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REVIEW ARTICLE

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Aspergillus fumigatus in cystic fibrosis: An update on immune interactions and molecular diagnostics in allergic bronchopulmonary aspergillosis

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Abstract

A wide spectrum of pathological conditions may result from the interaction of *Aspergillus fumigatus* and the immune system of its human host. Allergic bronchopulmonary aspergillosis is one of the most severe *A. fumigatus*-related diseases due to possible evolution toward pleuropulmonary fibrosis and respiratory failure. Allergic bronchopulmonary aspergillosis occurs almost exclusively in cystic fibrosis or asthmatic patients. An estimated 8%-10% of patients with cystic fibrosis experience this condition. The diagnosis of allergic bronchopulmonary aspergillosis relies on criteria first established in 1977. Progress in the understanding of host-pathogen interactions in *A. fumigatus* and patients with cystic fibrosis and the ongoing validation of novel laboratory tools concur to update and improve the diagnosis of allergic bronchopulmonary aspergillosis.

KEYWORDS

allergic bronchopulmonary aspergillosis, *Aspergillus fumigatus*, cystic fibrosis, immunoglobulin, molecular allergens

1 | INTRODUCTION

Aspergillus (*A.*) *fumigatus*, a ubiquitous airborne fungus, poses multiple threats in asthma, cystic fibrosis, and immunosuppressed hosts. Clinically, *A. fumigatus*-related conditions vary from so-called benign colonization to severe pulmonary or systemic diseases such as allergic bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis, invasive aspergillosis, and chronic lung allograft disease. Delineating *A. fumigatus* involvement in pulmonary disease is often difficult, especially in patients with cystic fibrosis (CF), and relies on a combination of clinical, radiological, immunological, and microbiological findings. ABPA or Hinson and Pepys' disease[1,2] is the most frequent form of human aspergilloses, mainly found on CF and asthma backgrounds. Criteria for ABPA diagnosis were first established in 1977.[3] Since then, significant progress has been achieved in the understanding of *A. fumigatus*-immune response crosstalk. Novel immunological and microbiological tools became available, and standardized laboratory tests are replacing older in-house methods. Our multidisciplinary approach aimed at reporting progress and current perspectives in *A. fumigatus* laboratory workup, with special emphasis on the challenging diagnosis of ABPA.

2 | *ASPERGILLUS FUMIGATUS* AND ITS INTERACTION WITH THE HUMAN HOST

The *Aspergillus* genus comprises 339 species of filamentous fungi ubiquitous all over the world in human environment, for example,

Abbreviations: ABPA, allergic bronchopulmonary aspergillosis; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; Ig, immunoglobulin; IL, interleukin; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism

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air, soil, cereals, fruit, plants, wet and decomposing organic matter. Airborne *Aspergillus* spp spores are present indoors and outdoors, with an estimated 10-1000 spores inhaled daily by each human.[4,5]

A growing number of *Aspergillus* species are recognized as pathogenic for animals and humans; yet *A. fumigatus* (Figure 1) is the most frequently involved in human medicine, with 80%-90% of human aspergilloses being caused by *A. fumigatus*.[6,7]

Virulence and subversion mechanisms of *A. fumigatus* are supported by multiple antigenic proteins and toxins which differentially become involved in host interaction depending on immune and environmental factors. *Aspergillus fumigatus* comprises around 200 antigenic proteins capable of inducing IgG responses and 80 allergenic proteins capable of inducing IgE responses.[8,9] There is partial overlap between the two categories, with extensive characterization still to be performed for most proteins. Intensive efforts for characterizing allergenic proteins resulted in 23 allergens currently included in the WHO/IUIS (International Union of Immunological Societies) database.[10] In comparison, the *Alternaria* genus possesses 12 characterized allergens, *Cladosporium herbarum* 8, and house dust mite *Dermatophagoides pteronyssinus*, one of the most prevalent causes of airborne allergy and asthma, 19.[10] Most of the *A. fumigatus* allergens display enzymatic activities, but structural components, such as antigenic mitochondrial components and allergen Asp f 34 of the fungal wall, are also reported.[8,11-13]

Aspergillus fumigatus interacts with the host's natural barriers and immune system, including airway mucus, airway epithelium, innate and adaptive immune cells. It behaves as an opportunistic pathogen, taking advantage of virtually any type of deficiency in host responses at various levels.

In healthy, immunocompetent hosts, inhaled *A. fumigatus* spores are eliminated by the mucociliary elevator and alveolar macrophages.[5,14-16] The ability of the host's immune system to mount an efficient response is crucial if the first line of defense is overwhelmed. If *A. fumigatus* manages to persist in the airways and enter the germination process, neutrophils sense and effectively target germ tubes.[17] *A. fumigatus* spores directly activate bronchial epithelial cells through binding of fungal beta-glucans to cellular dectin-1, resulting in the release of antimicrobial peptides such as pentraxin and mannose-binding lectin, and generation of reactive oxygen species and cytokines.[5,17]

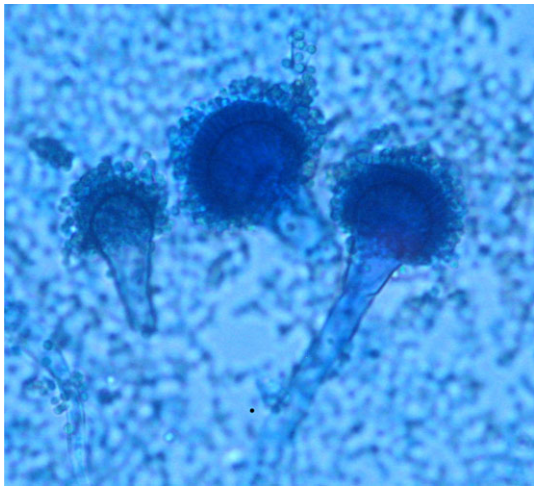

FIGURE 1 *Aspergillus fumigatus* smooth-walled conidiophores bearing subclavate vesicles and uniseriate conidiogenous cells producing numerous verrucose spherical conidias (Mycetblue™ staining, original magnification ×400)

Defective function of the cystic fibrosis transmembrane conductance regulator (CFTR) protein results in reduced chloride and bicarbonate secretion at the apical surface of epithelial cells, leading to more acid and viscous airway surface liquid stagnating in the lumen.[18] Trapped microorganisms are less likely to be eliminated, while the access of soluble antifungal mediators such as pentraxin-3 and mannose-binding lectin is impaired by increased mucus viscosity.[5,16,17] Thus, *A. fumigatus* stagnation and growth benefit from abnormal mucus microenvironment.

In patients with CF, this defect is not rescued by innate and adaptive immune cells, which are dysregulated. CFTR gene dysfunction directly impairs neutrophil generation of the microbicidal hypochlorous acid in phagolysosomes and favors skewing of T-lymphocyte anti-*A. fumigatus* responses toward interleukin (IL)-4, IL-13, and IgE production.[19,20] Secondarily acquired immune dysregulation includes the cleavage of membrane receptors by neutrophil-derived proteases in CF airways. Cleavage of macrophage phosphatidylserine receptors results in impaired removal of apoptotic cells in CF lungs, while cleavage of the costimulation molecule CD86 impairs dendritic cell maturation and activity.[21,22]

In addition to CFTR dysfunction, host's genetic background plays a role in the encounter with *A. fumigatus*. Patients carrying HLA-DR2 or HLA-DR5 phenotype are more susceptible to ABPA, while HLA-DQ2 subjects are relatively resistant.[23-26] The hypothesized mechanism is an increased production of regulatory IL-10 from dendritic cells expressing HLA-DR2/DR-5 with downstream inhibition of Th-1- and Th-17-type responses, which are instrumental for an effective response to *A. fumigatus*.[27,28] *A. fumigatus* by itself triggers Th2-type polarized responses, IgE production, lung eosinophilia, and airway hyperresponsiveness,[29-31] while excessive Th-2 responses to *A. fumigatus* are associated with ABPA.

Th2-polarized responses are further upregulated in subjects carrying gain-of-function single nucleotide polymorphisms (SNPs) of the IL-4 receptor. 95% of patients with ABPA display such SNPs, resulting in a lowered threshold of activation by ligand IL-4. Extracellular ile75val was the most frequently identified SNP, found in 80% of patients with ABPA. However, this SNP was also present in 54% of control subjects and was therefore not eligible as a susceptibility biomarker.[32]

Finally, healthy pulmonary and intestinal microbiomes might help preventing *A. fumigatus*-induced diseases, and alterations of the microbial populations might contribute to inefficient defenses. Normal gut microbiome inhibits systemic IgE responses through signals directed to toll-like receptors of innate immune cells including mast cells and basophils, while the production of short-chain fatty acids modulates dendritic cell responses.[33-35] In patients with CF, deteriorating lung function is associated with decreased phylogenetic variety of the bacterial airway microbiome

and enrichment in pulmonary pathogenic genera such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Haemophilus influenza*, and *Burkholderia cepacia*.[36-38] The proportion of saprophytic fungal species, which is <0.1% under normal conditions, is increased in CF patients' mycobiome and following antibiotic regimens.[36,39,40] Fungal communities were reported to be stable over time in patients with CF.[41] Persistent lower airway colonization is associated with increased microbial load, persistent inflammatory changes, and lung function loss.

Figure 2 summarizes the key events of the *A. fumigatus*-host encounter and crosstalk.

The pathophysiological continuum of *A. fumigatus*-host interaction is mirrored by laboratory and clinical frames of "asymptomatic" colonization, "benign" IgE sensitization to *A. fumigatus*, through *A. fumigatus*-induced cough and asthma, to aspergilloma and invasive aspergilloses. However, the first two stages lack consensus definitions. The growth of *A. fumigatus* in the airways, also termed "carriage" or "colonization," may be evidenced by culture-based methods, galactomannan detection in bronchoalveolar lavage liquid, PCR assays, and more recently new-generation sequencing methods and antigenemia measures.[9,37-39,42,43] The detection of "precipitins," which comprise mainly but not exclusively IgG, or anti-*A. fumigatus*-specific IgG, is sometimes considered as indicative of *A. fumigatus* carriage,[44] but most teams distinguish genuine *A. fumigatus* colonization from anti-*A. fumigatus* IgG detection. Serum anti-*A. fumigatus* IgG are found in most healthy individuals.[9,45-47] In patients with CF, these antibodies may be detected before the age of 2 and are virtually present in all patients aged 10 or older.[48] On the other hand, sensitization is defined by the detection of anti-*A. fumigatus*-specific IgE, through skin prick testing or serum specific IgE determination. Although the detection of *A. fumigatus* carriage and IgG and IgE responses against *A. fumigatus* may be clinically silent, they are associated with an accelerated loss of lung function in CF and asthmatic patients.[44,45,49,50] In lung transplant recipients, *A. fumigatus* colonization was linked to higher mortality,[51] and positive intraoperative *A. fumigatus* culture was associated with a higher risk of post-transplant invasive aspergillosis.[41]

3 | CYSTIC FIBROSIS AND ABPA: EPIDEMIOLOGY, NATURAL HISTORY, AND CURRENT DIAGNOSTIC GUIDELINES

The estimated prevalence of ABPA is 1%-2% among asthmatic patients (7%-14% in corticosteroid-dependent ones) and 8%-10% among patients with CF, with reported extremes of 1%-25% and slightly lower figures in children as compared to adult patients.[49,52,53] A meta-analysis determined the risk for ABPA at 40% in asthmatic patients displaying IgE sensitization to *A. fumigatus*, but only 12% in those without detectable IgE.[54] Similar figures are reported for ABPA occurring in patients with CF; of whom, up to 35% display IgE to *A. fumigatus*.[55] In patients with CF, the risk of developing an ABPA is increased in male patients and in cases of associated asthma or atopy, of more severely impaired respiratory function or nutritional status, of *Pseudomonas* spp carriage in sputum, and of prolonged treatment by azithromycin.[49,56]

In patients with CF, specific challenges need to be overcome during the diagnostic procedure, because of overlapping clinical features between ABPA and CF per se (cough, bronchospasm, intercurrent infections, radiological abnormalities), together with an increased frequency of *A. fumigatus* colonization and/or sensitization.[52] Moreover, in patients with CF, so-called benign colonization or sensitization by *A. fumigatus* has been shown to contribute to the deterioration of respiratory function, in pediatric and adult patients, and on native or transplanted lungs.[44,45,49-51]

The severity of ABPA resides in the worsening of respiratory and nutritional status.[44] Therapeutic options comprise associations of corticosteroids, systemic antifungals, and in some cases the monoclonal anti-IgE antibody omalizumab. The treatment is usually effective, provided it is initiated before the development of bronchiectasis and irreversible deterioration of respiratory function. Conversely, excess treatment poses specific risks. Corticosteroids inhibit the Th-17 branch of the adaptive immune response, which is needed for efficient antifungal responses and at the same time downregulated through direct effect of *A. fumigatus* on immune cells.[17,28-30] Antifungal molecules may select resistant strains of *Aspergillus* and other fungi and compromise the homeostasis of the patient's mycobiome.[5]

Allergic bronchopulmonary aspergillosis is a chronic disease with a succession of improvement and worsening episodes. Its diagnostic criteria are aimed at documenting an inflammatory and allergic response to *A. fumigatus*, while its clinical staging also takes into account the therapeutic response.[3,7,53,57,58] Table 1 summarizes the diagnostic criteria for ABPA.

Taken together, these scores allocate a preponderant role to immunological features. They aim at identifying an immune response, which (i) targets *A. fumigatus*; (ii) is IgE-mediated; and (iii) is accompanied by other concomitant, prominent abnormal findings. They were stated in 1977 according to the technical level of the day in immunology and radiology. However, technical progress accomplished in immunology since then has not been fully exploited for a more efficient ABPA diagnosis. The next paragraph will develop this idea.

4 | WHY SHOULD IMMUNOLOGICAL CRITERIA FOR ABPA DIAGNOSIS BE UPDATED?

A study based on the national registries of 30 countries concluded that ABPA is globally underdiagnosed in patients with CF, with barely 33% reported of the expected ABPA.[59] Notable differences in ABPA diagnosis rates are found even among European countries, with the lowest in Greece (14.5% reported vs expected number of ABPA cases) and the highest end in France (101%). Because, on the one hand, the first-line treatment of ABPA mainly relies on corticosteroids and, on the other hand, the consequences of a lack of ABPA treatment may be

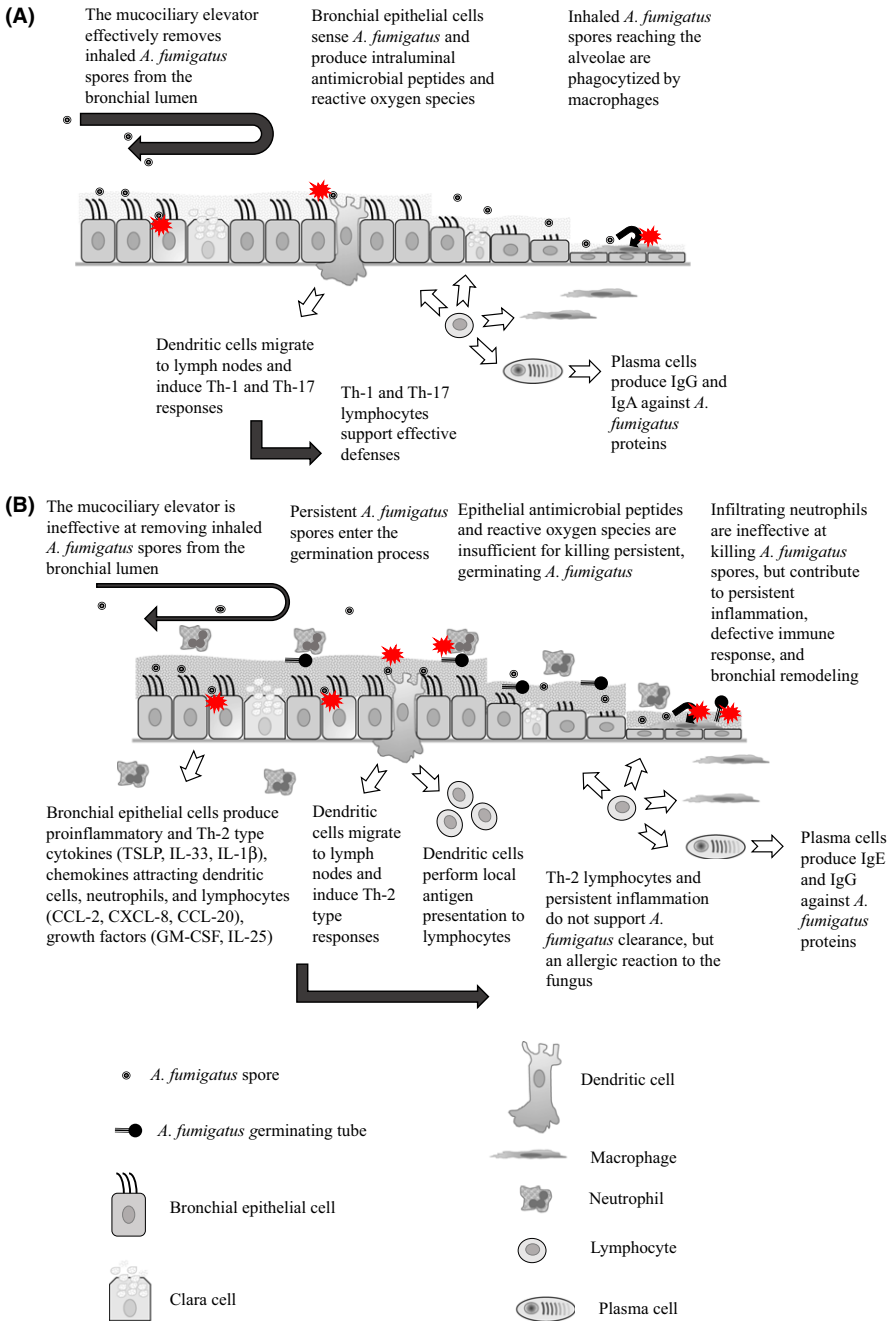

FIGURE 2 Key events of the interaction and immune crosstalk between *Aspergillus fumigatus* and the human host. (A) In immunocompetent hosts, inhaled spores are removed mainly by the mucociliary elevator. Alveolar macrophages phagocytize spores that reach lower airways, and bronchial epithelial cells respond to spores by means of reactive oxygen species and soluble antimicrobial peptides. Innate and adaptive immune responses are kept at a low level, and humoral responses are made of IgG and IgA against *A. fumigatus*. (B) In patients with cystic fibrosis, multiple defects lead to ineffective removal by the mucociliary elevator, epithelial soluble mediators, and alveolar macrophages. *Aspergillus fumigatus* persistence and growth initiates an important but ineffective inflammatory, Th-2 oriented, and remodeling response, with raised levels of IgE and IgG against *A. fumigatus*

irreversible, calls for updating the immunological criteria have been accumulating since the turn of the century.[47,52,60] These calls are supported by novel tools with enhanced performance. We will describe hereafter the issues associated with the current ABPA immunological diagnostic criteria including total serum IgE, *A. fumigatus*-specific IgE, and *A. fumigatus*-specific IgG and precipitins against *A. fumigatus*.

4.1 | Total IgE level

4.1.1 | Therapeutic interferences in quantification

According to classical ABPA criteria, a "normal" total IgE level excludes the diagnosis. This is not always appropriate, because total IgE level is altered by disease stages, disease severity, and therapeutic options, such as systemic corticosteroid therapy and omalizumab.[60-63]

TABLE 1 Diagnostic criteria for allergic bronchopulmonary aspergillosis (ABPA)

Hierarchy of criteria	Criteria for allergic bronchopulmonary diagnosis	Comments
Major criteria: Both are necessary, but not sufficient	Allergic sensitization to *Aspergillus fumigatus*: positive skin prick tests or detectable or "raised" sIgE to *A. fumigatus*	A cutoff of 0.35 kUA/L was suggested by an expert panel,[58] in replacement of the previous recommendation "raised serum specific IgE anti-*A. fumigatus* antibodies greater than twice non-ABPA IgE *A. fumigatus*-positive asthmatic serum pool"[67]
	Elevated total IgE levels >1000 kIU/L or >1000 μg/L depending on teams	1000 kIU/L=2400 μg/L Results may be expressed in IU, kIU, or μg according to local laboratory procedures. Cutoffs differ between children and adults
Minor criteria: Two of three are sufficient	*Aspergillus* IgG serology positive or "raised," detection of "anti-*Aspergillus* precipitins"	
	Radiology, according to the clinical stage: transient migratory pulmonary opacities to fixed central bronchiectasis	
	Elevated circulating eosinophils >0.5 or 1 G/L	This criterion is considered as outdated by most teams
Background	Asthma, cystic fibrosis	

Diagnostic criteria for ABPA proposed by Rosenberg et al.[3] and updates are presented in the left and middle columns, together with recent proposals and frequent drawbacks for the clinical practice.

4.1.2 | Ill-defined threshold level

Another limitation is that the threshold defining a "raised" level of total IgE is unclear, as the expression is available either in μg/L or in kIU/L, with 1000 μg/L corresponding to 400 kIU/L.[58,64,65] Moreover, reference values for total IgE levels in children are age dependent and cannot be compared to adult ranges before the age of 6,[66] prompting some teams to adopt local definitions of a raised total IgE level in children, such as twice the upper normal limit value for the age group.[49]

4.2 | Detection and measurement of specific IgE to *Aspergillus fumigatus*

4.2.1 | Laboratory methods

IgE-based criteria in the initial diagnostic scores were derived from the detection thresholds of the radioimmunological assays that were in use in the 1970-1980 period; they were phrased: "anti-*Aspergillus* allergic sensitization" and "serum specific IgE anti-*A. fumigatus* antibodies greater than twice non-ABPA IgE *A. fumigatus*-positive asthmatic serum pool".[67] The standard laboratory procedures in 2016 are based on standardized curve controls and calibrators, internationally validated cutoffs, and external quality controls. The use of in-house pools of local patient sera is no longer recommended.[9,68] Today's IgE measurements are automated, standardized, and support interlaboratory comparison.[68,69]

In 2013, the ISHAM experts proposed a 0.35 kUA/L threshold for defining the anti-*Aspergillus* sensitization, without detailing experimental evidence ("the expert group felt that a value >0.35 kUA/L should be used as a cutoff," in ref. 58). It should be remembered that 0.35 kUA/L was the historical threshold of the original radioallergosorbent test (RAST®) developed by Pharmacia (Uppsala, Sweden) more than 30 years ago. The cutoff of the three major clinical laboratory autoanalyzers for sIgE determination, Thermo Fisher, Hycor, and Siemens, has been set at the lower limit of quantification of the methods, that is, 0.10 kUA/L for more than 10 years. This 0.10 kUA/L cutoff has been accepted by the US Food and Drug Administration in 2008[69] and is now recommended by a 2015 consensus guidance document.[70] Good technical performances and improved sensitivity have been demonstrated in many allergic conditions, including *A. fumigatus* sensitization.[54,68,71-76] Thus, the rationale of using a higher threshold is neither technically nor medically founded.

4.2.2 | Skin prick tests vs specific IgE

Following a general rule in allergic sensitization diagnosis, both skin prick test with immediate (15-20 minutes) reading or the specific IgE blood level can be used for the diagnosis of fungal sensitization.[77] Skin prick test results are relatively poorly reproducible, heterogeneous among distinct centers in terms of procedures and interpretation, and further impeded by shortages in commercial fungal preparations. Some authors reported a decreased diagnostic sensitivity when compared to IgE detection.[54] Conversely, a wider use of automated IgE measurements results in a global tendency to adopt between-centers comparable procedures for detection and quantitation of *A. fumigatus* IgE sensitization.[9,68-70,74,76]

4.3 | Epidemiology of the antifungal and anti-*Aspergillus fumigatus* sensitization

Since the first ABPA diagnostic criteria were coined, it became clear that about 35%-50% of patients with CF display detectable sensitization to *A. fumigatus*. Thus, mere detection of IgE to *A. fumigatus* is not a suitable ABPA biomarker in this population. This explains the tentative proposal of "a raised level of sIgE to *A. fumigatus*" in more recent papers. Abundant experimental data support this view,[49,54,64,65,76,78] but a widely validated threshold

is lacking. Disparate cutoff values have been reported in the literature, from 1.91 kUA/L[54] to "class 2 or more"[49] to 29.8 kUA/L.[77] Patients with CF are frequently sensitized to fungal species cross-reacting with *A. fumigatus*, such as *Candida albicans* and *Alternaria alternata*.[77,79,80] *C. albicans* sensitization was demonstrated in 50%-60% of CF ABPA cohorts,[49,81] whereas *Al. alternata* sensitization occurred in 100% of ABPA in a pediatric cohort of patients with CF.[49] The clinical implications of these findings are currently unknown, even though genuine sensitization and antigenic cross-reactivity can influence therapeutic options.[55,77,82]

4.4 | Detection and measurement of specific anti-*Aspergillus fumigatus* IgG responses

Similar to IgE responses, IgG responses against *A. fumigatus* have been persistently measured with heterogeneous, in-house, methods displaying poor specificity and reproducibility, thus precluding interlaboratory comparison.[47,60] For instance, anti-*Aspergillus* precipitin detection, a method that is still considered as the gold standard, showed up to 30% of variation in the results assessed in the same serum sample.[58,83,84] Precipitins (Figure 3) consist of precipitating antibodies, which may belong to any antibody class, among which IgG is the most common isotype. Such poor diagnostic performances do not fit with the current good laboratory practices; they do not comply with the current clinical diagnostic assay standards, and disallow following-up a patient's IgG response. In contrast, automated platforms such as ImmunoCAP (ThermoFisher Scientific, Uppsala, Sweden) and Immulite (Siemens Munich, Germany) yield reproducible and interlaboratory comparable results. They are well suited for longitudinal follow-up.[9,46] We and others are currently analyzing the association of enhanced IgG detection and quantitation results with clinical *Aspergillus* disease presentations.[82-86] Indeed, the comparative assessment of quantitative IgE, IgG, and presence or absence of *A. fumigatus* is still to do in patients with CF, despite early studies showing clinically significant interplay of these features in CF patients with or without ABPA[45,48,87] and recent work uncovering a similar interplay in common airborne allergies of large cohorts.[88,89]

The next paragraph will present the advances in immunological methods, with an emphasis on IgE detection and quantification tools and their potential for improving ABPA diagnosis in patients with CF.

5 | COMPONENT-RESOLVED DIAGNOSTICS: MOLECULAR ASPECTS OF THE HUMORAL RESPONSE TO *ASPERGILLUS FUMIGATUS*

Component-resolved diagnostics was coined as a phrase almost 10 years ago, when IgE determination against distinct allergenic proteins (termed "components" or allergens in order to better distinguish individual allergenic proteins from allergenic extracts) became available for routine diagnostics of most of the airborne, food, and venom allergies.[90] It provided clinicians with a brand new tool for insight into the mechanisms and clinical implications of dozens of allergenic molecules, allowing to distinguish between genuine sensitization and cross-reactivity and to assess the nature and complexity of sensitization profiles. In less than a decade, component-resolved diagnostics revolutionized diagnostic accuracy, therapeutic choice, follow-up criteria, and prognostic prediction in allergic diseases.[82] Some of these proteins are currently under investigation for a combined follow-up of IgE and IgG[4] responses, both in humoral and in cellular assays with basophil activation tests.[78,91] Although their prophylactic and curative therapeutic use is intensively investigated, allergenic proteins are currently available only for in vitro procedures. Component-resolved diagnostics has already been tentatively applied to antifungal responses, particularly to *A. fumigatus* sensitization. The state of the art will be presented in the following paragraph.

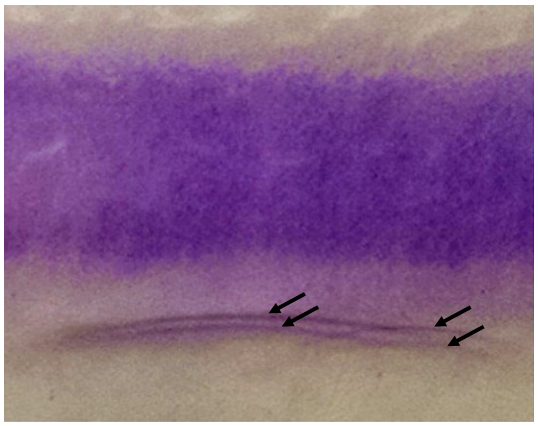

FIGURE 3 Immunoelectrophoresis showing four precipitin arcs (arrows) of anti-*Aspergillus fumigatus* antibodies

5.1 | Feasibility of *Aspergillus fumigatus* IgE and IgG component-resolved diagnostics

Of the 9926 genes of *A. fumigatus*, 23 proteins have been structurally and functionally characterized and introduced into the WHO/IUIS database of allergenic proteins.[10,82] Of these, five are commercially available as recombinant proteins for quantitative automated determination of specific IgE, IgG,[4] or IgA (Thermo Fisher Scientific). According to the international nomenclature, these proteins are named Asp f followed by a number: Asp f 1, Asp f 2, Asp f 3, Asp f 4, and Asp f 6. The Thermo Fisher quantitative method (ImmunoCAP®) is a widely used method for IgE determination. It is often considered as the reference method.[55] It comes with an abundant literature of IgE skin prick test and IgE clinical diagnosis correlates.[74,77,92] Moreover, it allows the determination of other antibody class responses (IgG, IgG4, IgA) with the same technical performances.[9,46,76] This method requires a minute volume of serum or plasma (40 μL) for assaying one specificity, that is, 240 μL for a complete *A. fumigatus* extract and determination of five allergens.

Measurement of anti-*A. fumigatus* IgE is comprised in the international quality control UK NEQAS program.[93] A multicentric study involving 25 hospital laboratories reported very good repeatability and interlaboratory reproducibility of IgE to *A. fumigatus* determination.[68] Other teams also reported very good performances of anti-*A. fumigatus* IgG determination with the same method or other automated methods.[46,74,76,84,94]

5.2 | Improved specificity for IgE to *Aspergillus fumigatus* vs other fungi

Some *A. fumigatus* proteins are specific of anti-*A. fumigatus* responses and can identify genuine sensitization, the best example being Asp f 1, while others, such as Asp f 6, share high sequence homology with proteins from other fungi and are thus more prone to cross-reactivity. At the patient level, cross-reactivity consists in the detection of IgE against multiple fungal extracts in either skin prick tests or in vitro assays. Cross-reactivity is a cause of lower specificity in the diagnostics of sensitization to *A. fumigatus*.[49,55,77] Therefore, *A. fumigatus* allergenic proteins perform better than extracts for accurate species diagnosis, thanks to their ability to distinguish genuine sensitization from cross-reactivity.[55,64,82] Table 2 introduces currently available *A. fumigatus* allergenic proteins.

5.3 | Improved specificity for differential diagnosis between "benign" sensitization to *A. fumigatus* and ABPA

Sensitization and raised levels of IgE to secreted Asp f 1 and to somatic Asp f 2 have been reported in 60%-100% of patients with ABPA and in 45%-75% of "benign" *Aspergillus* sensitization or other aspergillosis clinical presentations.[11,76,78,95,96] As a result, detection of IgE to Asp f 1 or Asp f 2 cannot identify patients with ABPA. Levels of IgE to Asp f 1 and Asp f 2 are reportedly discriminant, but the optimal threshold differs from one study to another.

Peroxisomal protein Asp f 3, allergen Asp f 4 of unknown function, and MnSOD Asp f 6 are somatic proteins and minor allergens (ie, <50% of non-ABPA patients sensitized to *A. fumigatus* display IgE to these proteins). They have been reported as specific for ABPA diagnosis in both CF and asthmatic patients.[11,13,64,65,92,96] Asp f 3 sensitization showed stable levels of IgE over time, despite ABPA flares and remission, thus enhancing the discrimination of patients with ABPA from non-ABPA patients.[64]

Some reports on Asp f 3, Asp f 4, and Asp f 6 warned against their cross-reactivity with homologues from other fungi, which should be considered during comorbid atopic dermatitis.[76,96-98] However, assessing sensitization to the complete panel of *A. fumigatus* allergens should avoid misinterpretation. Detection of IgE to species-specific Asp f 1 and Asp f 2 confirms genuine sensitization to *A. fumigatus*, while minor, ABPA-related, Asp f 3, Asp f 4, and Asp f 6 indicate the degree of complexity of the IgE response to *A. fumigatus*. Interestingly, Asp f 3, Asp f 4, and Asp f 6 are not secreted but somatic proteins, in contrast to extracellular Asp f 1 and Asp f 2.

TABLE 2 Molecular allergens of *Aspergillus fumigatus* currently available for in vitro diagnostics

Allergen name	MW	Function	Location	Species specific	Immunogenic potential	IgE response	IgG response	Fungal sensitization	ABPA	References
Asp f 1	18	Ribonuclease (ribotoxin, mitogillin family)	Secreted	Yes	+++ Absent from the spores. Produced during germination	+++ Major allergen	+++	+++	+++	8,9,11,13,63,76,78,87
Asp f 2	37	Fibrinogen-binding protein	Somatic	Yes	+++	+++ Major allergen	++	+++	+++	8,9,11,76,78
Asp f 3	19	Peroxisomal protein	Somatic	No Highly homologous of proteins from *Candida* and *Penicillium* genera	+	+ Minor allergen	+	++	+++	8,9,11,13,64,78,94
Asp f 4	30	Unknown	Somatic	Unknown	±	± Minor allergen	No	±	++	8,9,11,64,65,76,78,97
Asp f 6	26.5	Manganese superoxide dismutase	Somatic	No High cross-reactivity with fungal and human homologues	±	± Minor allergen	No	No	+	8,9,11,13,64,65,78,97,98

ABPA, allergic bronchopulmonary aspergillosis.
There are currently five commercially available molecular allergens of *A. fumigatus*. Their physicochemical properties, function, and humoral response are summarized.

Spreading of the IgE response from secreted (benign sensitization) to somatic (ABPA) might be indicative of the immune system fighting a higher load of *A. fumigatus* or of a more pronounced Th-2 skewing of the immune response to *A. fumigatus*. In both cases, a more complex IgE response to *A. fumigatus* could be predictive of a higher risk of clinical deterioration. The predictive value of complex sensitization profiles has been recently described in airborne and food allergies.[88,99]

For all allergens, further information is provided by quantitative comparison of IgE responses to individual *A. fumigatus* molecular allergens.[9,64,76,78] In this case, threshold levels depend on the control population, that is, healthy controls, asthmatic or CF patients, and therefore need validation prior to use in a given population of patients.

5.4 | Combined determination of IgE, IgG, and IgA against *Aspergillus fumigatus* individual proteins

Aspergillus fumigatus proteins may bind to one or more Ig classes, depending on the protein, the pathophysiologic background of the patient, and the time course of *A. fumigatus*-host interaction. *Aspergillus fumigatus* fractions with high (470 kDa), medium (250 kDa), and low (25-50 kDa) molecular weight were employed as early as 1985 for studying IgG, IgA, and IgE responses in 156 patients with CF.[45] Strong IgG and IgA responses to the 470-kDa fraction were found, even in the absence of *A. fumigatus* colonization defined as negative monthly sputum cultures over a 22-month period. IgG and IgA responses to the 25- to 50-kDa fraction were preferentially associated with persistent *A. fumigatus* colonization (positive sputum cultures during and prior to the study). The three *A. fumigatus* fractions also elicited specific IgE responses. Asp f 1 was later reported as a potent inducer of specific IgE and IgG responses in asthmatic and ABPA patients, but not in healthy controls, suggesting its utility as a prevalence and quantitative marker for ABPA.[87]

Preferential IgG or IgE responses were shown for individual *A. fumigatus* proteins,[8] suggesting that the overlap between antigenic, that is, IgG-inducing, and allergenic, that is, IgE-inducing molecules, is only partial in *A. fumigatus* and depends on both the protein and the immune status of the host. A shift between IgG and IgE responses to a given protein could mirror a pathophysiological change from chronic to allergic aspergillosis.[9]

Current reports on IgG responses to commercially available allergens from *A. fumigatus* are scarce and address mainly ABPA patients with an asthmatic background.[76] Our team found that IgG4 responses to *A. fumigatus* allergens did not distinguish between *A. fumigatus*-sensitized CF patients and active ABPA.[78] A first step toward an integrated IgE and IgG workup might be the use of a single method for head-to-head comparison. This is feasible with current routine autoanalyzers, with very good quantitative performances including interlaboratory comparison.[9,46,68,73,76,84,86]

Currently, the lack of commercially available IgG-skewed proteins and the limited number of commercially available allergens do not allow a comprehensive profiling of humoral responses to *A. fumigatus* proteins in health and disease.

6 | CONCLUSION

Currently unmet needs in the field of *A. fumigatus*-related diseases include differential diagnosis of benign vs pathogenic fungal sensitization in patients with CF. Among novel diagnostic tools, IgE and IgG markers of distinct humoral responses have benefitted from 40 years of technical progress and the validation of component-resolved diagnostics in the allergy field. Currently available IgE and IgG component-resolved diagnostics has been repeatedly reported as useful, but still awaits confirmation in large-scale multicenter studies evaluating patients with CF on the long term. We believe that an integrated analysis of IgE and IgG responses against *A. fumigatus* molecules would enhance the diagnosis of *Aspergillus*-induced diseases and especially that of ABPA. Moreover, IgE/IgG dichotomy of responses induced by individual proteins might lead to the identification of novel therapeutic targets.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

All authors wrote, proof-read, and approved the final version of the manuscript.

REFERENCES

1. Hinson KF, Moon AJ, Plummer NS. Broncho-pulmonary aspergillosis: a review and report of eight cases. *Thorax*. 1952;7:317-333.
2. Pepys J, Riddell RW, Citron KM, Clayton YM, Short EI. Clinical and immunologic significance of *Aspergillus fumigatus* in the sputum. *Am Rev Respir Dis*. 1959;80:167-180.
3. 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;86:405-414.
4. Rocchi S, Richaud-Thiriez B, Barrera C, et al. Evaluation of mold exposure in cystic fibrosis patients' dwellings and allergic bronchopulmonary risk. *J Cyst Fibros*. 2015;14:242-247.
5. Camargo JF, Husain S. Immune correlates of protection in human invasive aspergillosis. *Clin Infect Dis*. 2014;59:569-577.
6. Kosmidis C, Denning DW. The clinical spectrum of pulmonary aspergillosis. *Thorax*. 2015;70:270-277.
7. De Blay F, Delmas C, Molard A. Aspergillose broncho-pulmonaire allergique. *Encyclopédie Médico-Chirurgicale*. 2015;12:1-9.
8. Teutschbein J, Simon S, Lother J, et al. Proteomic profiling of serological responses to *Aspergillus fumigatus* antigens in patients with invasive aspergillosis. *J Proteome Res*. 2016;15:1580-1591.
9. Page ID, Richardson M, Denning DW. Antibody testing in aspergillosis – quo vadis? *Med Mycol*. 2015;53:417-439.
10. www.allergen.org. Accessed February 2, 2017.
11. Crameri R. Recombinant *Aspergillus fumigatus* allergens: from the nucleotide sequence to clinical applications. *Int Arch Allergy Immunol*. 1998;115:99-114.

12. Glaser AG, Kirsch AI, Zeller S, Menz G, Rhyner C, Crameri R. Molecular and immunological characterization of Asp f 34, a novel major cell wall allergen of *Aspergillus fumigatus*. *Allergy*. 2009;64:1144-1151.
13. Crameri R. Structural aspects of fungal allergens. *Semin Immunopathol*. 2015;37:117-121.
14. Balloy V, Chignard M. The innate immune response to *Aspergillus fumigatus*. *Microbes Infect*. 2009;11:919-927.
15. Cowley AC, Thornton DJ, Denning DW, Horsley A. Aspergillosis and the role of mucins in cystic fibrosis. *Pediatr Pulmonol*. 2017;52:548-555.
16. Bruscia EM, Bonfield TL. Innate and adaptive immunity in cystic fibrosis. *Clin Chest Med*. 2016;37:17-29.
17. Margalit A, Kavanagh K. The innate immune response to *Aspergillus fumigatus* at the alveolar surface. *FEMS Microbiol Rev*. 2015;39:670-687.
18. Kreda SM, Davis CW, Rose MC. CFTR, mucins, and mucus obstruction in cystic fibrosis. *Cold Spring Harb Perspect Med*. 2012;2:a009589.
19. Zhou Y, Song K, Painter RG, et al. Cystic fibrosis transmembrane conductance regulator recruitment to phagosomes in neutrophils. *J Innate Immun*. 2013;5:219-230.
20. Mueller C, Braag SA, Keeler A, Hodges C, Drumm M, Flotte TR. Lack of cystic fibrosis transmembrane conductance regulator in CD3+ lymphocytes leads to aberrant cytokine secretion and hyperinflammatory immune responses. *Am J Respir Cell Mol Biol*. 2011;44:922-929.
21. Vandivier RW, Fadok VA, Hoffmann PR, et al. Elastase-mediated phosphatidylserine receptor cleavage impairs apoptotic cell clearance in cystic fibrosis and bronchiectasis. *J Clin Invest*. 2002;109:661-670.
22. Bhattacharya S, Ray RM, Johnson LR. Decreased apoptosis in polyamine depleted IEC-6 cells depends on Akt-mediated NF-kappaB activation but not activity. *Apoptosis*. 2005;10:759-776.
23. Muro M, Mondejar-Lopez P, Moya-Quiles MR, et al. HLA-DRB1 and HLA-DQB1 genes on susceptibility to and protection from allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *Microbiol Immunol*. 2013;57:193-197.
24. Chauhan B, Santiago L, Kirschmann DA, et al. The association of HLA-DR alleles and T cell activation with allergic bronchopulmonary aspergillosis. *J Immunol*. 1997;159:4072-4076.
25. Aron Y, Bienvenu T, Hubert D, Dusser D, Dall'Ava J, Polla BS. HLA-DR polymorphism in allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol*. 1999;104:891-892.
26. Knutsen AP, Bush RK, Demain JG, et al. Fungi and allergic respiratory diseases. *J Allergy Clin Immunol*. 2012;129:280-291.
27. 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;191:1988-1991.
28. Murdock BJ, Shreiner AB, McDonald RA, et al. Coevolution of Th1, TH2, and TH17 responses during repeated pulmonary exposure to *Aspergillus fumigatus* conidia. *Infect Immun*. 2011;79:125-135.
29. Iijima K, Kobayashi T, Hara K, et al. IL-33 and thymic stromal lymphopoietin mediate immune pathology in response to chronic airborne allergen exposure. *J Immunol*. 2014;193:1549-1559.
30. Becker KL, Gresnigt MS, Smeekens SP, et al. Pattern recognition pathways leading to a Th2 cytokine bias in allergic bronchopulmonary aspergillosis patients. *Clin Exp Allergy*. 2015;45:423-437.
31. Lilly LM, Scopel M, Nelson MP, Burq AR, Dunaway CW, Steele C. Eosinophil deficiency compromises lung defense against *Aspergillus fumigatus*. *Infect Immun*. 2014;82:1315-1325.
32. Knutsen AP, Kariuki B, Consolino JD, Warrier MR. IL-4 alpha chain receptor (IL-4R alpha) polymorphisms in allergic bronchopulmonary aspergillosis. *Clin Mol Allergy*. 2006;4:3.
33. Geuking MB, Köller Y, Rupp S, McCoy KD. The interplay between the gut microbiota and the immune system. *Gut Microbes*. 2014;5:411-418.
34. Takahashi K. Interaction between the intestinal immune system and commensal bacteria and its effect on the regulation of allergic reactions. *Biosci Biotechnol Biochem*. 2010;74:691-695.
35. Kasakura K, Takahashi K, Itoh T, et al. Commensal bacteria directly suppress in vitro degranulation of mast cells. *Biosci Biotechnol Biochem*. 2014;78:1669-1676.
36. Kolwijck E, van de Veerdonck FL. The potential impact of the pulmonary microbiome on immunopathogenesis of *Aspergillus*-related lung disease. *Eur J Immunol*. 2014;44:3156-3165.
37. Lynch SV. The lung microbiome and airway disease. *Ann Am Thorac Soc*. 2016;13(Suppl 5):S462-S465.
38. Boutin S, Dalpke AH. Acquisition and adaptation of the airway microbiota in the early life of cystic fibrosis patients. *Mol Cell Pediatr*. 2017;4:1.
39. Delhaes L, Monchy S, Fréalle E, et al. The airway microbiota in cystic fibrosis: a complex fungal and bacterial community – implications for therapeutic management. *PLoS One*. 2012;7:e36313.
40. Krause R, Moissl-Eichinger C, Halwachs B, et al. Mycobiome in the lower respiratory tract – a clinical perspective. *Front Microbiol*. 2017;7:2169.
41. Kim SH, Clark ST, Surendra A, et al. Global analysis of the fungal microbiome in cystic fibrosis patients reveals loss of function of the transcriptional repressor Nrg1 as a mechanism of pathogen adaptation. *PloS Pathogen*. 2015;11:e1005308.
42. Agarwal R, Aggarwal AN, Sehgal IS, Dhooria S, Behera D, Chakrabarti A. Performance of serum galactomannan in patients with allergic bronchopulmonary aspergillosis. *Mycoses*. 2015;58:408-412.
43. Luong ML, Chaparro C, Stephenson A, et al. Pretransplant *Aspergillus* colonization of cystic fibrosis patients and the incidence of post-lung transplant invasive aspergillosis. *Transplantation*. 2014;97:351-357.
44. Fillaux J, Brémont F, Murris M, et al. *Aspergillus* sensitization or carriage in cystic fibrosis patients. *Pediatr Infect Dis J*. 2014;33:680-686.
45. Schønheyder H, Jensen T, Høiby N, Andersen P, Koch C. Frequency of *Aspergillus fumigatus* isolates and antibodies to *Aspergillus* antigens in cystic fibrosis. *Acta Pathol Microbiol Immunol Scand B*. 1985;93:105-112.
46. Page ID, Richardson MD, Denning DW. Comparison of six *Aspergillus*-specific IgG assays for the diagnosis of chronic pulmonary aspergillosis (CPA). *J Infect*. 2016;72:240-249.
47. Richardson MD, Page ID. *Aspergillus* serology: have we arrived yet? *Med Mycol*. 2017;55:48-55.
48. El-Dahr J, Fink R, Selden R, Arruda LK, Platts-Mills TA, Heymann PW. Development of immune responses to *Aspergillus* at an early age in children with cystic fibrosis. *Am J Respir Crit Care Med*. 1994;150:1513-1518.
49. Jubin V, Ranque S, Stremler Le Bel N, Sarles J, Dubus JC. Risk factors for *Aspergillus* colonization and allergic bronchopulmonary aspergillosis in children with cystic fibrosis. *Pediatr Pulmonol*. 2010;45:764-771.
50. Woolnough KF, Richardson M, Newby C, et al. The relationship between biomarkers of fungal allergy and lung damage in asthma. *Clin Exp Allergy*. 2017;47:48-56.
51. Felton TW, Roberts SA, Isalska B, et al. Isolation of *Aspergillus* species from the airway of lung transplant recipients is associated with excess mortality. *J Infect*. 2012;65:350-356.
52. Maturu VN, Agarwal R. Prevalence of *Aspergillus* sensitization and ABPA in cystic fibrosis: systematic review and meta-analysis. *Clin Exp Allergy*. 2015;45:1765-1778.
53. Kousha M, Tadi R, Soubani AO. Pulmonary aspergillosis: a clinical review. *Eur Respir Rev*. 2011;20:156-174.
54. Agarwal R, Aggarwal AN, Garg M, Saikia B, Chakrabarti A. Cut-off values of serum IgE (total and *A. fumigatus*-specific) and eosinophil

count in differentiating allergic bronchopulmonary aspergillosis from asthma. *Mycoses*. 2014;57:659-663.
55. Crameri R, Garbani M, Rhyner C, Huitema C. Fungi: the neglected allergenic sources. *Allergy*. 2014;69:176-185.
56. 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*. 2003;37(Suppl 3):S225-S264.
57. Knutsen AP. Allergic bronchopulmonary aspergillosis. *Clin Exp Allergy*. 2015;45:298-299.
58. 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;43:850-873.
59. Armstead J, Morris J, Denning DW. Multi-country estimates of different manifestations of aspergillosis in cystic fibrosis. *PLoS One*. 2014;9:e98502.
60. Patterson KC, Strek ME. Diagnosis and treatment of pulmonary aspergillosis syndromes. *Chest*. 2014;146:1358-1368.
61. Tillié-Leblond I, Germaud P, Leroyer C, et al. Allergic bronchopulmonary aspergillosis and omalizumab. *Allergy*. 2011;66:1254-1256.
62. Tanou K, Zintzara E, Kaditis AG. Omalizumab therapy for allergic bronchopulmonary aspergillosis in children with cystic fibrosis: a synthesis of published evidence. *Pediatr Pulmonol*. 2014;49:503-507.
63. Beam KT, Coop CA. Stroid sparing effect of omalizumab in seropositive allergic bronchopulmonary aspergillosis. *Allergy Rhinol*. 2015;6:143-145.
64. Knutsen AP, Hutcheson PS, Slavin RG, Kurup VP. IgE antibody to *Aspergillus fumigatus* recombinant allergens in cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *Allergy*. 2004;59:198-203.
65. 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;17:1330-1336.
66. Johansson SGO, Yman L. In vitro assays for immunoglobulin E. *Clin Rev Allergy*. 1988;6:93-139.
67. 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*. 1978;117:917-927.
68. Lambert C, Sarrat A, Bienvenu F, et al. The importance of EN ISO 15189 accreditation of allergen-specific IgE determination for reliable in vitro allergy diagnosis. *Allergy*. 2014;70:180-186.
69. Hamilton RG. Allergic sensitization is a key risk factor for but not synonymous with allergic disease. *J Allergy Clin Immunol*. 2014;134:360-361.
70. Hamilton RG. Clinical laboratories worldwide need to report IgE antibody results on clinical specimens as analytical results and not use differential positive thresholds. *J Allergy Clin Immunol*. 2015;136:811-812.
71. Pedrosa M, Boyano-Martinez T, Garcia-Ara MC, Caballero T, Quirce S. Peanut seed storage proteins are responsible for clinical reactivity in Spanish peanut-allergic children. *Pediatr Allergy Immunol*. 2012;23:654-659.
72. Agabriel C, Ghazouani O, Birnbaum J, et al. Ara h 2 and Ara h 6 sensitization predicts peanut allergy in Mediterranean pediatric patients. *Pediatr Allergy Immunol*. 2014;25:662-667.
73. Ollert M, Weissenbacher S, Rakoski J, Ring J. Allergen-specific IgE measured by a continuous random-access immunoanalyzer: interassay comparison and agreement with skin testing. *Clin Chem*. 2005;51:1241-1249.
74. 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:e61105.
75. Linden CC, Misiak RT, Wegienka G, et al. Analysis of allergen specific IgE cut points to cat and dog in the childhood allergy study. *Ann Allergy Asthma Immunol*. 2011;106:153-158.
76. Tanimoto H, Fukutomi Y, Yasueda H, et al. Molecular-based allergy diagnosis of allergic bronchopulmonary aspergillosis in *Aspergillus fumigatus*-sensitized Japanese patients. *Clin Exp Allergy*. 2016;46:381.
77. Mari A, Schneider P, Wally V, Breitenbach M, Simon-Nobbe B. Sensitization to fungi: epidemiology, comparative skin tests, and IgE reactivity to fungal extracts. *Clin Exp Allergy*. 2003;33:1429-1438.
78. Vitte J, Romain T, Carsin A, et al. *Aspergillus fumigatus* components distinguish IgE but not IgG4 profiles between fungal sensitization and allergic broncho-pulmonary aspergillosis. *Allergy*. 2016;71:1640-1643.
79. Esch RE. Allergen source materials and quality control of allergenic extracts. *Methods*. 1997;13:2-13.
80. Crameri R, Zeller S, Glaser AG, Vilhelmsson M, Rhyner C. Cross-reactivity among fungal allergens: a clinically relevant phenomenon? *Mycoses*. 2009;52:99-106.
81. Maiz L, Cuevas M, Quirce S, et al. Serologic IgE immune responses against *Aspergillus fumigatus* and *Candida albicans* in patients with cystic fibrosis. *Chest*. 2002;121:782-788.
82. Crameri R. Allergic bronchopulmonary aspergillosis (ABPA). Section B07. In: Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S et al.EAACI molecular allergology user's guide. *Pediatr Allergy Immunol*. 2016;27(Suppl 23):81-83.
83. Baxter CG, Denning DW, Joes 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*. 2013;19:E197-E204.
84. Oliva A, Flori P, Hennequin C, et al. Evaluation of the *Aspergillus* Western blot IgG kit for diagnosis of chronic aspergillosis. *J Clin Microbiol*. 2015;53:248-254.
85. Persat F, Hennequin C, Gangneux JP, Société Française de Mycologie Médicale. *Aspergillus* antibody detection: diagnostic strategy and technical considerations from the Société Française de Mycologie Médicale (French Society for Medical Mycology) expert committee. *Med Mycol*. 2017;55:302-307.
86. Agarwal R, Dua D, Choudhary H, et al. Role of *Aspergillus fumigatus*-specific IgG in diagnosis and monitoring treatment response in allergic bronchopulmonary aspergillosis. *Mycoses*. 2017;60:33-39.
87. Arruda LK, Platts-Mills TA, Longbottom JL, El-Dahr JM, Chapman MD. *Aspergillus fumigatus*: identification of 16,18, and 45 kDA antigens recognized by human IgG and IgE antibodies and murine monoclonal antibodies. *J Allergy Clin Immunol*. 1992;89:1166-1176.
88. Bousquet J, Anto JM, Akdis M, et al. Paving the way of systems biology and precision medicine in allergic diseases: the MeDALL success story. *Allergy*. 2016;71:1513-1525.
89. Siroux V, Lupinek C, Resch Y, et al. Specific IgE and IgG measured by the MeDALL allergen-chip depend on allergen and route of exposure: the EGEA study. *J Allergy Clin Immunol*. 2017;139:643-654.
90. Canonica GW, Ansotegui IJ, Pawankar R, et al. A WAO-ARIA-GA^{2}LEN consensus document on molecular-based allergy diagnostics. *World Allergy J*. 2013;6:17.
91. Santos AF, James LK, Bahnson HT, et al. IgG4 inhibits peanut-induced basophil and mast cell activation in peanut-tolerant children sensitized to peanut major allergens. *J Allergy Clin Immunol*. 2015;135:1249-1256.
92. Hemmann S, Nikolaizik WH, Schöni MH, Blaser K, Crameri R. Differential recognition of recombinant *Aspergillus fumigatus* allergens by cystic fibrosis patients with allergic bronchopulmonary aspergillosis or *Aspergillus* allergy. *Eur J Immunol*. 1998;28:1155-1160.
93. www.immqas.org. Accessed February 2, 2017.

94. Van Toorenenbergen AW. Between-laboratory quality control of automated analysis of IgG antibodies against *Aspergillus fumigatus*. *Diagn Microbiol Infect Dis*. 2012;74:278-281.
95. Kurup VP, Banerjee B, Hemmann S, Greenberger PA, Blaser K, Crameri R. Selected recombinant *Aspergillus fumigatus* allergens bind specifically to IgE in ABPA. *Clin Exp Allergy*. 2000;30:988-993.
96. 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*. 2006;4:11.
97. Ott H, Folster-Holst R, Merk HF, Baron JM. Allergen microarrays: a novel tool for high-resolution IgE profiling in adults with atopic dermatitis. *Eur J Dermatol*. 2010;20:54-61.
98. Gabriel MF, Postigo I, Gutierrez-Rodriguez A, et al. Characterisation of *Alternaria alternata* manganese-dependent superoxide dismutase, a cross-reactive allergen homologue to Asp f 6. *Immunobiology*. 2015;220:851-858.
99. Simpson A, Lazic N, Belgrave DC, et al. Patterns of IgE responses to multiple allergen components and clinical symptoms at age 11 years. *J Allergy Clin Immunol*. 2015;136:1224-1231.

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