RESEARCH ARTICLE

Allergic Bronchopulmonary Aspergillosis and Fungal Allergy in Severe Asthma

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ABSTRACT

Allergic hypersensitivity to fungi, especially to *Aspergillus fumigatus*, in asthmatic and cystic fibrosis patients may be complicated by the development of allergic bronchopulmonary aspergillosis (ABPA) or allergic fungal bronchopulmonary mycosis (ABPM). Recently, two fungal allergic disorders causing severe asthma have been described, allergic fungal asthma disease (AFAD) and severe asthma associated with fungal sensitivity (SAFS). In AFAD and SAFS, the principal fungal sensitivity is to *A. fumigatus*, and it has been proposed that AFAD and perhaps SAFS may be milder forms of ABPA. In this review, the clinical features, immunopathology, genetic risks, and treatment of ABPA, ABPM, AFAD and SAFS are discussed.

Abbreviations and Key Words

ABPA, allergic bronchopulmonary aspergillosis
AFAD, allergic fungal asthma disease
ABPM, allergic fungal bronchopulmonary mycosis
BALF, bronchoalveolar lavage fluid
BALT, bronchoalveolar lymphoid tissue
CF, cystic fibrosis
CFTR, cystic fibrosis transmembrane conductance regulator
HAM, high attenuated mucus
HRCT, high-resolution computed tomography
IL4RA, IL-4 receptor alpha chain
PAR2, protease associated receptor type 2
SAFS, severe asthma associated with fungal sensitivity
SNP, single-nucleotide polymorphism,

INTRODUCTION:

Allergic bronchopulmonary aspergillosis (ABPA) is the prototypic complication of *Aspergillus fumigatus* allergic hypersensitivity reaction in asthmatic and cystic fibrosis (CF) patients whose airways become colonized with *A. fumigatus*.[1-8] Though *A. fumigatus* predominates in ABPA, other *Aspergillus* species, *A. niger*, *A. terreus*, *A. flavus*, *A. oryzae*, and *A. ochraceus* may also cause ABPA. ABPA occurs in approximately 1-2% of asthmatic and 7-9% of CF patients. As seen in Table 1, other fungal species, such as *Candida*, *Bipolaris*, *Curvularia, Penicillium*, and *Alternaria* have been identified as causing symptoms similar to *Aspergillus fumigatus* classified as allergic bronchopulmonary mycosis (ABPM)(9). It is evident that fungal sensitivity is also a risk factor for development of severe persistent asthma, especially in adults with fixed airway obstruction. Denning et al[10, 11] have coined the term "severe asthma associated with fungal sensitivity (SAFS)" and Wardlaw et al[12, 13] have named it "allergic fungal asthma disease (AFAD)". SAFS and AFAD occur in a greater percentage of asthmatic patients. There are similarities of ABPA and AFAD and SAFS in terms of immunopathogenesis, immunogenetic risks, and fungal colonization; however, there are also differences. Thus, allergic sensitivity to fungi in asthmatic and CF patients displays a spectrum of phenotype from allergic asthma to SAFS, AFAD, ABPM, and ABPA.

ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

ABPA is a $CD4^+$ Th2 hypersensitivity lung disease caused by bronchial colonization with *Aspergillus fumigatus* (Table 1).[3, 8] Acute episodes of ABPA are characterized by worsening of asthma symptoms, worsening of lung function, recurrent transient pulmonary infiltrates, peripheral blood and pulmonary eosinophilia, elevated total serum IgE level, and elevated *A. fumigatus* specific IgE, IgG, and IgA antibody levels. During episodes of ABPA exacerbation, expectoration of thick brown mucoid sputum containing *A. fumigatus* hyphae may be present. Radiographically, there may be transient opacities of high attenuated mucus (HAM) that results in consolidation on chest radiograph, toothpaste/glove-in-finger opacities, tram-track opacities, ring shadow, and bronchiectasis may be present.[8] This is due to bronchi-filled hyperdense high attenuation mucus. Histologically, the bronchial airways are inflamed consisting of eosinophils, lymphocytes, and plasma cells. The airway lumen may be occluded by mucus containing *Aspergillus* hyphae and eosinophils. The bronchial epithelium demonstrates metaplasia and bronchocentric granuloma which may develop. With long-standing inflammations bronchiectasis, bronchial fibrosis and/or bronchiolitis obliterans may develop.

IMMUNOPATHOGENESIS of ABPA

In the pathogenesis of ABPA, *A. fumigatus* spores 3-5 microns in size are inhaled and germinate into the hyphae deep within the bronchi.[14] *Aspergillus* is thermotolerant and

grows well at body temperature. Other *Aspergillus* species, *A. niger*, *A. terreus*, *A. flavus*, *A. oryzae*, and *A. ochraceus*, may also cause ABPA or ABPA-like disease. The first line of defense against *Aspergillus* colonization in the lungs is macrophage and neutrophil killing of the conidia and the hyphae. There are several lines of evidence that the bronchoalveolar lymphoid tissue (BALT) initiates and maintains an immune response to *Aspergillus* with subsequent trafficking to the peripheral lymphoid system. Greenberger et al[15] demonstrated that IgE and IgA anti-*Aspergillus* antibodies were primarily synthesized within the BALT; whereas IgG anti-*Aspergillus* antibodies were produced in the peripheral lymphoid tissue Analysis of cells obtained from bronchoalveolar lavage fluid (BALF) in ABPA reveal an admixture of alveolar macrophages, eosinophils and lymphocytes, similar to that found in asthma on lung biopsy. Eosinophil infiltration predominated both in BALF and lung tissue as is evident in lung biopsy. In addition, eosinophils are activated and have released their mediators, such as major basic protein and eosinophil cationic protein. Th2 cytokine IL-5, which is essential for the activation of eosinophils, is essential in the development of ABPA. In addition, *Aspergillus* release a variety of proteins, including proteases, catalases, ribotoxin, superoxide dismutases, phosphatases, hemolysin, gliotoxin, and phthioic acid that have biologic as well as immunogenic properties.[14] In particular, *Aspergillus* proteases induce respiratory epithelial disruption and detachment and induce bronchial epithelial cell lines to produce pro-inflammatory chemokines and cytokines, such as IL-6, IL-8 and MCP-1. Proteases from *Alternaria* and *Cladosporium* also induce these same effects on the bronchial epithelia. These proteases also stimulate epithelial protease associated receptor type 2 (PAR2) that induce a Th2 inflammatory response.

Allergic bronchopulmonary mycosis (ABPM) is an ABPA-like disorder due to hypersensitivity reaction to fungi other than *A. fumigatus* (Table 1).[9] In the classification of asthma endotype and phenotypes, ABPM and ABPA are classified as ABPM endotype associated with an allergic eosinophilic phenotype.[16] ABPM is characterized by occurring in severe asthma, fungal colonization of the airway, eosinophilia, elevated serum IgE level, elevated specific anti-fungal antibody, less reversible or fixed airway disease, bronchiectasis, and bronchocentric granulomatosis. As seen in Table 1, the clinical, immunologic and radiographic findings in ABPM are similar to those seen in ABPA. *Candida albicans* is the predominant fungus identified, but other fungi, such as *Bipolaris, Schizophyllum, Curvularia*, and *Pseudallescheria*, have been identified. Two additional fungal sensitivities in severe asthma phenotypes should be considered in the ABPM endotype, namely AFAD and SAFS (Table 1). AFAD resembles ABPA in many ways. AFAD is caused by airway colonization of thermotolerant filamentous fungi, *Aspergillus* and *Penicillium*, and the yeast *Candida*.[13] Pulmonary function demonstrates reduced FEV-1 with fixed airway obstruction. Serum IgE levels are elevated but <1000 IU/ml, elevated

Aspergillus specific IgE and IgG antibody, eosinophilia, and radiographic evidence of HAM infiltrates and bronchiectasis. Woolnough et al[13] have proposed that severity of AFAD be divided into mild, moderate and severe with ABPA representing the severe form of AFAD. Denning et al[17] have characterized SAFS as severe asthma associated with fungal sensitivities, elevated serum IgE level but <1000 IU/ml and elevated fungal-specific IgE (Table 1). Chest radiograph abnormalities were not included in the criteria of SAFS. Pulmonary function also demonstrates reduced FEV-1. A variety of fungal sensitivities have been identified, but *A. fumigatus* occurs in 66% of patients. Other fungi that have been reported in SAFS include *Cladosporium* (52%), *Alternaria* (34%), *Penicillium* (48%), *Candida* (66%), *Trichophyton* (31%) and *Botrytis* (28%). As illustrated, fungal sensitivity in asthmatic patients may develop a spectrum of phenotypes. Recently, the International Society for Human and Animal Mycology (ISHAM) proposed revised diagnostic criteria of ABPA in asthmatic and CF patients,[8, 18] as seen in Table 2. 'Essential criteria' include serum IgE >1000 U/ml and elevated *Aspergillus* specific IgE. 'Additional criteria' (must have 2 of the 3) include eosinophilia ≥500 eosinophils/μL, *Aspergillus* specific IgG >27 mgA/ml, and chest radiograph and/or high resolution computed tomography (HRCT) consistent with ABPA. However, not included was the identification of *Aspergillus* or other fungi in the sputum, which was a minor criterion in the criteria proposed by Greenberger et al[4]. However, current technique of culturing *Aspergillus* from sputum is insensitive.[13] Woolnough et al[13] have proposed improved handling of sputum to increase the sensitivity of identifying *Aspergillus* as well as molecular techniques. As seen in Table 1, bronchial colonization with *Aspergillus* and/or other fungi is an important component in the immunopathogesis of ABPA, ABPM, AFAD, and SAFS. Furthermore, it is important especially in CF patients in differentiating between a CF flare versus an ABPA flare. Additionally, identification of the fungus would be important in differentiating ABPA versus ABPM. Also as seen in AFAD, there are similarities to ABPA with the only exception being an IgE <1000 IU/ml. Indeed, Woolnough et al[13] have proposed a spectrum of AFAD to ABPA. Identification of a fungus also may be beneficial in treating with antifungal medication. Thus, *Aspergillus* containing sputum is an important 'Additional Criteria' in the diagnosis and treatment of ABPA, as well as other disorders of ABPM, AFAD and SAFS.

GENETIC RISKS of ABPA

HLA-DR and HLA-DQ expression. Genetic risk factors have been identified in the development of ABPA (Table 3). Chauhan et al[19-21] reported that asthmatic and CF patients who expressed *HLA-DR2* and/or *HLA-DR5* and lacked *HLA-DQ2* were at increased risk to develop ABPA after exposure to *A. fumigatus.* Furthermore, within *HLA-DR2* and *HLA-DR5*, there were restricted genotypes. In particular, *HLA-DRB1*1501* and *HLA-DRB1*1503* were reported to produce high relative risk. On the other hand, 40% to 44% of non-ABPA

Table 1. Comparison of allergic bronchopulmonary aspergillosis, allergic bronchopulmonary mycosis, allergic fungal asthma disease, and severe asthma with fungal sensitivity.

Parameter	ABPA	ABPM	AFAD	SAFS
Patients at risk	Asthma, CF	Asthma, CF	Asthma	Asthma
Lung function	Worsening with exacerbation	Worsening with exacerbation	Decreased FEV-1 Fixed airway obstruction	Decreased FEV-1
Serum IgE	≥1000 IU/ml	≥1000 IU/ml	<1000 IU/ml	<1000 IU/ml
Fungal sIgE or Fungal prick skin test	*Aspergillus* sIgE >0.35 *Aspergillus* +	Fungal sIgE >0.35 +	*Aspergillus* sIgE >0.35 *Aspergillus* +	Fungal sIgE >0.35 +
Fungal sIgG	*Aspergillus* sIgG >27 mgA/ml	± Fungal sIgG	*Aspergillus* sIgG >40 mgA/ml in 40%	
Blood eosinophils	≥500 eosinophils/μL	≥500 eosinophils/μL	≥500 eosinophils/μL	
Chest Radiograph/HRCT	Fleeting opacities High attenuated mucus Bronchiectasis	Fleeting opacities Bronchiectasis	Infiltrates High attenuated mucus Bronchiectasis	
Bronchial fungal colonization	*Aspergillus fumigatus*	*Candida* – 60% *Bipolaris* – 13% *Schizophyllum* – 11% *Curvulaia* – 8% *Pseudallescheria* – 3% Rare *Alternaria*, *Fusarium*, *Penicillium*, *Cladosporium*, *Stemphylium*, *Rhizopus*, *Saccharomyces*, *Trichosporon*	*Aspergillus fumigatus* *Penicillium* chrysogenum *Candida albicans*	*Aspergillus* – 66% *Cladosporium* – 52% *Alternaria* – 34% *Penicillium* – 48% *Candida* – 66% *Trichophyton* – 31% *Botrytis* – 28%

Abbreviations: ABPA, allergic bronchopulmonary aspergillosis; ABPM, allergic bronchopulmonary mycosis; AFAD, allergic fungal asthma disease; SAFS, severe asthma associated with fungal sensitivity; sIgE, specific IgE; sIgG, specific IgG

atopic *Aspergillus*-sensitive individuals also have the *HLA-DR2* and/or *HLA-DR5* genotypes. Additional studies indicated that the presence of *HLA-DQ2*, especially *HLA-DQB1*0201*, provided protection from the development of ABPA even in the presence of *HLA-DR2* and/or *HLA-DR5*.

Table 2. International Society for Human and Animal Mycology (ISHAM) diagnostic criteria of allergic bronchopulmonary aspergillosis.

ISHAM CRITERIA
A. Patients with asthma or cystic fibrosis
i. Worsening lung function not attributable to another etiology
B. Essential Criteria (both must be met)
i. Total serum IgE ≥1000 IU/ml
ii. Positive skin prick test with *Aspergillus* species or *Aspergillus* specific IgE >0.35
C. Additional Criteria (at least 2 of the 3)
i. **Serum eosinophilia ≥500 eosinophils/µL**
ii. ***Serum Aspergillus* IgG >27 mgA/ml**
iii. **Chest radiograph/HRCT consistent with ABPA: New or recent abnormalities on chest radiography or chest CT that have not cleared with antibiotics and standard physiotherapy, such as nodules, consolidation, mucoid impaction, HAM, fleeting opacities, toothpaste/gloved-finger opacities, tram-track opacities) or permanent parallel lines, ring shadows, bronchiectasis, pleuropulmonary fibrosis**
iv. [#]***Aspergillus* containing mucus plugs**

[*]Adapted from Agarwal R et al[8, 18].
[#]Not a criterion in the ISHAM criteria.

IL4RA polymporphisms. Recently, we reported an increased frequency of single-nucleotide polymorphisms (SNP) of the IL-4 receptor alpha chain (*IL4RA*) in 92% of ABPA subjects, principally the IL-4-binding single-nucleotide polymorphism ile75val.[22-25] This was associated with increased sensitivity to *in vitro* IL-4 stimulation as measured by enhanced expression of the low-affinity IgE receptor (CD23) on B cells. This increased sensitivity to IL-4 is demonstrated by increased expression of CD23 and CD86 on B cells of ABPA subjects and increased CD23 expression during flares of ABPA. CD23 is expressed on a variety of cells, including B cells, natural killer cells, subpopulations of T cells, and a subpopulation of dendritic cells. T-cell CD23 and B-cell CD21 form a costimulatory pathway. T-cell CD28 and B-cells CD80 and CD86 costimulatory pathways activate both T and B cells, and CD28:CD86 is important in IgE synthesis. CD86 is also found on dendritic cells that

have the histamine receptor 2, which skews antigen-specific T cells to a Th2 response.

Examination of risk factors in AFAD and SAFS have not been extensively examined. However, in children with moderate-severe asthma sensitivities to *Alternaria* and *Aspergillus,* Knutsen et al[26, 27] reported HLA-DR restriction with increased frequency of HLA-DRB1*13 and HLA-DRB1*03. In addition, there was decreased frequency of HLA-DQB1*03, suggesting that HLA-DQB1*03 may be protective of the development of *Alternaria*/*Aspergillus* sensitive severe asthma (Table 3). Furthermore, there was significantly increased expression of the *IL4RA* ile75val SNP in 83% (0.388 allele frequency) in the fungal sensitive moderate-severe asthmatic children. This was associated with increased sensitivity to IL-4 stimulation measured by significantly increased IL-4 stimulated CD23 expression. There was also significantly increased IL-5 and IL-13 synthesis of lymphocytes stimulated by *Alternaria* and *Aspergillus*. Similar studies in adult asthmatics patients with either SAFS and/or AFAD need to be examined.

CFTR. Because ABPA is found in highest incidence among atopic patients with CF, Miller et al[28] examined mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) in subjects without CF. They reported that mutations were present at a higher frequency in asthmatic patients who developed ABPA, 6 of 21 (28.5%), versus control asthmatics, 2 of 43 (4.6%). These ABPA patients were heterozygous for the mutations (1 patient was compound heterozygote and reclassified as atypical CF), did not have a clinical diagnosis of CF, and had sweat chlorides <60mEq/L. Gamaletsou et al[29] subsequently confirmed these findings in a study of 189 ABPA patients. Heterozygous *CFTR* mutations were identified in 9.5% of patients and the major *CFTR* mutation was F508del mutation in 7.7% of ABPA patients (Table 3). Although the abnormal airway mucus in CF is thought to be a susceptibility factor for ABPA due to enhanced trapping of *Aspergillus* spores, it is unclear what effect heterozygous *CFTR* mutations may have on mucus quality in asthmatic airways. Recently, Fair et al[12] also reported heterozygous CFTR mutations in 7.5% of AFAD patients.

IL-10 promoter polymorphism. Brouard et al[30] reported that the *-1082GG* genotype of the IL-10 promoter was associated with *A. fumigatus* colonization and the development of ABPA in CF. The *-1082GG* polymorphism has been associated with increased IL-10 synthesis; whereas the *-1082A* allele has lower IL-10 synthesis. Our group[25] reported that the combination of increased expression of *HLA-DR2* and/or *HLA-DR5*, increased *IL-4RA* ile75val SNP expression, increased sensitivity to IL-4 stimulation, and increased *-1082GG* genotype was associated with increased risk in the development of ABPA with an odds ratio (OR) of 8.0 (3.0-21.7 95% CI interval, p <0.0001). Thus, these underlying genetic risks maybe responsible for skewing *Aspergillus*-specific Th2 responses in ABPA.

Table 3. Genetic risks of allergic bronchopulmonary aspergillosis and severe asthma associated with fungal sensitivity.

Genetic factor	ABPA	Fungal Sensitive	
		Adults with SAFS and/or AFAD	Children with moderate-severe asthma
HLA-DR restricted	HLA-DR2 (DRB1*15, B1*16) HLA-DR5 (DRB1*1, HLA-DRB1*12)		HLA-DR restricted
HLA-DQ protective	HLA-DQ2 (DQB1*02)		HLA-DQ3 (DQB1*03)
IL4RA SNP	rs1805010 (ile75val) rs1801275 (gln576arg) rs3024656 (intron)		rs1805010 (ile75val)
IL13 SNP	rs20541 (arg110gln)		
IL10 promoter SNP	-1082GG		
Surfactant protein A2	ala91pro, arg94arg 80% carrying both SNPs had ABPA Elevated total IgE levels and higher percentages of eosinophilia Binds *Aspergillus*		
CFTR gene	Heterozygous 9.5%, F508del 7.7%	Heterozygous 7.5% (AFAD)	
Toll-like receptor 9	Promoter T-1237C Decreases expression of TLR9		
Mannose binding lectin	*MBL2* G+1011 SNP Increased MBL levels Binds *Aspergillus*		

Abbreviations: ABPA, allergic bronchopulmonary aspergillosis; ABPM, allergic bronchopulmonary mycosis; AFAD, allergic fungal asthma disease; SAFS, severe asthma associated with fungal sensitivity; CFTR, cystic fibrosis transmembrane conductance regulator; IL4RA, IL-4 receptor alpha chain; MBL, mannose binding lectin; SNP, single nucleotide polymorphism; TLR, toll-like receptor.

Table 4. Clinical staging classification of allergic bronchopulmonary aspergillosis.

Stage	Definition	Features
0	Asymptomatic	• No previous diagnosis of ABPA • Controlled asthma (according to GINA/EPR-3 guidelines) • Fulfilling the diagnostic criteria of ABPA (Table 2)
1	Acute	• No previous diagnosis of ABPA • Uncontrolled asthma/symptoms consistent with ABPA • Meeting the diagnostic criteria of ABPA (Table 2)
1a	With mucoid impaction	• Mucoid impaction observed on chest radiograph or bronchoscopy
1b	Without mucoid impaction	• Absence of mucoid impaction observed on chest radiograph or bronchoscopy
2	Response	• Clinical and/or radiological improvement AND • Decline in IgE by ≥25% of baseline at 8 weeks
3	Exacerbation	• Clinical and/or radiological worsening AND • Increase in IgE by ≥50% from baseline established during Response/Remission stages
4	Remission	• Sustained clinical and radiological improvement • IgE levels persisting at or below baseline (or IgE level increase by <50% for ≥6 months off treatment)
5a	Treatment-dependent ABPA	• ≥2 Exacerbations within 6 months of stopping therapy OR • Worsening of clinical and/or radiological condition, along with immunological worsening (rise of IgE levels) on tapering oral glucocorticosteroids/azoles
5b	Corticosteroid-dependent asthma	• Systemic corticosteroids required for control of asthma while the ABPA activity is controlled as indicated by IgE levels and radiologic imaging
6	Advanced ABPA	• Extensive bronchiectasis due to ABPA on chest imaging • Complications (cor pulmonale and/or chronic type II respiratory failure)

Adapted from Agarwal R et al[8].

Abbreviations: ABPA, allergic bronchopulmonary aspergillosis; EPR-3, Expert Panel Report 3; GINA, Global Initiative for Asthma.

Surfactant protein A2. Saxena et al[31] reported that ABPA patients with polymorphisms (ala91pro, arg94arg) in the collagen region of pulmonary surfactant protein A2 (*SP-A2*) had increased total IgE levels and increased percentages of eosinophilia compared to those patients who lacked these SNPs (Table 3)..

Table 5. Treatment regimens of allergic bronchopulmonary aspergillosis.

Medication	Dose
Prednisone – Medium dose	*Adult*: 0.5 mg/kg/day for 2 weeks, Followed by 0.5 mg/kg/day on alternate days for 8 weeks, *Pediatrics*: 0.5-1.0mg/kg/day for 2 weeks, Followed by 0.5-1.0 mg/kg/day on alternate days for 8 weeks, Then taper by 5 mg every 2 weeks and discontinue after 3–5 months
Prednisone – High dose	*Adult*: 0.75 mg/kg/day for 6 weeks, Followed by 0.5 mg/kg/day for 6 weeks, *Pediatrics*: 0.5-1.0 mg/kg/day for 6 weeks, Followed by 0.5-1.0 mg/kg/day for 6 weeks, Then taper by 5 mg every 6 weeks and discontinue after 8–10 months
Pulse Methylprednisolone	15 mg/kg/dose (maximum 1 gram) for 3 consecutive days
Itraconazole	*Adult asthma*: 300-400 mg/day divided bid *Adult CF*: 500-1000 mg/day divided bid *Pediatrics*: 5-10-mg/kg/day divided bid if ≥200 mg bid
Voriconazole	*Adult*: 200-600 mg daily *Pediatrics <40 kg*: 100 mg bid *Pediatrics ≥40 kg*: 200 mg bid
Posaconazole	*Adults*: 800 mg daily *Pediatrics ≥13 years old*: 400 mg bid
Amphoteracin nebulized	Fungizon 5 mg/ml solution: dissolve 50 mg in 10 ml sterile H_20. Nebulize 10 mg (2 ml) jet nebulizer over 15 minutes BID x3 weekly for 4 months OR Ambisome Liposomal Amphotericin B 5 mg/ml solution: Nebulize 50 mg (10 ml) PARI turboboy nebulizer over 15 minutes q day x2 weekly
Omalizumab	Based on IgE level and BW in children ≥6 years
Mepolizumab	100 mg every 4 weeks subcutaneous in children ≥12 years
Benralizumab	Initial dose 30 mg subcutaneous q 4 weeks x3 doses in children ≥12 years Maintenance dose 30 subcutaneous mg q 8 weeks
Dupilumab	Initial dose 400 mg subcutaneous in children ≥12 years Maintenance dose 200 mg subcutaneous every 2 weeks OR Initial dose 600 mg subcutaneous Maintenance dose 300 mg subcutaneous every 2 weeks

Adapted from Agarwal et al[8, 18].

They also found that 80% of patients carrying both alleles had ABPA (p = 0.0079, OR = 10.4), while only 50% and 60% of patients carrying each allele individually were ABPA subjects, suggesting an additive effect. How these SNPs affect SP-A has not yet been elucidated, but the collagen region spanning both SNPs has been shown to associate with receptors of alveolar macrophages,[32] which are important in protecting against *Aspergillus* colonization.[33] It is theorized that changes in conformation or affinity of SP-A2 may decrease these interactions and compromise host defense.

Toll-like receptor polymorphisms. Wang et al[34] examined toll-like receptor (*TLR*) polymorphisms of *TLR2*, *TLR4*, and *TLR9* in cavitary pulmonary aspergillosis (CCPA), and severe asthma associated with fungal sensitization (SAFS). TLR-4 is among the major receptors for *Aspergillus* hyphae and plays an important part in innate host defense as TLR-4 deficient mice have increased susceptibility to invasive aspergillosis.[34] In CCPA patients, there was significantly increased frequency of the G allele of *TLR4* on asp299gly. ABPA patients had increased frequency of allele C for the *TLR9* T-1237C polymorphism compared to control patients. However, in SAFS patients who are predominantly *Aspergillus* sensitive, there was no association of polymorphisms of *TLR2*, *TLR4*, or *TLR9*. TLR-9 is a receptor that recognizes CpG motifs prevalent in bacterial and viral DNA. *Aspergillus* hyphae and conidia do signal through TLR-9 on murine neutrophils.[35] TLR-9 deficient mice demonstrate greater conidial and hyphal damage. In addition, Lazarus et al[36] reported that TLR9 polymorphisms have been associated with increased risk of asthma. Moreover, the *TLR9* C allele of T-1237C decreases expression of TLR-9. Thus, decreased TLR-9 protective function may be an underlying susceptibility in the development of ABPA.

Mannose binding lectin polymorphism. Vaid et al[37] reported increased frequency of intronic G1011A MBL SNPs of MBL in ABPA patients. This results in increased MBL levels and MBL binding *Aspergillus*.

TREATMENT of ABPA

The main treatment goals of ABPA are to resolve clinical symptoms, decrease recurrence of exacerbations, preserve lung function, and prevent development of bronchiectasis and/or fibrosis.[2-4, 7, 8, 38] Unfortunately, the natural history of ABPA is recurrent episodes of exacerbations of ABPA. Agarwal et al[8] has proposed a useful clinical staging classification of ABPA that is a modification of the original staging classification proposed by Greenberger et al[3]. The revised clinical staging classification (Table 4) has six stages: stage 1a, acute ABPA with mucoid impaction; stage 1b, acute ABPA without mucoid impaction; stage 2, response of ABPA to treatment; stage 3, exacerbation of ABPA; stage 4, remission of ABPA; stage 5a, treatment-dependent ABPA; stage 5b, corticosteroid-dependent asthma; and stage 6, advance ABPA manifested by bronchiectasis, cor pulmonale, pulmonary fibrosis. In stages 1 and 3 of acute and exacerbation of ABPA, the primary goals of treatment of is to reduce pulmonary

inflammation, control symptoms of asthma and ABPA, and reduce the development of pulmonary damage manifested as bronchiectasis, granulomata, and/or pulmonary fibrosis. In acute and exacerbations of ABPA, corticosteroids alone or in combination with antifungal medication are the most effective first-line treatment of ABPA. The goal of treatment of ABPA is also prevention of exacerbations as to avoid the pulmonary complications of ABPA. In order to prevent ABPA exacerbations, standard treatment of their asthma with inhaled corticosteroids with or without long-acting beta agonists, leukotriene receptor antagonists may not be sufficient. Likewise in stage 5a "Treatment-dependent ABPA" and in stage 5b "Corticoid-dependent asthma" step-up therapy with biological medications may be indicated. Thus, in ABPA patients with a history of ABPA exacerbation, treatment with biological medications that interfere with the allergic cascade and/or continuous antifungal therapy to prevent *Aspergillus* colonization may be needed.

Corticosteroid treatment. The primary treatment of ABPA is systemic corticosteroids (Table 5).[3, 4, 38-41] Corticosteroids decrease the inflammatory response elicited by *A. fumigatus* in the lung bronchial airway and parenchyma. There is general consensus on the use of corticosteroids for long periods of time in these patients. Traditionally, so-called "high dose prednisone" was recommended with a tapering course over 8 to 10 months (Table 5). However, recently, Agarwal et al[38] proposed a "medium dose prednisone" course tapering over 3 to 5 months (Table 5). The primary outcomes were exacerbation rates and development of stage 5b corticoid-dependent ABPA after 1 and 2 years, respectively, of treatment. Improvement in lung function and time to first exacerbation were similar in both groups. Adverse prednisone side effects were significantly decreased in the medium prednisone dose schedule. Alternatively in patients intolerant to prolonged corticosteroid treatment such as CF patients with pancreatic insufficiency, "Pulse Methylprednisolone" therapy (15 mg/kg/dose (maximum 1 gram))" over 3 consecutive days may be beneficial. Although there are no studies demonstrating the role of inhaled corticosteroids in treating ABPA even at high doses, they are important in the treatment of patient's underlying asthma.[38]

Antifungal treatment. The use of antifungal medications in ABPA has been studied and demonstrated to reduce the need for prolonged courses of corticosteroids (Table 5).[39, 40, 42, 43, 47] Studies have shown the antifungal medications itraconazole, voriconazole, and posaconazole can provide improvement in lung function, decrease in steroid dose, and reduced exacerbations. Chishimba et al[43] in a retrospective study of voriconazole and posaconazole in 20 patients with ABPA demonstrated a 70-75% clinical improvement. Levels should be monitored to ensure adequate absorption of the medication. It is important to review a patient's complete medication list to assess if any other medication may interfere with the absorption or metabolism of itraconazole. Proton-pump inhibitors and

H2 blockers can decrease the absorption of itraconazole. Recent studies have shown utility in inhaled amphotericin B to provide localized treatment of ABPA and thereby decrease side effects (Table 5).[45, 46] Recently, an inhaled itraconazole preparation to treat pulmonary *A. fumigatus* has been developed.[47] Pulmatrix has initiated a study to examine an inhaled itraconazole medication (Pulmazole, PUR1900) in treating ABPA in adults with asthma, to characterize its pharmacokinetics, and evaluate its effects on inflammation, pulmonary function, asthma symptoms and *Aspergillus* burden in sputum.

Studies treating with systemic antifungal therapy in SAFS and AFAD have yielded conflicting results. In the "*The Fungal Asthma Sensitization Trial (FAST)*" study, 29 patients with severe asthma sensitized to *A. fumigatus, C. herbarum, P. chrysogenum, C. albicans, T. mentagrophytes, A. alternata,* and/or *B. cinerea* were treated with oral itraconazole (200 mg twice daily) for 32 weeks, with follow-up for 16 weeks compared to 29 patients treated with placebo.[17] SAFS patient treated with itraconazole had a significant improvement of the Asthma Quality of Life Questionnaire (AQLQ) (increased 0.85), 27% decrease of IgE level from 187 IU/ml to136 IU/ml and an improvement of PF of 20.8 L/min. In a retrospective study of 5 SAFS patients who had failed itraconazole and subsequently treated with voriconazole or posaconazole, there was significant clinical improvement of asthma, reduced oral corticosteroid dose, and reduction of total and *Aspergillus* specific IgE.[43]. In AFAD patients, Agbetile et al[48] randomized 65 *Aspergillus* sensitized severe asthmatic patients to either 3 month treatment of voriconazole or placebo. There were no significant differences comparing voriconazole treatment versus placebo in the improvement of quality of life (QoL) or reduction in number of severe asthma exacerbations.

Biological treatments. ABPA, ABPM, SAFS and AFAD are $CD4^+$ Th2 driven "*Eosinophilic Asthma Phenotype*". When asthma symptoms and/or ABPA symptoms are not controlled and/or to prevent recurrence of asthma and/or ABPA exacerbations, step-up therapy with biological modifiers of omalizumab, mepolizumab, and duplilumab should be considered (Table 5). There have been a number of studies that have demonstrated benefit of omalizumab, a humanized monoclonal antibody that binds free IgE, in the management of ABPA.[49-51] Collins et al[49] reported the results of 21 ABPA patients who had received omalizumab for refractory ABPA. Omalizumab treatment resulted in decreased steroid use, decreased serum IgE levels, and improved symptoms. Tillie-Leblond et al[50] evaluated 16 asthmatic ABPA patients who received omalizumab for one year. With omalizumab treatment there were fewer ABPA exacerbation and reduction of oral steroids dose compared to the prior year. Recently, Voskamp et al[51] performed a randomized, double-blind, placebo-controlled, cross-over trial with 2 treatment phases of 4-month of omalizumab or placebo, followed by a 3 month wash-out period, and then a 4-month of omalizumab

or placebo cross-over period. There were significant reduction of ABPA exacerbations (2 versus 12 events), decreased fractional exhaled nitric oxide (FeNO)(30.5 to 17.1 ppb), decreased *A. fumigatus* stimulated basophil activation test, decreased basophils FcεR1 levels, and decreased basophil surface bound IgE levels.

More recently, additional biologics that block IL-5 (mepolizumab), IL-5 receptor alpha chain (IL-5RA) (benralizumab), and IL-4 receptor alpha chain (IL-4RA) (dupilumab) have become available in the treatment of '*Eosinophilic Asthma'*.[52] To date there are only case reports of treating ABPA with these agents. Terashima et al[53] reported a 64 year old woman with ABPA treated with mepolizumab. There was marked improvements in asthma symptoms, lung function, peripheral eosinophil counts, and disappearance of pulmonary infiltration and mucoid impaction. Soeda et al[54] reported successful treatment of ABPA with benralizumab in a 60 year old patient. There was marked improvement of radiologic findings of bronchial wall thickening, centrilobular nodules, air space consolidation, and mucoid impaction after 2 months of benralizumab treatment. Treatment of ABPA with dupilumab has a number of theoretical advantages over omalizumab, mepolizumab, and benralizumab. There are two IL-4 receptors: type I comprised of IL4 receptor alpha chain (IL-4Ra) and gamma chain; and type II comprised of IL-4Ra and IL-13 receptor alpha chain (IL-13Ra). IL-4 type I receptors are present on immune cells whereas type II receptors are present on lymphoid cells, respiratory epithelium cell, vascular endothelium, and smooth muscles. IL-4 stimulates both type I and II receptors; whereas IL-13 stimulates only type II receptors. Dupilumab by blocking IL-4Ra inhibits both IL-4 and IL-13 stimulations, blocking Th2 activity. Given the immunopathogenesis and immunogenetics of ABPA with increased Th2 stimulation, dupilumab may be very beneficial in the treatment of ABPA. In addition, dupilumab would probably be beneficial in the treatment of ABPM, AFAD, and SAFS.

In summary, allergic hypersensitivity reactions to *Aspergillus fumigatus* occurring in asthmatic and/or cystic fibrosis patients may lead to ABPA and devastating pulmonary damage. Though much is known about the immunopathogenesis and genetic risk factors in the development of ABPA, much more research is needed to optimize treatment to control asthma, prevent ABPA flares and prevent further lung damage. Though it is hypothesized that AFAD and SAFS may be similar to ABPA, similar studies examining the immunopathogesis and genetic risk factors need to be examined. Likewise, more research is needed to optimize treatment of AFAD and SAFS. As all of these fungal sensitive conditions may be classified as 'Eosinophilic Asthma' phenotype, treatment with biologic agents, such as mepolizumab, benralizumab and/or dupilumab, needs to be evaluated.

REFERENCES:

1. Kanj A, Abdallah N. The spectrum of pulmonary aspergillosis. Respir Med 2018; 141:121-131. PMID 30053957

2. Knutsen AP, Bush RK, Demain JG, et al. Fungi and allergic lower respiratory tract disease. J Allergy Clin Immunol 2012; 129(2):280-291. PMID 22284927

3. Rodriquez J, Caruthers C. Diagnosis and optimal management of fungal-associated asthma and rhinitis. Exp Rev of Clin Immunol 2016; 12(5):531-550, 2016. PMID 26776889

4. Greenberger PA, Bush RK. Allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol In Pract 2014; 2:703-708. PMID 25439360

5. Stevens DA, Moss R, Kurup VP, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis: Cystic Fibrosis Foundation Consensus Conference. Clin Infect Dis 2003; 37(Suppl 3):S225-264. PMID 12975753

6. Baxter CG, Dunn G, Jones AM, et al. Novel immunologic classification of aspergillosis in adult cystic fibrosis. J Allergy Clin Immunol 2013; 132(3):560-566. PMID 23726262

7. Agarwal R. Allergic bronchopulmonary aspergillosis. Chest 2009; 135(3):805-826. PMID 19265090

8. Agarwal R, Sehgal IS. Developments in the diagnosis and treatment of allergic bronchopulmonary aspergillosis. Exp Rev Respir Med 2016; 10(12):1317-1334. PMID 27744712

9. Chowdhary A, Agarwal K. Allergic bronchopulmonary mycosis due to fungi other than Aspergillus: a global overview. Crit Rev Microbiol 2014; 40(1):30-48. PMID 23383677

10. Denning DW, Pashley C, Hartl D, et al. Fungal allergy in asthma-state of the art and research needs. Clin Transl Allergy 2014; 4:1-23. PMID 24735832

11. Denning DW, O'Driscoll BR. The link between fungi and severe asthma: a summary of the evidence. Eur Respir J 2006; 27(3):615-626. PMID 16507864

12. Fairs A, Agbetile J, Hargadon B, et al. IgE sensitization to Aspergillus fumigatus is associated with reduced lung function in asthma. Am J Respir Crit Care Med 2010; 82(11):1362-1368. PMID 20639442

13. Woolnough K, Fairs A. Allergic fungal airway disease: pathophysiologic and diagnostic considerations. Curr Opin Pulm Med 2015; 21(1):39-47. PMID 25415407

14. Knutsen AP. Immune responses in allergic bronchopulmonary aspergillosis and fungal allergy. In: Mold Allergy, Biology and Pathogenesis, Editor Viswanath P. Kurup, Biological Sciences, Research Signpost, 2005: 209-235.

15. Greenberger PA, Smith LJ. Analysis of bronchoalveolar lavage in allergic bronchopulmonary aspergillosis: divergent responses of antigen-specific antibodies and total IgE. J Allergy Clin Immunol 1988; 82(2):164-170. PMID 3042837

16. Lotvall J, Akdis CA, Bacharier LB, et al. Asthma endotypes: a new approach

to classification of disease entities within the asthma syndrome. J Allergy Clin Immunol 2011; 127:355-360. PMID 21281866

17. 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 2009; 179(1):11-18. PMID 1894842

18. Agarwal R, Shah A CA, Gupta D, et al. Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. Clin Exp Allergy 2013; 43(8):850-873. PMID 23889240

19. Chauhan BA, Knutsen AP. T cell subsets, epitope mapping, and HLA-restriction in patients with allergic bronchopulmonary aspergillosis. J Clin Invest1996; 97(10):2324-2331. PMID 8636413

20. Chauhan B, Santiago L, Hauptfield V, et al. The association of HLA-DR alleles, T-cell activation, and allergic bronchopulmonary aspergillosis. J Immunol 1997; 159(8):4072-4076. PMID 9378997

21. Chauhan B, Santiago L, Hutcheson PS, et al. Evidence for the involvement of two different MHC class II regions in susceptibility or protection in allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol 2000; 106(4):723-729. PMID 11031343

22. Khan S, McClellan JS. Increased sensitivity to IL-4 in patients with allergic bronchopulmonary aspergillosis. Int Arch Allergy Immunol 2000; 123(4): 319–326. PMID 11146389

23. Knutsen AP, Hutchinson PS. Increased sensitivity to IL-4 in cystic fibrosis patients with allergic bronchopulmonary aspergillosis. Allergy 2004; 59(1):81–87. PMID 14674938

24. Knutsen AP, Kariuki B. IL-4 alpha chain receptor (IL-4Ra) polymorphisms in allergic bronchopulmonary aspergillosis. Clin Mol Allergy 2006; 4(3):1-6 PMC1403796

25. Knutsen AP, Kariuki B. HLA-DR, IL-4RA, and IL-10: Genetic risk factors in allergic bronchopulmonary aspergillosis. Pediatr Asthma Allergy Immunol 2009; 21(4):185-190. doi.org/10.1089

26. Knutsen AP, Vijay HM, Kariuki B, et al. Mold-sensitivity in children with moderate-severe asthma is associated with HLA-DR and HLA-DQ. Allergy 2010; 65(11):1367-1375. PMID 20486920

27. Knutsen AP, Vijay HM, Kariuki B, et al. Association of IL-4RA single nucleotide polymorphisms, HLA-DR and HLA-DQ in children with *Alternaria*-sensitive moderate-severe asthma. Clin Mol Allergy 2010; 8:5. PMID 20298583

28. Miller PW, Hamosh A, Macek M Jr, et al. Cystic fibrosis transmembrane

conductance regulator (CFTR) gene mutations in allergic bronchopulmonary aspergillosis. Am J Hum Genet. 1996; 59(1):45-51. PMID 8659542

29. Gamaletsou MN, Hayes G. F508del CFTR gene mutation in patients with allergic bronchopulmonary aspergillosis. J Asthma 2018; 55(8):837-843. PMID 29035608

30. Brouard J, Knauer N, Boelle PY, et al. Influence of interleukin-10 on Aspergillus fumigatus infection in patients with cystic fibrosis. J Infect Dis. 2005; 191(11):1988-1991. PMID 15871134

31. Saxena S, Madan T. Association of polymorphisms in the collagen region of SP-A2 with increased levels of total IgE antibodies and eosinophilia in patients with allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol 2003; 111(5):1001-1007. PMID 12743564

32. Weikert LF, Edwards K. SP-A enhances uptake of bacillus Calmette-Guérin by macrophages through a specific SP-A receptor. Am J Physiol 1997; 272(5 Pt 1):L989-95. PMID 9176265

33. Schaffner A, Douglas H. Selective protection against conidia by mononuclear and against mycelia by polymorphonuclear phagocytes in resistance to aspergillus. Observations on these two lines of defense in vivo and in vitro with human and mouse phagocytes. J Clin Invest 1982; 69(3):617–631. PMID 7037853

34. Wang JE, Warris A, Ellingsen EA, et al. Involvement of CD14 and toll-like receptors in activation of human monocytes by Aspergillus fumigatus hyphae. Infect Immunol 2001; 69(4):2402-2406. PMID 11254600

35. Bellocchio S, Moretti S, Perruccio K, et al. TLRs govern neutrophil activity in aspergillosis. J Immunol 2004; 173(12):7406-7415. PMID 15585866

36. Lazarus R, Klimecki WT, Raby BA, et al. Single-nucleotide polymorphisms in the Toll-like receptor 9 gene (TLR9): frequencies, pairwise linkage disequilibrium, and haplotypes in three U.S. ethnic groups and exploratory case-control disease association studies. Genomics 2003; 81(1):85-91. PMID 12573264

37. Vaid M, Kaur S. Distinct alleles of mannose-binding lectin (MBL) and surfactant proteins A (SP-A) in patients with chronic cavitary pulmonary aspergillosis and allergic bronchopulmonary aspergillosis. Clin Chem Lab Med 2007; 45(2):183-186. PMID 17311505

38. Agarwal R, Aggarwal AN, Dhooria S, A randomised trial of glucocorticoids in acute-stage allergic bronchopulmonary aspergillosis complicating asthma. Eur Respir J 2016; 47(2): 490-498. PMID 26585431

39. Agarwal R, Dhooria S, Sehgal IS, et al. A randomized trial of itraconazole vs prednisolone in acute-stage allergic bronchopulmonary aspergillosis

complicating asthma. Chest 2018; 153(3):656-664. PMID 29331473

40. Agarwal R, Dhooria S, Sehgal IS, et al. A randomised trial of voriconazole and prednisolone monotherapy in acute-stage allergic bronchopulmonary aspergillosis complicating asthma. Eur Respir J 2018; 52(3). PMID 30049743

41. Agarwal R, Khan A. Role of inhaled corticosteroids in the management of serological allergic bronchopulmonary aspergillosis (ABPA). Intern Med 2011; 50(8):855-860. PMID 21498933

42. Stevens DA, Schwartz HJ, Lee JY, et al. A randomized trial of itraconazole in allergic bronchopulmonary aspergillosis. N Engl J Med 2000; 342(11):756-762. PMID 10717010

43. Chishimba L, Niven RM. Voriconazole and posaconazole improve asthma severity in allergic bronchopulmonary aspergillosis and severe asthma with fungal sensitization. J Asthma 2012; 49(4):423-433. PMID 22380765

44. Periselneris J, Nwankwo L, Schelenz S, et al. Posaconazole for the treatment of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. J Antimicrob Chemother 2019. Epub ahead of print. PMID 30805605

45. Ram B, Aggarwal AN, Dhooria S, et al. A pilot randomized trial of nebulized amphotericin in patients with allergic bronchopulmonary aspergillosis. J Asthma 2016; 53(5): 517–524. PMID 26666774

46. Proesmans M, Vermeulen F. Use of Nebulized Amphotericin B in the treatment of allergic bronchopulmonary aspergillosis in cystic fibrosis. Inter J Pediatr 2010; 2010:376287. PMID 21234103

47. Wlaź P, Knaga S, Kasperek K, et al. Activity and safety of inhaled itraconazole nanosuspension in a model pulmonary *Aspergillus fumigatus* infection in inoculated young quails. Mycopathologia 2015; 180(1-2): 35–42. PMID 25790942

48. Agbetile J, Bourne M, Fairs A, et al. Effectiveness of voriconazole in the treatment of Aspergillus fumigatus-associated asthma (EVITA3 study). J Allergy Clin Immunol 2014; 134(1):33-39. PMID 24290286

49. Collins J, deVos G. Allergic bronchopulmonary aspergillosis treated successfully for one year with omalizumab. J Asthma Allergy 2012; 5: 65-70. PMID 23204847

50. Tillie-Leblond I, Germaud P, Leroyer C, et al. Allergic bronchopulmonary aspergillosis and omalizumab. Allergy 2011; 66(9):1254-1256. PMID 21517902

51. Voskamp AL, Gillman A, Symons K, et al. Clinical efficacy and immunologic effects of omalizumab in allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol Pract 2015; 3(2):192-199. PMID 25640470

52. Casale TB. Biologics and biomarkers for asthma, urticaria, and nasal polyposis. J Allergy Clin Immunol

2017; 139(5):1411-1421. PMID 28477720

53. Terashima T, Shinozaki T. A case of allergic bronchopulmonary aspergillosis successfully treated with mepolizumab. BMC Pulm Med. 2018; 18(1):53. PMID 29587693

54. Soeda S, Kono Y, Tsuzuki R, et al. Allergic bronchopulmonary aspergillosis successfully treated with benralizumab. J Allergy Clin Immunol Pract 2018; Epub ahead of print. PMID 30513359