labeled as ABPA-S [39,46].

What criteria should be used for the diagnosis & classification of *Aspergillus*-related hypersensitivity phenomena in asthma?

The Patterson criteria proposed more than three decades ago have been the basis for diagnosis of ABPA till date. These criteria however have certain limitations [73]: they place equal importance to all the components while some parameters such as IgE levels are more important than others; there is no consensus on the number of major or minor criteria necessary for the diagnosis. For example, if one makes the diagnosis of ABPA using at least five criteria then there is a fall in specificity while if we use at least seven criteria there is a fall in sensitivity [8]; the optimum cutoff values for IgE levels and eosinophil count are not specified; and the minor criteria seem to be redundant. The recent consensus-based guidelines have taken cognizance of the deficiencies of the previous criteria in the light of



Figure 1. High-resolution computed tomography of the thorax (lung windows) demonstrating extensive areas of bronchiectasis involving the right lung.

the knowledge accumulated over the years and have revised these criteria (**Box 6**) [7]. The criteria are now simpler and more evidence based. Bronchiectasis has been removed from the diagnostic criteria as ABPA may present without bronchiectasis. The cutoff value of IgE has been proposed as 1000 IU/ml pending further validation, as it can help in differentiating severe asthma with fungal sensitization from ABPA-S. Minor criteria have been removed. The diagnostic algorithm shown in **Figure 3** may be followed for arriving at the specific diagnosis. Patients who do not fulfill all the criteria for diagnosis may be categorized as 'ABPA-at risk', and should be kept under close followup (**Box 7**).

What should be the basis for staging ABPA?

ABPA is staged both clinically and radiologically, the former has therapeutic and the latter prognostic implications [45]. Patterson *et al.* has classified ABPA into five clinical stages [36]; however, this staging was limited by the lack of precise definitions at each stage. The latest guidelines have recommended a new staging system (**Table 1**) to circumvent the ambiguities of the previous system. Certain points deserve special mention. ABPA is now classified into seven stages with stages 1 and 5 having sub-stages. The stages do not represent sequential temporal regression and a patient may revert to a lower stage with treatment or time. Up to half of the patients may experience an exacerbation of the disease usually within 1–2 years [36–37,39]. It is important to note that IgE levels do not return to normal values even in patients with remission [67]. Thus, repeated evaluation of IgE should be performed to characterize the 'new baseline' value, which is then used for defining exacerbations. A prolonged remission does not imply a cure as exacerbations of the disease can occur several years after remission. Even in stage 6 (advanced ABPA), patients may require therapy as the disease can remain clinically and immunologically active [74].

Patterson *et al.* have classified ABPA radiologically into two stages: ABPA-S and ABPA with central bronchiectasis (ABPA-CB), with ABPA-S representing patients without pulmonary damage [37]. Kumar *et al.* divided cases of ABPA into ABPA-S, ABPA-CB and ABPA with other radiologic findings (ABPA-CB-ORF) based on findings on CT chest scan [75]. In a large study, no difference was found among

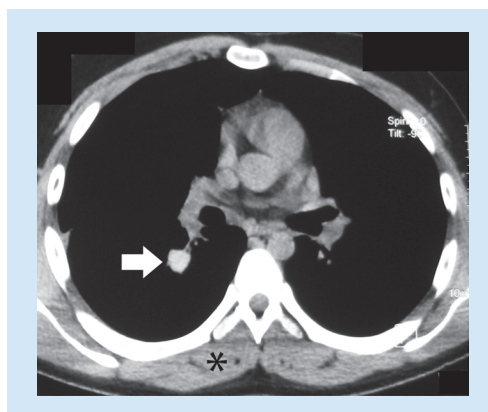


Figure 2. High-resolution computed tomography of the chest (mediastinal windows) demonstrating high attenuation mucus (arrow).

*The mucoid impaction is visually denser than the paraspinal skeletal muscle.

the radiologic stages of ABPA in terms of duration of illness, severity of asthma and serologic findings [39]. However, severity of bronchiectasis (based on the number of segments affected by bronchiectasis on HRCT) and the presence of HAM were predictors of relapse and failure to achieve complete remission [13]. These findings prompted Agarwal *et al.* to propose a new classification scheme based on HAM, thus creating three classes: ABPA-S, ABPA-CB and ABPA-CB-HAM [9]. In this study, the older classification schemes were compared with the one based on HAM and it was found that HAM most consistently predicted immunological severity in ABPA [9]. The long-term outcome of ABPA-S is good (despite frequent relapses), and in one study no patient developed bronchiectasis with appropriate management [76]. The radiologic classification proposed in the recent guidelines takes cognizance of all the classification schemes and divides ABPA radiologically into four stages (**Table 2**).

Is there any role of spirometry in the diagnosis of ABPA?

Spirometry may help in the diagnosis and severity assessment of asthma. However, asthma patients with or without ABPA may have a normal spirometry. Thus, a normal spirometry does not exclude the diagnosis of ABPA [39]. Bronchial provocation testing with *Aspergillus* antigens are generally not advisable as it can cause severe bronchospasm [77].

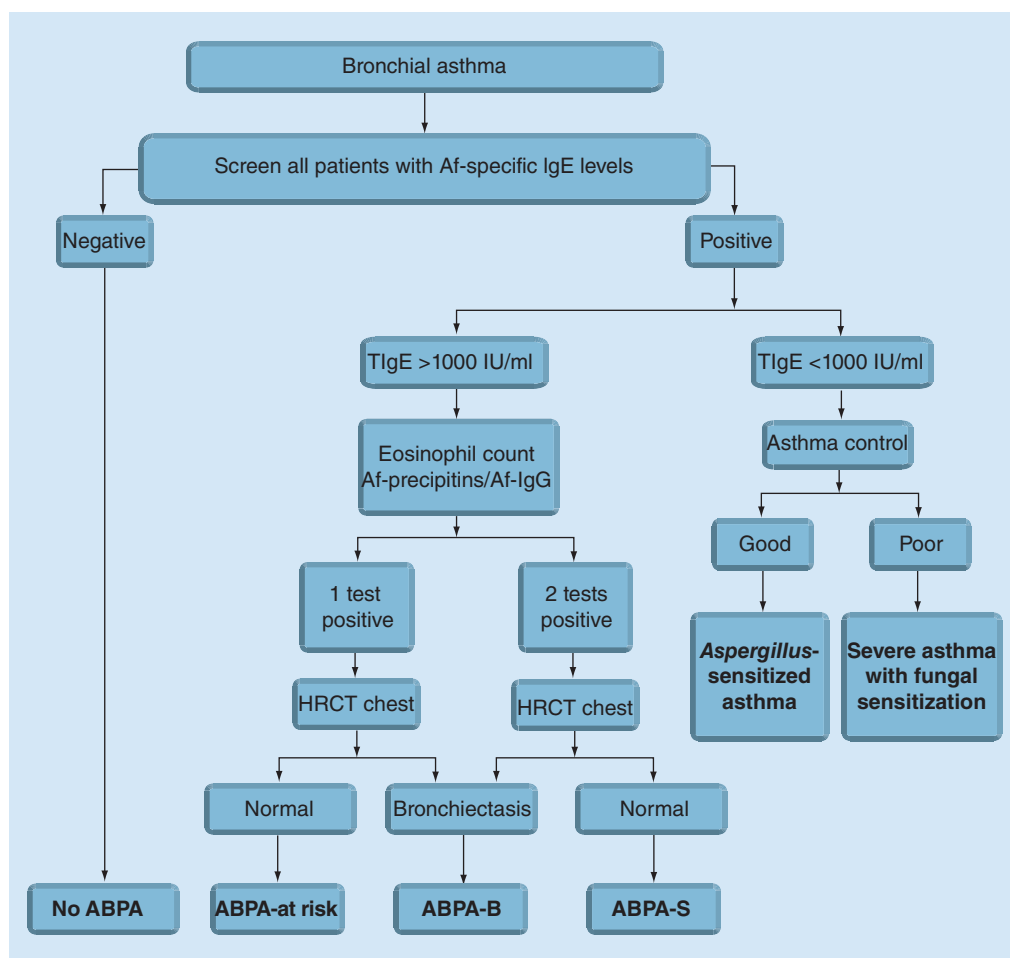


Figure 3. Algorithm for the diagnosis of allergic bronchopulmonary aspergillosis in asthma.

ABPA: Allergic bronchopulmonary aspergillosis; ABPA-B: Allergic bronchopulmonary aspergillosis with bronchiectasis; ABPA-S: Serological allergic bronchopulmonary aspergillosis; Af: *Aspergillus fumigatus*; HRCT: High-resolution computed tomography; SAFS: Severe asthma with fungal sensitization; TIgE: Total IgE.

Do sputum cultures for *A. fumigatus* have any significance in diagnosing ABPA?

Growth of *A. fumigatus* in respiratory secretions is not diagnostic of ABPA because the fungus can also be cultured in other pulmonary diseases due to its ubiquitous nature [78]. Sputum cultures may grow *A. fumigatus* in 40–60% of ABPA patients [4,17,77]. A better technique for sputum processing employing separation of sputum plugs from saliva before inoculation may increase the yield of cultures [79]. Interestingly, the vast majority of ABPA patients have detectable *A. fumigatus* DNA in their sputum despite negative cultures, attesting to its definite presence in the lung [80]. The role of culture (if an isolate can be obtained) mainly lies in susceptibility testing and/or real-time

molecular testing for resistance to antifungal agents [80].

What vision do we have for 2020 in the field of ABPA diagnostics?

New markers like recombinant *Aspergillus* antigens, thymus- and activation-regulated chemokine (TARC) and the FACS basophil CD203c assay are being evaluated for use in the diagnosis and followup of ABPA. They have the potential to become a routine tool in the armamentarium of ‘ABPAologists’ in 2020.

• Recombinant *A. fumigatus* antigens

Recombinant antigens are synthesized from cDNA libraries of *A. fumigatus* and can be produced as highly purified proteins. They may

Box 7. Case commentary: final diagnosis.

- Case 1: The patient has *Aspergillus* sensitization, positive precipitins, high IgE levels and raised eosinophil count. She also has bronchiectasis and high attenuation mucus (HAM) on high-resolution computed tomography. According to the algorithm shown in **Figure 3**, and the radiological classification listed in **Table 2**, she fits into the classical picture of allergic bronchopulmonary aspergillosis (ABPA)-HAM. If there were only bronchiectasis without HAM, then this patient would be classified as ABPA with bronchiectasis.
- Case 2: This patient had a negative *Aspergillus* skin test but had all other features of ABPA on further investigation. In view of no abnormality on a chest high-resolution computed tomography, he is diagnosed as serological ABPA.
- Cases 3 and 4 reveal positive precipitins and normal peripheral blood eosinophil counts. Now, it is further confirmed that they have *Aspergillus*-sensitized asthma and severe asthma with fungal sensitization, respectively.

contribute to significant improvement in the diagnostic specificity and reproducibility as compared with crude extracts [81]. In ABPA, these antigens have been evaluated both for skin testing and measurement of specific IgE levels [82–84]. The most widely studied recombinant *A. fumigatus* antigens include rAsp f1 (secreted ribotoxin), rAsp f2 (unknown function), rAsp f3 (peroxisomal protein), rAsp f4 (unknown function) and rAsp f6 (cellular manganese superoxide dismutase) [85]. Studies suggest that rAsp f4 and f6 represent specific markers for ABPA and allow a differentiation from *Aspergillus*-sensitized asthma [85,86]. The proposed reason for the specificity of rAsp f4 and f6 is that they are intracellular nonsecreted proteins [87]. Therefore,

only patients suffering from ABPA get exposed to these proteins, as a result of fungal damage related to cellular defense mechanisms [88]. In contrast, *A. fumigatus*-sensitized asthmatics mount an IgE response only to secreted proteins (rAsp f1 and f3) produced shortly after spore germination [89]. One study suggested that serial determinations of IgE to rAsp f3 may provide improved distinction between stages of flares and remission compared with changes in IgE to a crude *Aspergillus* extract [90]. A combination of these antigens is more useful rather than using a single antigen [91,92]. Commercial automated assays for determination of specific IgE levels against recombinant *A. fumigatus* antigens are available [92–94]. Although detection of IgE

Table 1. Clinical staging of allergic bronchopulmonary aspergillosis in patients with asthma.

Stage	Definition	Features
0	Asymptomatic	Never diagnosed to have ABPA in the past, presentation with controlled asthma (according to GINA guidelines) and meeting the diagnostic criteria of ABPA (Box 6)
1	Acute	Never diagnosed to have ABPA in the past, presentation with uncontrolled asthma/constitutional symptoms and meeting the diagnostic criteria of ABPA
1a	With mucoid impaction	Presence of mucoid impaction on thoracic imaging or bronchoscopy
1b	Without mucoid impaction	No mucoid impaction on thoracic imaging or bronchoscopy
2	Response	Clinical and/or radiological improvement [†] and fall in IgE by ≥25% of baseline at 8 weeks
3	Exacerbation	Clinical and/or radiological worsening accompanied by an increase in IgE by ≥50% from the previous baseline
4	Remission	Sustained clinoradiological improvement with IgE levels remaining at or below baseline (or increase by <50%) for ≥6 months off therapy with oral steroids
5a	Treatment-dependent ABPA	Two or more relapses within 6 months of stopping treatment or deterioration in clinical and/or radiological condition and/or immunological worsening on tapering oral steroids/azoles
5b	Glucocorticoid-dependent asthma	Systemic corticosteroids required for asthma control while the ABPA activity is controlled (as indicated by IgE levels and thoracic imaging)
6	Advanced ABPA	Presence of complications (cor pulmonale and/or chronic type II respiratory failure) along with presence of extensive bronchiectasis consistent with ABPA on thoracic radiology after excluding alternate causes

[†]For example, resolution of mucoid impaction and consolidation. Permanent changes such as bronchiectasis may persist.

ABPA: Allergic bronchopulmonary aspergillosis; GINA: Global initiative for asthma.

Adapted with permission from [7].

Table 2. Revised radiologic classification of allergic bronchopulmonary aspergillosis based on findings on a high-resolution computed tomography of the chest.

Classification	Features
ABPA-S	Fulfils the diagnostic criteria of ABPA (Box 6) with absence of any radiological finding of ABPA on HRCT of the thorax
ABPA-B	Satisfies the diagnostic requirements of ABPA along with presence of bronchiectasis
ABPA-HAM	ABPA along with presence of high attenuation mucus on HRCT of the thorax
ABPA-CPF	Fulfils the diagnostic criteria of ABPA with at least two radiological features suggestive of fibrosis [†] without presence of mucoid impaction (or HAM)

[†]Includes fibrocavitary lesions, pulmonary fibrosis, pleural thickening and others.
ABPA: Allergic bronchopulmonary aspergillosis; ABPA-S: Serological ABPA; B: Bronchiectasis; CPF: Chronic pleuropulmonary fibrosis; HAM: High attenuation mucus; HRCT: High-resolution computed tomography.
Adapted with permission from [7].

response to recombinant *Aspergillus* antigens looks promising, there is literature that questions this claim [86,95]. Therefore, further scrutiny in larger controlled studies is obligatory before this test gets incorporated into the routine diagnostic workup in the clinic [96].

• **Thymus- & activation-regulated chemokine/CCL17**

ABPA is characterized by a Th2 type of immune response [97]. TARC acts as a chemoattractant to Th2 type of cells acting through CCR4 and CCR8 receptor present on these cells. TARC has been shown to have a better diagnostic accuracy as a sole marker than specific IgE to crude or recombinant *A. fumigatus* antigens with sensitivity of 100% and a specificity of 97.2% for ABPA diagnosis in CF [98]. It may also indicate exacerbations of ABPA better than IgE levels if serial determination is performed [99]. Large studies are required to evaluate the role of this novel marker in the diagnosis and followup of ABPA complicating asthma.

• **FACS basophil CD203c assay**

Patients with CF complicated by ABPA display characteristic increases in blood basophil CD203c levels both at baseline and following *in vitro* stimulation with an allergenic extract of *A. fumigatus* compared with patients with CF alone and in CF colonized with *A. fumigatus* [100]. As with TARC, the role of this modality needs to be investigated in ABPA complicating asthma.

Conclusion & future perspective

In summary, the article provides the current evidence-based approach towards a diagnosis of ABPA. Asthmatic patients presenting to special asthma or chest clinics should be routinely screened for ABPA, with *A. fumigatus* specific IgE levels. It is imperative that the diagnosis of ABPA is secured as early as possible, as untreated disease usually culminates in bronchiectasis and pulmonary fibrosis, all manifestations of end-stage lung disease. More than six decades have elapsed since the original description of this disorder, however

EXECUTIVE SUMMARY

- All patients with persistent asthma should be screened for allergic bronchopulmonary aspergillosis (ABPA) irrespective of the the level of control.
- The recommended test for screening asthmatic patients for ABPA is the *A. fumigatus*-specific IgE.
- *Aspergillus* skin prick test is less sensitive than *A. fumigatus*-specific IgE level and can potentially miss one in ten asthmatic patients being screened for ABPA.
- High-attenuation mucus is the most specific test in diagnosis of ABPA but is unfortunately encountered only in 20% of ABPA patients.
- Recombinant antigen-based immunological tests are the most promising investigations in the diagnosis of ABPA but require more evaluation before routine use in the clinic setting.
- The new criteria for diagnosis, staging and classification are currently the most evidence-based and precise definitions. The utilization of these criteria would not only help in making an accurate diagnosis, but also allow rigorous stratification of disease during management.

the diagnosis of ABPA still remains elusive. There is an urgent need for defining the cutoff values of various investigations such as IgE levels (total and *A. fumigatus* specific). Recombinant *Aspergillus* antigens, TARC and the FACS basophil CD203c assay seem promising tools in the diagnosis and possibly followup of ABPA, and need to be investigated preferably in a multicentric approach. There is also a dire need to study the natural history of patients with asthma and fungal sensitization so as to properly define the temporal change in various parameters (such as

skin test, IgE levels and others) in these patients.

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References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

- Agarwal R, Chakrabarti A. Allergic bronchopulmonary aspergillosis in asthma: epidemiological, clinical and therapeutic issues. *Future Microbiol.* 8(11), 1463–1474 (2013).
- Agarwal R. Allergic bronchopulmonary aspergillosis. *Chest* 135(3), 805–826 (2009).
- **Comprehensive review on allergic bronchopulmonary aspergillosis (ABPA).**
- Greenberger PA. When to suspect and work up allergic bronchopulmonary aspergillosis. *Ann. Allergy Asthma Immunol.* 111(1), 1–4 (2013).
- Rosenberg M, Patterson R, Mintzer R, Cooper BJ, Roberts M, Harris KE. Clinical and immunologic criteria for the diagnosis of allergic bronchopulmonary aspergillosis. *Ann. Intern. Med.* 86(4), 405–414 (1977).
- **First paper to elaborate the criteria for diagnosis of ABPA.**
- Stevens DA, Moss RB, Kurup VP *et al.* Allergic bronchopulmonary aspergillosis in cystic fibrosis – state of the art: Cystic Fibrosis Foundation Consensus Conference. *Clin. Infect. Dis.* 37(Suppl. 3), S225–S264 (2003).
- **Proceedings of an international workshop on ABPA complicating cystic fibrosis.**
- Agarwal R, Hazarika B, Gupta D, Aggarwal AN, Chakrabarti A, Jindal SK. *Aspergillus* hypersensitivity in patients with chronic obstructive pulmonary disease: COPD as a risk factor for ABPA? *Med. Mycol.* 48(7), 988–994 (2010).
- Agarwal R, Chakrabarti A, Shah A *et al.* Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. *Clin. Exp. Allergy* 43(8), 850–873 (2013).
- **Proceedings of an international workshop on ABPA; provides a new criteria for diagnosis and staging of ABPA.**
- Agarwal R, Maskey D, Aggarwal AN *et al.* Diagnostic performance of various tests and criteria employed in allergic bronchopulmonary aspergillosis: a latent class analysis. *PLoS ONE* 8(4), e61105 (2013).
- **First paper to describe the sensitivity and specificity of various investigations utilized in ABPA using the methodology of latent-class analysis.**
- Agarwal R, Khan A, Gupta D, Aggarwal AN, Saxena AK, Chakrabarti A. An alternate method of classifying allergic bronchopulmonary aspergillosis based on high-attenuation mucus. *PLoS ONE* 5(12), e15346 (2010).
- **First paper to provide evidence regarding various radiological classifications used in ABPA. Found high-attenuation mucus-based classification to be the best classification.**
- Mastella G, Rainisio M, Harms HK *et al.* Allergic bronchopulmonary aspergillosis in cystic fibrosis. A European epidemiological study. *Epidemiologic Registry of Cystic Fibrosis. Eur. Respir. J.* 16(3), 464–471 (2000).
- Kim JH, Jin HJ, Nam YH, Hwang EK, Ye YM, Park HS. Clinical features of allergic bronchopulmonary aspergillosis in Korea. *Allergy Asthma Immunol. Res.* 4(5), 305–308 (2012).
- Shah A, Kala J, Sahay S, Panjabi C. Frequency of familial occurrence in 164 patients with allergic bronchopulmonary aspergillosis. *Ann. Allergy Asthma Immunol.* 101(4), 363–369 (2008).
- Agarwal R, Gupta D, Aggarwal AN, Saxena AK, Chakrabarti A, Jindal SK. Clinical significance of hyperattenuating mucoid impaction in allergic bronchopulmonary aspergillosis: an analysis of 155 patients. *Chest* 132(4), 1183–1190 (2007).
- **First paper to define high-attenuation mucus as a prognostic marker in ABPA.**
- Agarwal R, Aggarwal AN, Gupta D, Bal A, Das A. Case report: a rare cause of miliary nodules – allergic bronchopulmonary aspergillosis. *Br. J. Radiol.* 82(980), e151–e154 (2009).
- Ye F, Zhang NF, Zhong NS. [Clinical and pathological analysis of allergic bronchopulmonary aspergillosis in China]. *Zhonghua Jie He He Hu Xi Za Zhi*, 32(6), 434–438 (2009).
- McCarthy DS, Pepys S. Allergic bronchopulmonary aspergillosis. Clinical immunology. 1. Clinical features. *Clin. Allergy* 1(1), 261–286 (1971).
- Chakrabarti A, Sethi S, Raman DS, Behera D. Eight-year study of allergic bronchopulmonary aspergillosis in an Indian teaching hospital. *Mycoses* 45(8), 295–299 (2002).
- Kumar R, Gaur SN. Prevalence of allergic bronchopulmonary aspergillosis in patients with bronchial asthma. *Asian Pac. J. Allergy Immunol.* 18(4), 181–185 (2000).
- Patterson R. Allergic bronchopulmonary aspergillosis and hypersensitivity reactions to fungi. In: *Fishman's Pulmonary Diseases and Disorders*. Fishman AP, Elias JA, Fishman JA, Grippi MA, Kaiser LR, Senior RM (Eds). McGraw Hill, NY, USA, 777–782 (1998).
- Denning DW, Pleuvry A, Cole DC. Global burden of allergic bronchopulmonary aspergillosis with asthma and its complication chronic pulmonary aspergillosis in adults. *Med. Mycol.* 51(4), 361–370 (2013).
- **Scoping review on the global burden on ABPA in asthma.**
- Agarwal R, Aggarwal AN, Gupta D, Jindal SK. *Aspergillus* hypersensitivity and allergic bronchopulmonary aspergillosis in patients with bronchial asthma: systematic review and meta-analysis. *Int. J. Tuberc. Lung Dis.* 13(8), 936–944 (2009).
- Hogan C, Denning DW. Allergic bronchopulmonary aspergillosis and related

- allergic syndromes. *Semin. Respir. Crit. Care Med.* 32(6), 682–692 (2011).
- 23 Patterson R, Roberts M. IgE and IgG antibodies against *Aspergillus fumigatus* in sera of patients with bronchopulmonary allergic aspergillosis. *Int. Arch. Allergy Appl. Immunol.* 46(1), 150–160 (1974).
- 24 Wang JL, Patterson R, Rosenberg M, Roberts M, Cooper BJ. Serum IgE and IgG antibody activity against *Aspergillus fumigatus* as a diagnostic aid in allergic bronchopulmonary aspergillosis. *Am. Rev. Respir. Dis.* 117(5), 917–927 (1978).
- 25 Patterson R, Greenberger PA, Ricketti AJ, Roberts M. A radioimmunoassay index for allergic bronchopulmonary aspergillosis. *Ann. Intern. Med.* 99(1), 18–22 (1983).
- 26 Heinzerling L, Mari A, Bergmann KC *et al.* The skin prick test – European standards. *Clin. Transl. Allergy* 3(1), 3 (2013).
- 27 Ownby DR. Diagnostic tests in allergy. In: *Allergic Diseases: Diagnosis and Treatment*. Lieberman P, Anderson JA (Eds). Humana Press, Totowa, NJ, USA, 27–38 (2007).
- 28 Agarwal R, Noel V, Aggarwal AN, Gupta D, Chakrabarti A. Clinical significance of *Aspergillus* sensitisation in bronchial asthma. *Mycoses* 54(5), e531–e538 (2011).
- 29 Agarwal R, Gupta D. Severe asthma and fungi: current evidence. *Med. Mycol.* 49 (Suppl. 1), S150–S157 (2011).
- 30 Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. *Eur. Respir. J.* 27(3), 615–626 (2006).
- **Outstanding review on the link between fungi and severe asthma.**
- 31 Denning DW, O'Driscoll BR, Powell G *et al.* Randomized controlled trial of oral antifungal treatment for severe asthma with fungal sensitization: The Fungal Asthma Sensitization Trial (FAST) study. *Am. J. Respir. Crit. Care Med.* 179(1), 11–18 (2009).
- 32 Agarwal R. Severe asthma with fungal sensitization. *Curr. Allergy Asthma Rep.* 11(5), 403–413 (2011).
- 33 Mahdavinia M, Grammer LC. Management of allergic bronchopulmonary aspergillosis: a review and update. *Ther. Adv. Respir. Dis.* 6(3), 173–187 (2012).
- 34 Malo JL, Longbottom J, Mitchell J, Hawkins R, Pepys J. Studies in chronic allergic bronchopulmonary aspergillosis. 3. Immunological findings. *Thorax* 32(3), 269–274 (1977).
- 35 Heiner DC, Rose B. Elevated levels of gamma-E (IgE) in conditions other than classical allergy. *J. Allergy* 45(1), 30–42 (1970).
- 36 Patterson R, Greenberger PA, Radin RC, Roberts M. Allergic bronchopulmonary aspergillosis: staging as an aid to management. *Ann. Intern. Med.* 96(3), 286–291 (1982).
- **First paper to formulate clinical staging of ABPA.**
- 37 Patterson R, Greenberger PA, Halwig JM, Liotta JL, Roberts M. Allergic bronchopulmonary aspergillosis. Natural history and classification of early disease by serologic and roentgenographic studies. *Arch. Intern. Med.* 146(5), 916–918 (1986).
- **First paper to provide radiological classification of ABPA.**
- 38 Patterson R, Greenberger PA, Harris KE. Allergic bronchopulmonary aspergillosis. *Chest* 118(1), 7–8 (2000).
- 39 Agarwal R, Gupta D, Aggarwal AN, Behera D, Jindal SK. Allergic bronchopulmonary aspergillosis: lessons from 126 patients attending a chest clinic in north India. *Chest* 130(2), 442–448 (2006).
- 40 Wark PA, Hensley MJ, Saltos N *et al.* Anti-inflammatory effect of itraconazole in stable allergic bronchopulmonary aspergillosis: a randomized controlled trial. *J. Allergy Clin. Immunol.* 111(5), 952–957 (2003).
- 41 Agarwal R, Khan A, Aggarwal AN *et al.* Clinical relevance of peripheral blood eosinophil count in allergic bronchopulmonary aspergillosis. *J. Infect. Public Health* 4(5–6), 235–243 (2011).
- 42 Baxter CG, Denning DW, Jones AM, Todd A, Moore CB, Richardson MD. Performance of two *Aspergillus* IgG EIA assays compared with the precipitin test in chronic and allergic aspergillosis. *Clin. Microbiol. Infect.* 19(4), E197–E204 (2013).
- 43 Van Hoeyveld E, Dupont L, Bossuyt X. Quantification of IgG antibodies to *Aspergillus fumigatus* and pigeon antigens by ImmunoCAP technology: an alternative to the precipitation technique? *Clin. Chem.* 52(9), 1785–1793 (2006).
- 44 Smith NL, Denning DW. Underlying conditions in chronic pulmonary aspergillosis including simple aspergilloma. *Eur. Respir. J.* 37(4), 865–872 (2011).
- 45 Agarwal R, Khan A, Garg M, Aggarwal AN, Gupta D. Pictorial essay: allergic bronchopulmonary aspergillosis. *Indian J. Radiol. Imaging* 21(4), 242–252 (2011).
- 46 Agarwal R, Khan A, Garg M, Aggarwal AN, Gupta D. Chest radiographic and computed tomographic manifestations in allergic bronchopulmonary aspergillosis. *World J. Radiol.* 4(4), 141–150 (2012).
- 47 Malo JL, Pepys J, Simon G. Studies in chronic allergic bronchopulmonary aspergillosis. 2. Radiological findings. *Thorax* 32(3), 262–268 (1977).
- 48 McCarthy DS, Simon G, Hargreave FE. The radiological appearances in allergic broncho-pulmonary aspergillosis. *Clin. Radiol.* 21(4), 366–375 (1970).
- 49 Shah A. Allergic bronchopulmonary and sinus aspergillosis: the roentgenologic spectrum. *Front. Biosci.* 8, e138–e146 (2003).
- 50 Mintzer RA, Rogers LF, Kruglik GD, Rosenberg M, Neiman HL, Patterson R. The spectrum of radiologic findings in allergic bronchopulmonary aspergillosis. *Radiology* 127(2), 301–307 (1978).
- 51 Currie DC, Goldman JM, Cole PJ, Strickland B. Comparison of narrow section computed tomography and plain chest radiography in chronic allergic bronchopulmonary aspergillosis. *Clin. Radiol.* 38(6), 593–596 (1987).
- 52 Sandhu M, Mukhopadhyay S, Sharma SK. Allergic bronchopulmonary aspergillosis: a comparative evaluation of computed tomography with plain chest radiography. *Australas. Radiol.* 38(4), 288–293 (1994).
- 53 Panchal N, Pant C, Bhagat R, Shah A. Central bronchiectasis in allergic bronchopulmonary aspergillosis: comparative evaluation of computed tomography of the thorax with bronchography. *Eur. Respir. J.* 7(7), 1290–1293 (1994).
- 54 Ward S, Heyneman L, Lee MJ, Leung AN, Hansell DM, Muller NL. Accuracy of CT in the diagnosis of allergic bronchopulmonary aspergillosis in asthmatic patients. *AJR Am. J. Roentgenol.* 173(4), 937–942 (1999).
- 55 Neeld DA, Goodman LR, Gurney JW, Greenberger PA, Fink JN. Computerized tomography in the evaluation of allergic bronchopulmonary aspergillosis. *Am. Rev. Respir. Dis.* 142(5), 1200–1205 (1990).
- 56 Panchal N, Bhagat R, Pant C, Shah A. Allergic bronchopulmonary aspergillosis: the spectrum of computed tomography appearances. *Respir. Med.* 91(4), 213–219 (1997).
- 57 Angus RM, Davies ML, Cowan MD, McSharry C, Thomson NC. Computed tomographic scanning of the lung in patients with allergic bronchopulmonary aspergillosis and in asthmatic patients with a positive skin

- test to *Aspergillus fumigatus*. *Thorax* 49(6), 586–589 (1994).
- 58 Fairs A, Agbetele J, Hargadon B *et al*. IgE sensitisation to *Aspergillus fumigatus* is associated with reduced lung function in asthma. *Am. J. Respir. Crit. Care Med.* 182(11), 1362–1368 (2010).
- 59 Goyal R, White CS, Templeton PA, Britt EJ, Rubin LJ. High attenuation mucous plugs in allergic bronchopulmonary aspergillosis: CT appearance. *J. Comput. Assist. Tomogr.* 16(4), 649–650 (1992).
- 60 Logan PM, Muller NL. High-attenuation mucous plugging in allergic bronchopulmonary aspergillosis. *Can. Assoc. Radiol. J.* 47(5), 374–377 (1996).
- 61 Agarwal R, Aggarwal AN, Gupta D. High-attenuation mucus in allergic bronchopulmonary aspergillosis: another cause of diffuse high-attenuation pulmonary abnormality. *AJR Am. J. Roentgenol.* 186(3), 904 (2006).
- 62 Agarwal R. High attenuation mucoid impaction in allergic bronchopulmonary aspergillosis. *World J. Radiol.* 2(1), 41–43 (2010).
- 63 Agarwal R. Allergic bronchopulmonary aspergillosis: lessons for the busy radiologist. *World J. Radiol.* 3(7), 178–181 (2011).
- 64 Karunaratne N, Baraket M, Lim S, Ridley L. Case quiz. Thoracic CT illustrating hyperdense bronchial mucous plugging: allergic bronchopulmonary aspergillosis. *Australas. Radiol.* 47(3), 336–338 (2003).
- 65 Kopp W, Fotter R, Steiner H, Beaufort F, Stammberger H. Aspergillosis of the paranasal sinuses. *Radiology* 156(3), 715–716 (1985).
- 66 Dillon WP, Som PM, Fullerton GD. Hypointense MR signal in chronically inspissated sinonasal secretions. *Radiology* 174(1), 73–78 (1990).
- 67 Agarwal R, Gupta D, Aggarwal AN *et al*. Clinical significance of decline in serum IgE Levels in allergic bronchopulmonary aspergillosis. *Respir. Med.* 104(2), 204–210 (2010).
- 68 Madan K, Bal A, Agarwal R. Pleural effusion in a patient with allergic bronchopulmonary aspergillosis. *Respir. Care* 57(9), 1509–1513 (2012).
- 69 Agarwal R, Paul AS, Aggarwal AN, Gupta D, Jindal SK. Efficacy of cosmetic talc vs. iodopovidone for chemical pleurodesis: a prospective randomized controlled trial. *Respirology* 16(7), 1064–1069 (2011).
- 70 Agarwal R, Srinivas R, Agarwal AN, Saxena AK. Pulmonary masses in allergic bronchopulmonary aspergillosis: mechanistic explanations. *Respir. Care* 53(12), 1744–1748 (2008).
- 71 Agarwal R, Aggarwal AN, Garg M, Saikia B, Gupta D, Chakrabarti A. Allergic bronchopulmonary aspergillosis with aspergilloma: an immunologically severe disease with poor outcome. *Mycopathologia* 174(3), 193–201 (2012).
- 72 Agarwal R, Aggarwal AN, Gupta N, Gupta D. A rare cause of acute respiratory failure-allergic bronchopulmonary aspergillosis. *Mycoses* 54(4), e223–e227 (2011).
- 73 Agarwal R. Controversies in allergic bronchopulmonary aspergillosis. *Int. J. Respir. Care* 6(2), 56–63 (2010).
- 74 Lee TM, Greenberger PA, Patterson R, Roberts M, Liotta JL. Stage V (fibrotic) allergic bronchopulmonary aspergillosis. A review of 17 cases followed from diagnosis. *Arch. Intern. Med.* 147(2), 319–323 (1987).
- 75 Kumar R. Mild, moderate, and severe forms of allergic bronchopulmonary aspergillosis: a clinical and serologic evaluation. *Chest* 124(3), 890–892 (2003).
- 76 Agarwal R, Garg M, Aggarwal AN, Saikia B, Gupta D, Chakrabarti A. Serologic allergic bronchopulmonary aspergillosis (ABPA-S): Long-term outcomes. *Respir. Med.* 106(7), 942–947 (2012).
- 77 McCarthy DS, Pepys J. Allergic bronchopulmonary aspergillosis. Clinical immunology. 2. Skin, nasal and bronchial tests. *Clin. Allergy* 1(4), 415–432 (1971).
- 78 Campbell MJ, Clayton YM. Bronchopulmonary aspergillosis. A correlation of the clinical and laboratory findings in 272 patients investigated for bronchopulmonary aspergillosis. *Am. Rev. Respir. Dis.* 89, 186–196 (1964).
- 79 Pashley CH, Fairs A, Morley JP *et al*. Routine processing procedures for isolating filamentous fungi from respiratory sputum samples may underestimate fungal prevalence. *Med. Mycol.* 50(4), 433–438 (2012).
- 80 Denning DW, Park S, Lass-Flörl C *et al*. High-frequency triazole resistance found in nonculturable *Aspergillus fumigatus* from lungs of patients with chronic fungal disease. *Clin. Infect. Dis.* 52(9), 1123–1129 (2011).
- 81 Cramer R, Lidholm J, Menz G, Gronlund H, Blaser K. Automated serology with recombinant allergens. A feasibility study. *Adv. Exp. Med. Biol.* 409, 111–116 (1996).
- 82 Moser M, Cramer R, Brust E, Suter M, Menz G. Diagnostic value of recombinant *Aspergillus fumigatus* allergen I/a for skin testing and serology. *J. Allergy Clin. Immunol.* 93(1 Pt 1), 1–11 (1994).
- 83 Nikolaizik WH, Moser M, Cramer R *et al*. Identification of allergic bronchopulmonary aspergillosis in cystic fibrosis patients by recombinant *Aspergillus fumigatus* I/a-specific serology. *Am. J. Respir. Crit. Care Med.* 152(2), 634–639 (1995).
- 84 Nikolaizik WH, Weichel M, Blaser K, Cramer R. Intracutaneous tests with recombinant allergens in cystic fibrosis patients with allergic bronchopulmonary aspergillosis and Aspergillus allergy. *Am. J. Respir. Crit. Care Med.* 165(7), 916–921 (2002).
- 85 Hemmann S, Nikolaizik WH, Schoni MH, Blaser K, Cramer R. Differential IgE recognition of recombinant *Aspergillus fumigatus* allergens by cystic fibrosis patients with allergic bronchopulmonary aspergillosis or Aspergillus allergy. *Eur. J. Immunol.* 28(4), 1155–1160 (1998).
- 86 Kurup VP, Banerjee B, Hemmann S, Greenberger PA, Blaser K, Cramer R. Selected recombinant *Aspergillus fumigatus* allergens bind specifically to IgE in ABPA. *Clin. Exp. Allergy* 30(7), 988–993 (2000).
- 87 Cramer R. Recombinant *Aspergillus fumigatus* allergens: from the nucleotide sequences to clinical applications. *Int. Arch. Allergy. Immunol.* 115(2), 99–114 (1998).
- 88 Cramer R, Hemmann S, Ismail C, Menz G, Blaser K. Disease-specific recombinant allergens for the diagnosis of allergic bronchopulmonary aspergillosis. *Int. Immunol.* 10(8), 1211–1216 (1998).
- 89 Arruda LK, Mann BJ, Chapman MD. Selective expression of a major allergen and cytotoxin, Asp f I, in *Aspergillus fumigatus*. Implications for the immunopathogenesis of Aspergillus-related diseases. *J. Immunol.* 149(10), 3354–3359 (1992).
- 90 Knutsen AP, Hutcheson PS, Slavin RG, Kurup VP. IgE antibody to Aspergillus fumigatus recombinant allergens in cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *Allergy* 59(2), 198–203 (2004).
- 91 Casaulta C, Fluckiger S, Cramer R, Blaser K, Schoeni MH. Time course of antibody response to recombinant *Aspergillus fumigatus* antigens in cystic fibrosis with and without ABPA. *Pediatr. Allergy Immunol.* 16(3), 217–225 (2005).
- 92 Kurup VP, Knutsen AP, Moss RB, Bansal NK. Specific antibodies to recombinant allergens of *Aspergillus fumigatus* in cystic fibrosis patients with ABPA. *Clin. Mol. Allergy* 4, 11 (2006).

- 93 Cramer R, Lidholm J, Gronlund H, Stuber D, Blaser K, Menz G. Automated specific IgE assay with recombinant allergens: evaluation of the recombinant *Aspergillus fumigatus* allergen I in the Pharmacia Cap System. *Clin. Exp. Allergy* 26(12), 1411–1419 (1996).
- 94 Hemmann S, Ismail C, Blaser K, Menz G, Cramer R. Skin-test reactivity and isotype-specific immune responses to recombinant Asp f 3, a major allergen of *Aspergillus fumigatus*. *Clin. Exp. Allergy* 28(7), 860–867 (1998).
- 95 de Oliveira E, Giavina-Bianchi P, Fonseca LA, Franca AT, Kalil J. Allergic broncho-pulmonary aspergillosis' diagnosis remains a challenge. *Respir. Med.* 101(11), 2352–2357 (2007).
- 96 Denning DW, Pashley C, Hartl D *et al.* Fungal allergy in asthma-state of the art and research needs. *Clin. Transl. Allergy*, 4, 14 (2014).
- 97 Kauffman HF. Immunopathogenesis of allergic bronchopulmonary aspergillosis and airway remodeling. *Front. Biosci.* 8, e190–e196 (2003).
- 98 Latzin P, Hartl D, Regamey N, Frey U, Schoeni MH, Casaulta C. Comparison of serum markers for allergic bronchopulmonary aspergillosis in cystic fibrosis. *Eur. Respir. J.* 31(1), 36–42 (2008).
- 99 Hartl D, Latzin P, Zissel G, Krane M, Krauss-Etschmann S, Griesse M. Chemokines indicate allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 173(12), 1370–1376 (2006).
- 100 Gernez Y, Dunn CE, Everson C *et al.* Blood basophils from cystic fibrosis patients with allergic bronchopulmonary aspergillosis are primed and hyper-responsive to stimulation by *Aspergillus* allergens. *J. Cyst. Fibros.* 11(6), 502–510 (2012).