



Asthma and Fungus: Role in Allergic Bronchopulmonary Aspergillosis (ABPA) and Other Conditions

Meenu Singh¹ · Nandini Paul¹ · Shreya Singh² · Gyan Ranjan Nayak³

Received: 27 September 2017 / Accepted: 13 February 2018
© Dr. K C Chaudhuri Foundation 2018

Abstract

Asthma is an allergic, respiratory disorder characterized by hyper responsiveness of the airway to external stimuli. Considerable research is currently being directed towards understanding the role of environmental and genetic factors contributing to the development of asthma and its severity. Recent years have seen a substantial rise in evidence linking fungi to asthma. Few major clinical conditions associated with fungal sensitization and hypersensitive immune response are Allergic bronchopulmonary aspergillosis (ABPA), Allergic fungal rhinosinusitis (AFRS) and Severe asthma with fungal sensitization (SAFS). The most common fungi implicated in these conditions belong to genus *Aspergillus*, although an association with several other fungi has been described. In this review authors discuss the varying clinical characteristics of fungus induced respiratory complications in individuals with asthma. They also highlight the epidemiology of these conditions including their prevalence in children and their fungal etiological profile. Laboratory diagnostic methods and clinical case definitions have also been discussed. Future studies evaluating the role of fungal exposure and susceptibility to asthma are required. Till date there are no guidelines for the diagnosis and treatment of ABPA in pediatric population, thus it is also imperative to establish validated clinical definitions of fungal allergic manifestations in pediatric patients with asthma to fully understand this complex interaction.

Keywords Asthma · Fungus · Allergic bronchopulmonary aspergillosis (ABPA)

Introduction

Asthma is an allergic disorder of the respiratory system associated with recurrent symptoms of wheeze, chest tightness, airway remodelling and bronchial hyperresponsiveness. Two endotypes of asthma have been described *i.e.*, allergic asthma and intrinsic asthma, both differing in pathophysiology and treatment. Allergic asthma is predominant in children and is characterized by an elevated Th2 immune response. In children, it develops as an atopic march starting from allergic sensitization through eczema and allergic rhinitis to asthma.

The overproduction of IgE antibodies triggered by signalling cascade driven by interleukins (IL-4, IL-13 and IL-5) and activation of B cells to produce antibodies is the hallmark of Th2 immune response in asthma [1]. Most of the patients with asthma present with mild symptoms which are controlled with anti-inflammatory and bronchodilator therapy; a few cases (upto 10% of patients with asthma) suffer from severe obstruction and inflammation of the airways. Severe asthma is a phenotype of asthma presenting with difficult-to-treat symptoms. Several environmental factors including house dust mite, animal dander and environmental fungi are known to worsen the asthma control. Severe asthma has been found to be strongly associated with fungal atopy resulting in increased asthma severity, hospital admissions and intensive care admissions in adults and increased bronchial reactivity in children [2]. The link between severe asthma and fungi has been known for centuries but is yet to be clearly understood. Severe asthma with fungal sensitization (SAFS) has been recently described as a new phenotype of asthma, representing a march from allergic asthma to SAFS and finally the development of allergic bronchopulmonary aspergillosis (ABPA) [3].

Meenu Singh and Nandini Paul will act as first authors for this manuscript

✉ Meenu Singh
meenusingh4@gmail.com

¹ Advanced Pediatrics Centre, PGIMER, Chandigarh 160012, India

² Department of Microbiology, PGIMER, Chandigarh, India

³ Department of Otolaryngology, PGIMER, Chandigarh, India

Severe Asthma and Fungi

Fungal pathogens are amongst the most common factors contributing in severe forms of allergic asthma in India. Fungal exposure is universal, but the sensitization to fungi is not common. Fungi those are able to grow at body temperature cause infection in the lungs and lead to pulmonary disorders and include yeasts, *Aspergillus* and *Penicillium* genera. The most common fungal species affecting the lungs is *Aspergillus fumigatus*, although other species are also capable of causing lung infection [4]. *Aspergillus fumigatus*, a mould, found in indoor spaces and environment, sewage treatment facilities, moist decaying organic matter, potting soil and compost piles is the most prevalent organism responsible for complicating the allergic airway disease in developing countries [5]. The association of severe asthma and fungi results into multiple clinical conditions such as allergic fungal rhinosinusitis (AFRS), severe asthma with fungal sensitization (SAFS), allergic bronchopulmonary aspergillosis (ABPA) or allergic bronchopulmonary mycosis (ABPM) [6]. The first evidence of systematic study on the association of *Aspergillus* sensitization and severity of asthma was published in 1978 based on a survey conducted in Cleveland and London by using identical methods and study groups. Immediate hypersensitive immune response among patients with asthma was observed to be 28% in Cleveland and 23% in London whereas the frequencies of serum precipitins to *Aspergillus* were 7.5% in Cleveland and 10.5% in London [7]. This was the only study till 2005 when another study was conducted to understand the association among adults with severe asthma and *Aspergillus* sensitization in Indian tertiary healthcare centre. The frequency of *Aspergillus* sensitization among adult asthmatic subjects was 28.5% and of serum precipitins was 7.6% [8]. A few other studies have reported the frequency of *Aspergillus* sensitization and positivity to serum precipitins in asthmatic patients, 38% & 9% and 24% & 13%, respectively [9, 10]. Recently, a study conducted in South East Asian population with severe asthma groups including 61.2% Chinese, 16.5% Malay, 14.1% Indian and 8.3% other ethnic groups, has estimated 18.3% prevalence of *Aspergillus* sensitization in uncontrolled asthma and 4.1% in controlled asthma [11].

Allergic Bronchopulmonary Aspergillosis

ABPA is an allergic respiratory disorder characterized by airway hyperreactivity to the antigens of *Aspergillus fumigatus*, elevated serum biomarkers of fungal allergy including total IgE and *Aspergillus*-specific IgE and complicated asthma phenotype. After the first description of three cases of ABPA by Hinson et al. in 1952 and second report from United States in 1967, ABPA is not considered a rare entity throughout the world today. Various groups from around the world including UK, US,

Australia and India, are trying to resolve the complexity of its phenotypic and genomic diversity. Till now, UK has come up with 111 cases, US with 118 and two groups from India with 155 and 164 cases forming the large cohorts of ABPA among asthmatics [12]. There are a few reports of ABPA in children by Chetty et al. and Singh et al. and some case reports in India [13, 14]. Early diagnosis and management of ABPA can help to put a check on the severe outcomes and permanent lung damage due to fibrosis and failure to thrive.

Immediate hypersensitive immune response to *Aspergillus fumigatus* antigens and complicating asthma or cystic fibrosis are the characteristic features of allergic bronchopulmonary aspergillosis. Clinical features of ABPA include “poorly controlled asthma, recurrent wheeze, productive cough, hemoptysis, low grade fever, weight loss, malaise and fatigue.” Brownish black mucus plugs, consolidation and atelectasis have also been reported in a few studies. The diagnosis of ABPA is based on clinical, serological and radiological features. The diagnostic criteria includes the presence of asthma, hypersensitivity to *A. fumigatus* antigens, increased total IgE levels (>1000 IU/mL), increased *aspergillus*-specific IgE levels (>0.35 kUA/L) and serum precipitins, eosinophilia and proximal bronchiectasis [15]. These features lack the specificity and vary in different stages of ABPA. Recently, new diagnostic and staging criteria have been proposed by Agarwal et al. in international conference organized by International Society For Human and Animal Mycology (ISHAM). It included immediate hypersensitivity to *A. fumigatus* antigens, elevated IgE levels and *Aspergillus*-specific IgE levels as obligatory criteria, excluding central bronchiectasis [16]. Different stages of ABPA have been described as acute, remission, exacerbation, corticosteroid dependent asthma and fibrotic stage. These stages do not follow each other sequentially and have different features of diagnostic criteria, thus requiring different treatment strategies [17]. Later, Agarwal et al. has proposed new stages introducing asymptomatic stage.

Diagnosis and Classification of ABPA

Sensitisation and reactivity to *A. fumigatus* can be predicted by skin prick test. Various parameters are followed to confirm the diagnosis of ABPA, including serological and radiological findings. Antigens of *Aspergillus fumigatus* (Af) are found on the surface of epithelium and penetrate it by releasing proteases and escape the killing by mononuclear cells and eosinophils [18]. Positive sputum culture does not indicate the pathogenetic trait of *aspergillus*, and its correlation with severity of disease is yet to be established. Radin et al. conducted a study and demonstrated that there is a positive correlation between level of exposure to *Aspergillus* and exacerbations in ABPA [19]. The diagnosis of ABPA is made with clinical,

immunological and radiological characteristics. Criteria established by Rosenberg and Patterson is the most commonly used for diagnosis, whereas Agarwal et al. has also come up with a modified criteria for diagnosis of ABPA (Table 1).

Laboratory Tests

The main immunological parameters for the diagnosis of ABPA include: *Aspergillus* skin test, elevated total serum IgE levels, increased *Aspergillus*-specific IgE and IgG levels. Other than these, positive serum precipitins to *A. fumigatus*, peripheral eosinophilia are also included in the laboratory screening tests. *Aspergillus* skin test is the first choice of physicians for the diagnosis of ABPA. It is performed by using antigens of *A. fumigatus*, commercial or locally prepared. A positive immediate cutaneous hypersensitivity test to the antigens of *A. fumigatus* is the characteristic feature of ABPA and represents the presence of specific IgE antibodies to *A. fumigatus* (type I hypersensitivity). A late reactivity of *A. fumigatus* antigens detects the presence of type III hypersensitivity *i.e.* the formation of immune complexes. Total serum IgE levels ≥ 1000 IU/ml suggests the diagnosis of ABPA. These levels are altered significantly during the treatment with glucocorticoids and antifungal drugs. Measurement of the *aspergillus*-specific IgG antibodies further confirms the diagnosis. *Aspergillus fumigatus* specific IgG₁, IgG₂, and IgG₄, have been found to be raised in patients with ABPA but not IgG₃ antibody levels. *Aspergillus fumigatus* specific IgG₄ was the most specific among IgG subtypes for the confirmed diagnosis of ABPA [21].

Table 1 Criteria for the diagnosis of Allergic bronchopulmonary aspergillosis in asthma [20]

Patterson criteria

- Asthma, Immediate cutaneous hyperreactivity on *Aspergillus* skin test (type I reaction), Elevated serum IgE (>417 IU/mL), Elevated serum *A. fumigatus* specific IgE (>0.35 kUA/L), Precipitating antibodies (IgG) in serum against *A. fumigatus*, Eosinophilia (>1000 cells/mL), Central bronchiectasis, Transient or fixed pulmonary opacities.
- Diagnosis of ABPA is made on the presence of any five, six, seven or eight criteria.

Agarwal criteria

- Obligatory criteria: Asthma, Elevated total IgE levels (>1000 IU/mL), Elevated IgE against *A. fumigatus* (>0.35 kUA/L)
- Other criteria: Type I *Aspergillus* skin test positive, Presence of serum precipitating antibodies against *A. fumigatus*, Radiographic pulmonary opacities (fixed/transient), Total eosinophil count >1000 cells/mL, Central bronchiectasis on high resolution computed tomography of the chest.
- Diagnosis of ABPA is made on the presence of all obligatory criteria and three of five other criteria.

Classification of ABPA has been proposed by Patterson et al. [22], Kumar et al. [23] and Agarwal et al. [24] contributing to understand the complexity of disease. Patterson et al. proposed two categories, ABPA-S (seropositive), ABPA-CB (with central bronchiectasis); Kumar et al. introduced ABPA-CB-ORF and Agarwal et al. added ABPA-HAM (high attenuation mucus). The classification scheme is based on CT examination of chest and has been found useful in assessing severity and outcome in ABPA (Table 2).

Presence of bronchiectasis had been defined as the severe form of ABPA previously; recently, HAM is found to be associated to the immunological parameters to a greater extent [25]. ABPA-S fulfils the diagnostic criteria but there is absence of central bronchiectasis whereas, ABPA-CB shows the presence of central bronchiectasis on chest computed tomography [17]. Further, mild, moderate and severe forms were classified as ABP-S, ABPA-CB and ABPA-CB-ORF respectively and were based on the clinical, serological and radiological features of patients. ABPA-CB-ORF was the most severe form of ABPA with highest levels of serum total IgE, *Aspergillus*-specific IgE and having radiological features of pulmonary fibrosis, blebs, pneumothorax, pleural effusion and thickening along with central bronchiectasis [26]. The latest classification proposed by Agarwal group showed ABPA-CB-HAM characterized by greater density of mucus in comparison to paraspinal skeletal muscle, is the most severe form. Recurrent relapses, total and *Aspergillus*-specific IgE levels and eosinophil count were higher in HAM than in other forms of ABPA [24].

Allergic Fungal Rhinosinusitis

Allergic fungal rhinosinusitis (AFRS) is a distinct clinical entity and differs from other forms of rhinosinusitis and resembles allergic broncho-pulmonary aspergillosis (ABPA) [27, 28]. It was first recognised by Miller and later on described by Katzenstein [27]. It is characterized by type 1 hypersensitivity, eosinophilic mucin and trapped fungi within the sinuses. The organisms

Table 2 Classification criteria for ABPA by different groups

Author	Categories
Patterson et al. [22]	ABPA-S ABPA-CB
Kumar et al. [23]	ABPA-S (Mild) ABPA-CB (Moderate) ABPA-CB-ORF (Severe)
Agarwal et al. [24]	ABPA-S ABPA-CB ABPA-CB-HAM

responsible are *Aspergillus* and dematiaceous fungi like *Alternaria*, *Bipolaris*, *Curvularia*, and *Fusarium*. Bent and Kuhn developed the diagnostic criteria for AFRS which includes 5 major and 6 minor criteria. The major criteria includes type I hypersensitivity, nasal polyposis, characteristic radiologic finding, allergic mucin and positive fungal stain. Minor criteria are asthma, unilateral disease, Charcot-Leyden crystals, bony erosion on radiology, fungal culture and peripheral eosinophilia [29]. The hallmark of AFRS is “allergic mucin” which contains layers of necrotic and degranulating eosinophils, Charcot-Leyden crystals and fungal hyphae.

Clinically, patient is adolescent or young adult with complaints of nasal obstruction, nasal discharge, anosmia and headache. Approximately 50% of AFRS patients were noted to have associated bronchial asthma. Pediatric patients can also present with telecanthus and proptosis. Final diagnosis is only after histopathological evidence of allergic mucin. Treatment includes perioperative steroids and endoscopic surgical clearance of sinuses with serial follow up.

Immune Response in ABPA

Aspergillus fumigatus is universal in distribution and its exposure is unavoidable. The small spores of 3–5 µm can easily deposit in lower bronchial airways whereas, they are cleared from the airways of immunocompetent individuals [30]. The fungal spores become swollen and start germination in the airways resulting in the recognition of pathogen associated molecular patterns (PAMPs) present on the surface of *A. fumigatus* and binding to pattern recognition receptors *i.e.* Toll-like receptors (TLRs), C-type lectins (CTLs), on the epithelial cells of the host [31]. This is the first step in the generation of immune response in ABPA and leads to the activation of signalling cascade responsible for the production of inflammatory cytokines, prostaglandins and reactive oxygen species. In addition, the fungal metabolism results in the production of primary and secondary metabolites, exoproteases and other fungal products [32]. This results in compromised fungal clearance, breaching of the epithelium and activation of immune response. The innate and adaptive immune response is active against these allergens in ABPA. Alveolar macrophages serve as the first line of innate defense in ABPA, that ingest and phagocytose the inhaled conidia and prevent their germination to hyphae. Resident lung dendritic cells serve as the early effector cells against *Aspergillus* conidia and hyphae, and subsequently result in the activation of adaptive immune response [33].

ABPA is characterised by a pronounced CD4⁺ Th-2 lymphocyte response. This Th2 immune response is evidenced by enhanced immunoglobulin E (IgE), eosinophils, and inflammatory infiltrates [34]. This hypersensitivity immune response is associated with inflammatory destruction of the airways in response to *Aspergillus* allergens. Thus, prolonged

colonization of the airways with *Aspergillus* induces strong inflammatory responses and high IgE levels. The factors responsible for ABPA are not clearly understood yet, but it is believed that *Aspergillus*-specific IgE-mediated type-I hypersensitivity reactions and specific IgG-mediated type III hypersensitivity reactions play central roles in the pathogenesis of ABPA [35]. A number of genetic risk factors have recently been identified in the development of ABPA. These include polymorphism of HLA-DR and HLA-DQ, IL-4 receptor alpha chain (IL-4RA), IL-10-1082GA promoter, surfactant protein A2 (SP-A2), Mannose binding lectins (MBL) and cystic fibrosis transmembrane conductance regulator (CFTR) mutations. *Aspergillus* antigens mediated activation of T and B cells has been shown in humans and animal models. CD4⁺ Th-2 lymphocyte activation is thought to be the major factor involved in ABPA. In murine models of ABPA, the number of GM-CSF, IL-4 and IL-5 positive cells was found to be higher as compared to controls and IL-10 seemed to be a natural suppressor of proinflammatory cytokine production [19].

Regulatory T cells (T regs) have been shown to be critical in the maintenance of immune responses and T cell homeostasis. The natural T regulatory cells (nTregs) are self antigen specific CD4⁺ T cells that express CD25 in high levels and FoxP3. The adaptive or induced T regulatory cells arise as a result of the activation of mature T cells in the absence of optimal antigen exposure or co-stimulation or in the presence of certain inhibitory cytokines [36].

Several aspects of this problem remain unexplored specially in children necessitating more studies. Since fungal sensitization of children with asthma is increasing, there is a need for having more studies specially in different parts of the country.

Treatment

Early diagnosis and treatment of ABPA are important to prevent serious and potentially irreversible lung damage [37]. If ABPA is diagnosed early and treated promptly, the respiratory symptoms may be reduced, lung function may be improved and as a result, unnecessary treatments such as intravenous antibiotics may be avoided. This may also be beneficial in preventing long-term damage to the airways such as bronchiectasis and fibrosis.

The primary basis of ABPA treatment is either the suppression of airway hypersensitivity in response to the fungus by suppression of immune response and/or trying to reduce the fungal burden in the airways thus, reducing the allergic stimulus. Corticosteroids are used for the suppression of the immune response for 2–6 mo period with the most initial treatment with oral prednisolone of 0.5 mg/kg daily for 1–2 wk, then on alternate days for 6–8 wk, followed by tapering dose of 5–10 mg every 2 wk [30]. Treatment varies by stage, in that oral glucocorticosteroids are indicated in acute (stage 1) cases, may be tapered and withdrawn when the patient is in remission (stage

2), and addition of itraconazole is appropriate for patients with relapse (stage 3) or corticosteroid-dependent asthma (stage 4). Detecting pulmonary exacerbations is essential for limiting the destruction of airways, but the long term use of systemic corticosteroids has not been recommended, as there is no proof of this treatment in preventing progressive bronchial destruction.

The effectiveness of itraconazole as a steroid sparing and anti-inflammatory agent, presumably *via* reduction in the amount of fungus in the airway, has been convincingly demonstrated. Similar data are not available for other antifungal agents and inhaled corticosteroids. It is not clear if any therapy is effective in patients with end-stage pulmonary fibrosis (stage-5) but this stage is rarely, if ever, seen in pediatric cases.

Various antifungal agents are also used for the treatment of ABPA *i.e.*, itraconazole, voriconazole, ketoconazole and amphotericin B. But for these antifungal agents, long-term studies are required to demonstrate that treatment modifies the progressive decline in lung function seen with the disease [38]. Itraconazole in combination with inhaled corticosteroids has recently been shown to be useful for reducing eosinophilic airway inflammation, reducing systemic immune activation and reducing severe exacerbations over a period of 16 wk in adults with ABPA complicating asthma. The use of itraconazole in children is less well studied [39]. Itraconazole is an inhibitor of cytochrome P450 3A4. It increases exposure to methylprednisolone and dexamethasone but not prednisolone or endogenous cortisol. Concomitant use of itraconazole and inhaled budesonide can result in adrenal suppression and even Cushing syndrome. Itraconazole can be given at an adult dose of 200 mg twice daily or a pediatric dose of 5 mg/kg once daily (up to 200 mg). Itraconazole blood levels (target, $\geq 1 \mu\text{g/mL}$) to document adequate absorption can be helpful in patients with a poor response. Administration of itraconazole with cola beverage 1 h before meals or gastric acid suppression will improve absorption. Some patients need higher dosing or switching from capsules to the more bioavailable liquid cyclodextrin suspension (100 mg/5 mL) [35].

Voriconazole is the newest of the azole antifungal agents and it has been found to be effective in the treatment of invasive pulmonary aspergillosis in immunosuppressed people. But till now, its use has not been documented in the treatment of ABPA [40]. Glucocorticoids have been found to be effective in controlling asthma but in case of ABPA these are less effective. Environmental exposure may be associated with the development of the disorder but it has not been well studied. Areas with high concentrations of mold should be avoided (crawl spaces, decaying organic material, *etc*). Immunotherapy in ABPA has not been studied much, however fungal immunotherapy has serious side effects and dosage needs to be optimized.

Long term corticosteroid treatment is associated with various side effects such as weight gain, psychosis, gastric ulcers, hypertension, bone disorders, immune suppression, growth or development related disorders. Corticosteroid treatment

should be associated with pneumococcus and influenza vaccination and supplementation with calcium and vitamin D for monitoring bone disorders. ABPA patients receiving treatment with corticosteroids may develop invasive pulmonary aspergillosis and central nervous system disease as has been reported in some cases [41].

Various side effects of azole therapy include nausea, vomiting, diarrhea, fever, rash, and headache. Prolonged use of azoles for the treatment of ABPA affects liver function and causes elevated transaminase levels. Drug interactions with other drugs have been reported such as midazolam, cyclosporine and methylprednisolone but not prednisone [41].

New therapeutic treatments may come from specific antiproteases, that are currently being developed, and that will inhibit proteases from both fungal origin and those derived from the inflammatory response (eosinophils, mast cells). Furthermore, studies suggest that immune modulation with medications such as anti-IgE, anti-IL-4, anti-IL-13 monoclonal antibodies may be helpful in the treatment of ABPA. Reports demonstrating improvement in ABPA with omalizumab, an anti-IgE suggest that this therapy may be beneficial. However it is associated with a risk of anaphylaxis and an increased frequency of cardiac/ thromboembolic events and thus this treatment is not recommended as yet due to limited evidence of safety [42].

Although *Aspergillus* is ubiquitous in distribution, the identification of environmental niches supporting its active growth may be advised and remediation considered. Theoretically, this may reduce the ongoing exposure to fungal antigens however, it may not be practically possible and evidence of definite benefit is lacking [41].

Surgical management has a limited role but may be necessary in case of progression to aspergilloma or necrotizing pneumonia and surgical resection may be considered in case of heavy bleeds from these necrotic areas.

Contributions MS: Article conceived and drafted. NP: Literature search and writing; SS and GN: Literature search and review. MS will act as guarantor for this paper.

Compliance with Ethical Standards

Conflict of Interest None.

Source of Funding ICMR, New Delhi has supported the fellowship of Ms. Nandini Paul.

References

1. Lambrecht BN, Hammad H. The immunology of asthma. *Nat Immunol.* 2015;16:45–56.
2. 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.* 2006;27:615–26.

3. Agarwal R. Severe asthma with fungal sensitization. *Curr Allergy Asthma Rep.* 2011;11:403–13.
4. Hedayati MT, Pasqualotto AC, Warn PA, Bowyer P, Denning DW. *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. *Microbiology.* 2007;153:1677–92.
5. Kradin RL, Mark EJ. The pathology of pulmonary disorders due to *aspergillus* spp. *Arch Pathol Lab Med.* 2008;132:606–14.
6. Rick EM, Woolnough K, Pashley CH, Wardlaw AJ. Allergic fungal airway disease. *J Investig Allergol Clin Immunol.* 2016;26:344–54.
7. Schwartz HJ, Citron KM, Chester EH, et al. A comparison of the prevalence of sensitization to *aspergillus* antigens among asthmatics in Cleveland and London. *J Allergy Clin Immunol.* 1978;62:9–14.
8. Maurya V, Gu gnani HC, Sarma PU, Madan T, Shah A. Sensitization to *aspergillus* antigens and occurrence of allergic bronchopulmonary aspergillosis in patients with asthma. *Chest.* 2005;127:1252–9.
9. Longbottom JL, Pepys J. Pulmonary aspergillosis: diagnostic and immunological significance of antigens and c-substance in *aspergillus fumigatus* XXXVIII in the serum of patients suffering from pulmonary aspergillosis. *J Pathol Bacteriol.* 1964;88:141–51.
10. Hendrick DJ, Davies RJ, D'Souza MF, Pepys J. An analysis of skin prick test reactions in 656 asthmatic patients. *Thorax.* 1975;30:2–8.
11. Goh KJ, Chau A, Yui A, Lapperre TS, Chan AKW, Chew FT. Sensitization to *aspergillus* species is associated with frequent exacerbations in severe asthma. *J Asthma Allergy.* 2017;10:131–40.
12. Agarwal R, Gupta D, Aggarwal AN, et al. Clinical significance of decline in serum IgE levels in allergic bronchopulmonary aspergillosis. *Respir Med.* 2010;104:204–10.
13. Chetty A, Bhargava S, Jain RK. Allergic bronchopulmonary aspergillosis in Indian children with bronchial asthma. *Ann Allergy.* 1985;54:46–9.
14. Singh M, Das S, Chauhan A, et al. The diagnostic criteria for allergic bronchopulmonary aspergillosis in children with poorly controlled asthma need to be re-evaluated. *Acta Paediatr.* 2015;105:e206–9.
15. 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.* 1977;86:405–14.
16. 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.* 2013;43:850–73.
17. Patterson R, Greenberger PA, Radin RC, Roberts M. Allergic bronchopulmonary aspergillosis: staging as an aid to management. *Ann Intern Med.* 1982;96:286–91.
18. Kauffman HF. Immunopathogenesis of allergic bronchopulmonary aspergillosis and airway remodeling. *Front Biosci.* 2003;8:e190–6.
19. Radin RC, Greenberger PA, Patterson R, Ghory A. Mould counts and exacerbations of allergic bronchopulmonary aspergillosis. *Clin Allergy.* 1983;13:271–5.
20. 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.* 2013;8:1–7.
21. Thia LP, Balfour Lynn IM. Diagnosing allergic bronchopulmonary aspergillosis in children with cystic fibrosis. *Paediatr Respir Rev.* 2009;10:37–42.
22. 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.* 1986;146:916–8.
23. Kumar R. Mild, moderate and severe forms of allergic bronchopulmonary aspergillosis. A clinical and serologic evaluation. *Chest.* 2003;124:890–2.
24. 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.* 2010;5:1–9.
25. Agarwal R. Pictorial essay: allergic bronchopulmonary aspergillosis. *World J Radiol.* 2011;3:178–81.
26. Kumar R. Mild, moderate, and severe forms of allergic bronchopulmonary aspergillosis: a clinical and serologic evaluation. *Chest.* 2003;124:890–2.
27. Katzenstein ALA, Sale SR, Greenberger PA. Allergic *aspergillus* sinusitis: a newly recognized form of sinusitis. *J Allergy Clin Immunol.* 1983;72:89–93.
28. Safirstein BH. Allergic bronchopulmonary aspergillosis with obstruction of the upper respiratory tract. *Chest.* 1976;70:788–90.
29. Bent JP, Kuhn FA. Diagnosis of allergic fungal sinusitis. *Otolaryngol Head Neck Surg.* 1994;111:580–8.
30. Tracy MC, Okorie CUA, Foley EA, Moss RB. Allergic bronchopulmonary aspergillosis. *J Fungi.* 2016;2:17.
31. Gresnigt MS, Netea MG, Van de Veerdonk FL. Pattern recognition receptors and their role in invasive aspergillosis. *Ann N Y Acad Sci.* 2012;1273:60–7.
32. Blanco JL, Garcia ME. Immune response to fungal infections. *Vet Immunol Immunopathol.* 2008;125:47–70.
33. Rapaka RR, Kolls JK. Pathogenesis of allergic bronchopulmonary aspergillosis in cystic fibrosis: current understanding and future directions. *Med Mycol.* 2009;47:S331–7.
34. Kurup VP, Raju R, Manickam P. Profile of gene expression in a murine model of allergic bronchopulmonary aspergillosis. *Society.* 2005;73:4381–4.
35. Fricker-Hidalgo H, Coltey B, Llerena C, et al. Recombinant allergens combined with biological markers in the diagnosis of allergic bronchopulmonary aspergillosis in cystic fibrosis patients. *Clin Vaccine Immunol.* 2010;17:1330–6.
36. Nouri-Aria KT, Durham SR. Regulatory T cells and allergic disease. *Inflamm Allergy Drug Targets.* 2008;7:237–52.
37. Kraemer R, Deloséa N, Ballinari P, Gallati S, Cramer R. Effect of allergic bronchopulmonary aspergillosis on lung function in children with cystic fibrosis. *Am J Respir Crit Care Med.* 2006;174:1211–20.
38. Nandakumar S, Miller CW, Kumaraguru U. T regulatory cells: an overview and intervention techniques to modulate allergy outcome. *Clin Mol Allergy.* 2009;7:5.
39. Skowronski E, Fitzgerald DA. Life-threatening allergic bronchopulmonary aspergillosis in a well child with cystic fibrosis. *Med J Aust.* 2005;182:482–3.
40. Wark P. Pathogenesis of allergic bronchopulmonary aspergillosis and an evidence-based review of azoles in treatment. *Respir Med.* 2004;98:915–23.
41. Patterson K, Strek ME. Allergic bronchopulmonary aspergillosis. *Proc Am Thorac Soc.* 2010;7:237–44.
42. FDA Safety Notice. FDA issues early communication concerning safety of asthma drug Xolair. *MD Consult* July 17, 2009.