

Review Article

Serum markers for allergic bronchopulmonary aspergillosis in cystic fibrosis: State of the art and further challenges

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Allergic bronchopulmonary aspergillosis (ABPA), which results from hypersensitivity, primarily to *Aspergillus*, represents a severe complication in patients suffering from asthma or cystic fibrosis (CF). Since early treatment of ABPA is supposed to prevent long-term damages, ABPA has to be diagnosed promptly. However, this diagnosis is not straightforward due to clinical and radiological features of ABPA overlapping with those of CF. Despite ABPA specific diagnosis criteria proposed by the Cystic Fibrosis Foundation in 2003, making a definitive ABPA diagnosis in CF patients remains a challenge. Recent advances in the immunopathogenesis of ABPA have initiated the development of new serological tests, such as the recently reported detection of specific IgE to recombinant *A. fumigatus* allergens, or Thymus- and activation-regulated chemokine (TARC / CCL17), both of which are of value in the diagnosis of ABPA. We review in this paper the serum markers that can advance ABPA diagnosis in CF patients, ranging from the well known criteria (anti-*A. fumigatus* IgE, IgG, and precipitins) to the recent biomarkers (IgE towards recombinant *A. fumigatus* allergens or TARC detection). Taking into account the up-dated physiopathology of ABPA, we discuss their place and their usefulness, especially TARC, to improve early ABPA detection and monitoring in CF patients.

Keywords cystic fibrosis, ABPA, biomarkers, serodiagnosis, TARC

Introduction

Aspergillus fumigatus is the main causal agent of human aspergillosis (80% of clinical infections). It can induce aspergilloma in patients with pre-existing lung-cavities, chronic necrotizing aspergillosis or invasive infection in mildly or severely immunocompromised patients, and finally allergic responses in sensitized individuals such as those with allergic bronchopulmonary aspergillosis (ABPA) [1,2]. ABPA frequently occurs in patients suffering from asthma or cystic fibrosis (CF), and can contribute

significantly to the morbidity of CF lung disease [1,2]. It represents an important CF complication with an overall prevalence of 7.8% in Europe [3]. However, this prevalence rate ranges from 0.95 to 10.9% (see Table 1 in Pihet *et al.* [4]) depending on the criteria used in the diagnosis of ABPA in CF patients, which vary considerably among countries, centres, and studies.

ABPA is an allergic late-phase inflammatory response to numerous antigens of *Aspergillus*, mainly of *A. fumigatus* but sometimes to those of other species such as *A. flavus* [5]. A similar disease entity can be caused by other moulds such as *Scedosporium apiospermum* and referred to more generally as allergic bronchopulmonary mycosis (ABPM). This lung complication, resulting from hypersensitivity, is clinically characterized by impaired mucociliary clearance, mucoid impaction with airway obstruction, and pulmonary infiltrates. Since early treatment of ABPA is

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Table 1 Cystic Fibrosis Foundation Consensus Conference's criteria for the diagnosis of ABPA and guidelines for screening criteria [7].

Criteria for ABPA diagnosis

- Acute or subacute clinical deterioration
- Serum total IgE > 1000 IU/ml
- Immediate positive skin test to *A. fumigatus* > 3 mm or positive specific IgE to *A. fumigatus*
- *A. fumigatus* positive precipitins or presence of anti-*A. fumigatus* IgG
- New or recent changes in chest radiograph/CT

Guidelines for screening criteria

- Persistence of clinical suspicion for ABPA in patients > 6 years old
- Measure total serum IgE annually:
If IgE > 500 IU/ml, determine immediate cutaneous reactivity to *A. fumigatus* and consider diagnosis
If IgE < 500 IU/ml, repeat IgE measurement and perform further diagnostic tests

supposed to reduce symptoms, to improve lung function, and to prevent long-term damage such as bronchiectasis and fibrosis, ABPA has to be diagnosed promptly. However, due to its overlapping clinical and radiological features with those of CF exacerbations, ABPA diagnosis is known to be difficult and must be confirmed through a combination of clinical, radiological and laboratory data.

Different sets of diagnostic criteria, as well as guidelines for screening these criteria, were established for ABPA in CF patients by the UK cystic fibrosis trust in 2002 [6] and the US cystic fibrosis foundation consensus conference in 2003 [7]. In 2003, five criteria were proposed to describe classic ABPA (Table 1), associated with a set of four minimal diagnostic criteria including; (i) clinical deterioration, (ii) total serum IgE concentration >500 IU/ml, (iii) positive immediate cutaneous reactivity to *A. fumigatus* or raised specific anti-*A. fumigatus* IgE, and (iv) IgG precipitins, but these are not mandatory and can be replaced by radiological or CT-scan changes [7]. In the UK, published diagnosis criteria are slightly different since they include blood eosinophilia >500/mm³, total IgE >500 IU/ml or four-fold increase in IgE titres, and recovery of *Aspergillus* in culture from sputum or observation of fungal hyphae upon direct examination of clinical samples [6]. Despite these clearly defined criteria for the diagnosis of ABPA in CF patients, making a definitive ABPA determination can still, on occasion, be challenging. Recent advances from experimental studies of the immunopathogenesis of ABPA in CF have resulted in the development of new serological tests, such as detection of specific IgE to recombinant *A. fumigatus* allergens (rAsp f), macrophage-derived chemokine (MDC), or Thymus activation-regulated chemokine (TARC/CCL17) which may contribute to ABPA diagnosis [8–14].

The aim of this paper is to review serum markers that can advance ABPA diagnosis in CF patients, from the well known criteria (anti-*A. fumigatus* IgE, IgG precipitins) to the recent biomarkers, i.e., IgE to rAsp f or MDC,

or TARC detection. Taking into account the up-dated information on the physiopathology of ABPA, we discuss their place and usefulness, especially TARC, in improving early ABPA detection and monitoring of CF patients.

Current physiopathology of ABPA in CF patients and clinical significance of biomarkers in the course of ABPA

The physiopathologic processes involved in ABPA are complex, not fully understood, and probably linked to multiple factors including genetic aspects of the host, as well as individual responses to allergens and antigens and environmental conditions. Since recent reviews have largely detailed the pathogenesis of ABPA in CF patients [15,16], we will only focus on those physiopathology points that can contribute to a better understanding of biomarkers. ABPA is still considered to be the result of type I, III and IVb responses to *Aspergillus* compounds [1,7,17,18]. In CF patients, the presence of excessive thick and sticky bronchial mucus in airways plays an important role by facilitating the entrapment of airborne fungal conidia and providing a suitable environment for the growth of these microorganisms. The conidia subsequently germinate and continuously release antigens, as well as specific allergens (such as rAsp f6, the manganese-dependent superoxide dismutase produced during fungal growth [19]), which are responsible for mainly a Th2-mediated immune host response [1,18,20] and can contribute to ABPA diagnosis as highly specific serum markers [10,12,21], or allergens for skin test [22,23]. As reviewed recently [1,2,7,11], *Aspergillus* antigens activate both T and B cells, but high levels of IL-4 and IL-10 result in a predominant Th2 immune response, with an enhancement of both IgE production and attraction of eosinophils. The activation of B cells in CF patients who are developing ABPA, leads to high levels not only of IgE (including total IgE and specific

IgE towards *A. fumigatus* allergens and recombinant antigens), but also of IgA and IgG, except the IgG3 subclass [1,24]. Dendritic cells (DCs) in the respiratory tract are currently considered as one of the major cell types [15,16] which initiate the adaptative immune response to pulmonary pathogens [2,25]. DCs have also been reported to be able to (a) internalize *A. fumigatus* conidia and hyphae via their Toll-like receptor [2,25,26], (b) promote Th2 response, (c) attract regulatory T cells (Treg), (d) as well as to secrete thymus- and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC) [16,20,26–28]. TARC and MDC are two CC-chemokine ligands (CCL17 and CCL22 respectively), able to recruit Th2 and Treg cells via a common receptor, i.e., the CC-chemokine receptor 4 (CCR4). The different hypotheses about immunological mechanisms involving CCR4 receptor and TARC have been detailed in recent reviews [15,16,29]. While regulatory processes of CF are probably highly complex and remain unclear due to numerous interactions between human immune system, genetic disease, therapies, variability in fungal protein expression and interaction with frequent bacterial colonizations or infections, it is highly speculated that TARC plays a role in the ABPA process. The association between elevated serum levels of TARC and the alteration in lung function (decreased FEV₁) might support this pathophysiological role for TARC in Th2 response in ABPA [8,11]. More recently, Th17 cell population expressing CCR4 (TARC receptor) has been described in the lung of CF patients, but it is still matter of debate whether Th17 response is protective or not (for review [15]). Finally, the acute inflammatory response during ABPA peak could progressively lead to irreversible fibrosis with destruction of nearby lung tissue, rationalizing an early appropriate therapy.

To summarize, the strong immunologic responses are key determinants in the pathogenesis of ABPA. While the relationship between sensitization to *A. fumigatus* and progression of pulmonary dysfunction in CF patients with ABPA is still unclear (with conflicting studies and few that are longitudinal [6]), we might improve our ability to differentiate *A. fumigatus* sensitization from ABPA for its quick diagnosis and treatment to prevent serious and potentially irreversible lung damages. Beside the lack of test standardization, some improvements have been made in this area, such as the production of recombinant *A. fumigatus* antigens (rAsp f) involved in ABPA immune process, and which are of interest in the diagnosis of ABPA exacerbation, especially rAsp f4, and rAsp f6. Some authors [8] have suggested combining total serum IgE, with specific IgE to rAsp f to differentiate ABPA from sensitization. On the immunology side, TARC levels in serum currently appear to be the best way to differentiate CF patients with and without ABPA even if

its role within the complex immune response remains unclear [8].

All these serological markers are summarized in Table 2. Regarding the current understanding of ABPA pathogenesis in CF, their place to support ABPA diagnosis and evolution is now discussed.

IgE immune response against *A. fumigatus* in CF patients

Current place of total IgE detection

Total IgE level is one of the major criteria in the diagnosis of ABPA in CF patients [7]. Since IgEs are key molecules in immediate and late hypersensitization phases, total IgE detection is included in the screening tests with the suggestions for the annual measurement of total serum IgE, associated with other diagnostic tests, i.e., specific IgE and IgG detection, if titers are >500 IU/ml. Other diagnostic tests (Table 1) and a new total IgE measurement are strongly recommended if the level is <500 IU/ml [6,7]. Nevertheless, the importance of total IgE in ABPA diagnosis cannot be overemphasized, taking into account its poor specificity and the individual variability in immune response. IgE is not exclusively directed towards *Aspergillus* allergens but is highly stimulated by the IL-4/Th2 response in CF, and remains non-specific.

Furthermore, the intense and repetitive immune stimulation by fungal allergens may lead to an increase of total IgE in CF even in the absence of ABPA [30]. Alternatively, ABPA has been diagnosed in some rare patients with clinical suspicion but low total IgE levels (<500 IU/ml) [31–33]. It could be potentially linked to some differences in the ability to class switching to IgE in B cells of the bronchial mucosa as proposed in asthma [34]. The value of this criterion varies markedly according to the ABPA stages (Table 2) [17,35], the atopic background [7] and the delay in IgE dosage (half-life of IgE is about 2.5 days). Total IgE antibodies are present in blood but the level is not correlated with the extent of bronchiectasis [36]. Conversely, total IgE level in blood decreased in response to corticosteroid therapy [37,38]. Despite some limitations [14], the total IgE level is still recommended for ABPA screening and follow-up purposes in CF patients [13,18,39].

Specific IgE responses towards *A. fumigatus* allergens and recombinant antigens

Complex mixtures of allergens derived from *A. fumigatus* cultures have been described and used to measure specific immune response using different methods (ELISA Kit, Homemade ELISA technique, and ImmunoCAP system), with cut-offs ranging from 0.35 kUA/l (corresponding to

Table 2 Place of biomarkers according to the five stages of ABPA.

ABPA Stages [35]	Clinical features	Radiology	Biology in serum					TARC [8]
			Blood eosinophilia [17]	Total IgE [17]	Specific IgE to <i>A. fumigatus</i> [13]	Anti- <i>A. fumigatus</i> precipitins or IgG [24]	Anti- <i>A. fumigatus</i> IgA [8,80,88]	Specific IgE to recombinant allergens [13]
I: acute	Fever, cough, chest pain, haemoptysis, sputum	Pulmonary infiltrate(s)	+	+++	++	+ excepted IgG ₃	++	+++ rAsp f2 +++ rAsp f3 +++ rAsp f4
II: remission	Asymptomatic or stable asthma	No infiltrate	–	+	+/-	+/-		+++ rAsp f6 +++ rAsp f3 +++ rAsp f4
III: exacerbation	Symptoms mimicking the acute stage or asymptomatic	Pulmonary infiltrate(s)	+	+++	+	+	++	+++ rAsp f1 +++ rAsp f2 +++ rAsp f3 +++ rAsp f6
IV: cortico-dependent asthma	Persistent severe asthma	+/- pulmonary infiltrate(s), +/- proximal bronchiectasis	+/-	+++	+/-	+/-		
V: fibrosis (end-stage)	Cyanosis, severe dyspnea	Cavitary lesions, Extensive bronchiectasis, Fibrosis	–	+	+/-	+/-	+	

class 1 [10,12,40,41]) to higher than 3.7 kUA/l (corresponding to class 3 or 4 [9]). Crude allergen fractions, for which variability in antigenic content has been described in commercial extracts [42], are commonly used in laboratory practices. Despite this lack of standardization, high levels of anti-*A. fumigatus* IgE are often linked to ABPA in patients with *Aspergillus* sensitization and developing ABPA [31]. The mean value is generally more elevated in CF-ABPA patients than those with CF or atopic patients without ABPA [12,13,43]. However, since *A. fumigatus* secretes numerous allergenic compounds, including enzymes, IgE towards *A. fumigatus* recombinant antigens have been recently described as being more suitable than crude *A. fumigatus* antigens [7,12,23]. Purified but non commercialized *A. fumigatus* allergens (such as rAsp f13, rAsp f16, rAsp f34) have been tested, but their usefulness in ABPA diagnosis should be explored and confirmed on larger populations of CF patients [12,44–48]. Currently, five recombinant allergens, i.e., rAsp f1, f2, f3, f4 and rAsp f6, have been commercialized and studied. Briefly, two of them, that is rAsp f1 (a ribotoxin) and rAsp f3 (related to a peroxisomal protein) seem to be related to fungal sensitization, but rAsp f3 was also found to be of interest in ABPA diagnosis [13]. Two other recombinant allergens, i.e., rAsp f4 (an endocellular protein of unknown function) and rAsp f6 (a manganese-dependent superoxide dismutase produced during fungal growth [19]) may be more specific for ABPA diagnosis, whatever their use as serum markers [10,12,21] or in intracutaneous tests [22,23]. The major fungal allergen, rAsp f2 a fibrinogen binding protein has been reported to bind specifically with IgE in ABPA sera [49]. While the relevance of using these five commercial recombinants in ABPA diagnosis is still matter of debate [14,50], the measurement of specific antibodies to rAsp f4 and rAsp f6 in association with total IgE determination could be helpful in discriminating CF patients with ABPA from *Aspergillus* sensitized CF patients without ABPA [8,10,12,13,51,52]. The reported specificity of these two recombinants is higher than their sensitivity, particularly for rAsp f6 [14,40], and using the ImmunoCap method rather than ELISA [12]. None of these criteria have been studied according to ABPA Patterson's stages, but Knutsen *et al.* [13] compared results of recombinant allergens from ABPA-CF patients during flares and remission, and found a level of Asp f3 significantly higher during flares of ABPA-CF patients (Table 2). While it has been demonstrated that neither one single recombinant allergen nor one class of antibody can differentiate ABPA from non-ABPA CF patients [12], an evaluation of the binding to different antibody isotypes (IgA, IgG, and IgE) of several recombinant allergens has been proposed to be better able to discriminate CF patients with ABPA.

IgG Immune response against *A. fumigatus* in CF patients

Since precipitins and anti-*A. fumigatus* IgG detection are also important criteria in the diagnosis of ABPA [6,7], a large number of studies have been published to document this class of antibodies. Precipitating antibodies were described as sensitive markers in CF patients [52,53], but their level fluctuates over time and they may also be absent in some CF patients with ABPA [53,54]. Furthermore, presence of precipitins in CF patients without ABPA suggests they cannot differentiate sensitization from ABPA. Their detection is based on a semi-quantitative test, performed by immunoprecipitation methods (double immunodiffusion and counterimmunoelectrophoresis are the most common methods used) with crude antigen extracts. One precipitin line is usually considered as significant [53,55], but there is a wide variety of cut-off criteria used to define the significance of precipitin test results. Revelation of specific enzymatic activities linked to fungal catalases and chymotrypsin could be useful in the serodiagnosis of aspergillosis in immunocompetent hosts, including ABPA [54,56,57]. More recently, anti-*A. fumigatus* IgG detection by ELISA has been developed. ELISA methods have many advantages, i.e., they are simple, sensitive, can be automated, target crude extract, purified/semi purified or recombinant antigens, and allow quantification of the antibody response [58]. Although few commercial ELISA kits have been developed [9,55], precipitin and anti-*A. fumigatus* IgG detection methods are generally homemade and lack standardization.

Different antigens used

Crude extracts from mycelia or spores (somatic antigens) and culture filtrates (metabolic antigens) are the most commonly used antigens [52]. In invasive aspergillosis, even if the use of different antigen extracts in the IgG testing for *Aspergillus* antibody detection is not well worked out and standardized, one study reported that somatic antigens seem to be related to early detection whereas metabolic antigens could detect a higher proportion of positive antibody responses [59].

Regarding the considerable strain variation of their composition and reactivity, as well as the culture conditions, the preparation method and the batch [52,54], relevant components of crude antigenic extracts have been identified and purified, in order to improve antibody detection and to obtain standardized immunoassays. Semi purified antigens with reactivity to IgG from CF-ABPA and non CF-ABPA sera include the following; a 250-kDa fraction with catalase activity [56], 73-kDa and 43-kDa fractions with acid protease activity [60], or Ag7 and Ag13 [61]. A few studies compared IgG detection in ABPA patients those with

aspergilloma or invasive pulmonary aspergillosis (IPA) and reported the identification of ABPA specific antibodies. First, a Western blot approach with crude *A. fumigatus* extracts revealed that, as opposed to sera from patients with aspergilloma, ABPA sera (from patients with unknown underlying disease) frequently reacted with low-molecular-weight (<14-kDa) components, suggesting these could be more specifically related to ABPA, even if their precise composition widely varied from patient to patient [62]. Secondly, sequential exploration of antibody response to fractionated *A. fumigatus* antigens (mixed somatic and metabolic) in CF patients revealed that two proteins (>900-kDa and 170-kDa) were the most recognized by human IgG antibodies in sera from CF patients with ABPA. Increasing titers to these fractions could be correlated with exacerbations of the condition. But only the 170-kDa fraction and another fraction (88-kDa) were found to be significantly different between ABPA and non-ABPA patients [63].

More recently, recombinant antigens have been produced (in *Pichia pastoris* or *Escherichia coli* [64–66]) and evaluated for the diagnosis of ABPA. Only four out of eight recombinant antigens (the 18-kDa ribonuclease (RNU), the 360-kDa catalase (CAT), the dipeptidylpeptidase V (DPPV) and the dipeptidylpeptidase IV (DPPIV)) were able to discriminate between sensitized and ABPA patients. Using a combination of RNU and DPPV yielded a high diagnostic value (88% sensitivity), and antibody level follow-up in the same patient revealed that an increase in titers was more often associated with ABPA episodes whereas a decrease was correlated with an improvement of the patient [65]. Other recombinant antigens, such as mitogillin [66] or cell wall galactomannoprotein [64] have been shown to improve the diagnosis of aspergilloma and IPA (100% and 33–64% sensitivity, respectively), but their contribution to ABPA diagnosis has not yet been evaluated.

IgG versus precipitins for the diagnosis of ABPA in CF patients

Studies evaluating the contribution of IgG detection by ELISA to the diagnosis of ABPA in CF patients are summarized in Table 3. They report highly variable sensitivity and specificity, which reach 45–100% and 39–84%, respectively, and result from huge variation both in ELISA method (antigen, cut-off), and population selection (ABPA and controls) (Table 3).

When concordance between ELISA and agar gel double diffusion (AGDD) was analyzed, a clear relation has been reported in non-CF patients. Sera that react in AGDD also react in ELISA [67] and patients with a higher number of precipitating lines (three or more) have higher ELISA titers than patients with two or fewer precipitin lines [68]. Similarly, in CF patients, ELISA methods correlate approximately

Table 3 Sensitivity and specificity of anti-*Aspergillus fumigatus* IgG detection by ELISA for ABPA serodiagnosis in CF patients. Comparison with precipitins.

ELISA IgG detection	Antigen composition	CF ABPA patients			Control patients			Sensitivity of ELISA	Sensitivity Specificity of DGD [†] using A _f [‡] extracts
		Cut-Off	Number	Mean value of ELISA (or median if indicated)	Number and type	Mean value of ELISA (or median if indicated)			
Homemade	Mixture of somatic and culture filtrate	>12% of PC*	3 ABPA, 4 probable	53 ± 28% of PC 70 ± 59% of PC	23 CF sensitized 37 CF controls	43 ± 69% of PC 6 ± 3% of PC	100% 67%	86% 90%	[63]
Homemade	Extract from Diakit (Greer Laboratories) and private source	mean OD ≥ twice control pool**	6 ABPA	NA [§]	112 CF without ABPA	NA	NA 39%	100% NA	[53]
Homemade	NA	NA	626 ABPA	14.3 ± 6.5 g/l	5693 CF without ABPA	12.4 ± 6.3 g/l	NA	Not done	[3]
Homemade	Standardized DPC antigens (Diagnostic Products Corporation, Los Angeles, CA)	50 or 100 kU/l	16 ABPA	NA	36 CF without sensitization or ABPA	NA	100% 86%	Not done	[21]
Homemade	<i>A. fumigatus</i> extract	140 kU/l	12 ABPA	NA	36 CF without ABPA	NA	54% 84%	Not done	[8]
ImmunoCAP (Phadia)	NA	35mgA/l [#] (or 70 mgA/l)	11 ABPA	13.7 mgA/l (median)	42 healthy controls 112 CF without ABPA	25.7 mgA/l (median) 33.4 mgA/l (median)	45% 61% (or 9%–86%)	NA (Concordance of DGD with ImmunoCap 86%)	[55]
ImmunoCAP (Phadia)	NA	NA	7 ABPA	132.5 mgA/l	21 CF sensitized 59 CF controls	53.9 mgA/l 9.0 mgA/l	100% 74% (90% if cut-off = 90 mgA/l)	Not done	[9]

[†]DGD = Double Gel Diffusion; [‡]A_f = *A. fumigatus*; *PC = Positive Control; [§]NA = Not Available; **Control pool = sera from skin-test negative and precipitin negative subjects; [#]mgA/L = milligrams of antigen-specific antibodies per liter.

with precipitin titers [9]. A concordance of 86% between ImmunoCAP and precipitins has been recently reported in patients with bronchial asthma, bronchiectasis, CF-ABPA, non-CF ABPA, or other pulmonary diseases [55]. In these patients, Van Hoeyveld *et al.* also observed that samples with a high number of precipitation lines generally have high IgG antibody concentrations with ImmunoCAP, with few discordant cases [55]. The absence of a systematic correlation between IgG titers with the number of bands detected by gel diffusion has also been reported in patients with ABPA (with unknown underlying disease) [62]. Some studies even reported a higher sensitivity of ELISA, which could detect antibodies against non-precipitating antigenic components in addition to the antibodies detected by AGDD [69,70]. A moderate concordance may also be partly explained by differences in assay detection limit, quality and composition of the antigen extract, which are in agreement with the variations between ELISA methods recently observed by Barton *et al.* (a lower sensitivity of a homemade EIA test, with a failure to detect IgG in sera where high level had been detected by ImmunoCAP) [9]. Interestingly, Barton *et al.* also reported higher IgG levels in CF-ABPA patients compared with non-ABPA patients and suggested that ImmunoCAP higher cut-offs could be used to improve the specificity of the diagnosis of ABPA, a suggestion in agreement with the Mastella *et al.* study [3]. However, these results are contradictory with Van Hoeyvelde *et al.*'s study, which reported a high IgG level in patients with IA or aspergillosis and non-CF ABPA patients but not in CF-ABPA patients [55]. All these data need to be confirmed in large multicenter studies, which could contribute in the evaluation of the specificity and sensitivity of both precipitins and IgG detection, and clarify their contribution to the diagnosis of ABPA, especially in the CF patient population. A consensus method, based on standard antigens, is also essential to get a reliable laboratory diagnosis.

IgG subclasses, IgM and IgA immune response against *A. fumigatus*

Few studies have focused on IgM, IgG subclasses, or IgA response against *A. fumigatus* in ABPA. Most of the IgM studies were performed in non-CF patients, demonstrating that IgM antibodies tend to be less specific [71–73].

In CF patients, the usefulness of specific IgG subclasses in the diagnosis of ABPA has been suggested [12,24,74–76]. Higher levels of IgG and IgG subclass antibodies to *A. fumigatus* have been reported in CF patients compared with healthy controls [24]. In particular, the IgG₄ level was elevated in CF patients (specificity of 88%; [24]), whatever the antigen used, i.e., crude antigen or recombinant allergens (Asp f1, Asp f2, Asp f23, Asp f4, and Asp f6) [12]. This result is in agreement with the high specificity of

elevated IgG₄ levels found in non-CF patients with ABPA [24,77,78]. No significant difference was noticed in the IgG₃ levels, while the use of IgG₁, as well as IgG₂ levels gave inconclusive results [12,24]. In addition, anti-*A. fumigatus* IgG subclass levels seem to be influenced by gamma-light chain allotypes in CF patients [76]. Thus, determination of IgG and IgG subclass antibody levels might be more suitable in excluding the diagnosis of ABPA [24,76].

Regarding IgA and IgA subclass antibody response [12,75,79,80], crude *Aspergillus* antigen gave higher IgA levels in sera than rAsp f1, f2, f3, f4 and f6 [12]. Although no significant IgA response was shown in numerous studies on ABPA [12,81], a marked IgA₁ response against crude and recombinant antigens has been recently described in CF patients with ABPA [12]. This is in agreement with previous results of elevated serum anti-*Aspergillus* IgA especially IgA₁ in the acute, exacerbation, and fibrotic stages of ABPA [80]. As the level of IgA is higher in bronchoalveolar fluid (BAL) than in blood [73,79], the determination of specific IgA antibody response in serum is also of limited value, especially in the follow-up of treated ABPA patients [14].

In conclusion, measurements of IgG and IgG subclasses (especially IgG₄) antibodies against *A. fumigatus* seem to be of interest in CF patients, as a screening test to exclude the ABPA diagnosis, while IgA measurement could be useful to determine patient's ABPA stage (Table 2).

New biomarkers: chemokines especially TARC

In CF patients with chronic colonization, *A. fumigatus* antigens are continuously stimulating the immune response, resulting in a large inflammatory response including Th17, as well as Treg cells with a central role of Th2 pathway (for review see [15,16,20]). *De facto* fungal allergens are increasing the production of immunological mediators that could be considered as probable biomarkers regarding their relevance (in terms of specificity and sensitivity) to discriminate sensitization from ABPA [29]. Among them, TARC and MDC are two newly identified mediators which have been suggested to modulate the pulmonary immune response in ABPA.

Place of thymus activation and regulated chemokine (TARC)

In a cross sectional study, higher levels of CCR4 receptor and of TARC were observed in sera of 16 CF patients with ABPA [11]. A longitudinal study [8] confirmed this first conclusion through the use of clinical and biological follow-up of 48 CF patients including 12 with ABPA. TARC levels (analyzed by sandwich ELISA) increased early in the course of ABPA, before total IgE elevation [8], with an inverse correlation between TARC levels and spirometric

parameters (FEV₁) identified in CF patient with ABPA [11]. Elevated TARC level was strongly correlated with the level of rAsp f4 in ABPA patients, while no association was found with the other recombinants (rAsp f1, f2, f3 and f6) [11]. This biomarker showed a greater test accuracy for ABPA diagnosis than all other biological markers tested (total IgE, specific IgG and antibodies against recombinant allergens rAsp f1, rAsp f3, rAsp f4 and rAsp f6) [29]. It seems to be able to differentiate CF patients with ABPA from those colonized or sensitized to *A. fumigatus* [11]. Furthermore, corticotherapy decreases the TARC secretion, producing a rapid decrease of serum TARC level that can be used to follow patient evolution as well as the effect of corticosteroid therapy in ABPA [8,11].

Place of macrophage-derived chemokine (MDC)

In ABPA-CF patients, high serum levels of MDC have been reported and were associated with IgE peaks. MDC serum levels were not correlated with rAsp f4, rAsp f6, or FEV₁ levels, or with corticosteroid therapy [29].

Other chemokines

Detection and profiling of other chemokines (in particular IL-8 and IL-10) have been done to analyze their significance in the evolution of fungal pulmonary diseases during immunological studies. IL-8 and IL-17 levels in sputum are increased in ABPA and could be a key mediator of tissue damage [82,83], while IL-10 produced in large amounts by activated Treg cells is supposed to regulate the immune response [15]. High levels of IL-10 has been found in patients with *A. fumigatus*-related disease [84]. Especially, high serum levels of IL-10 were found in *Aspergillus* colonized CF patients carrying the IL-10-1082GG genotype [84,85].

Taken as a whole, the data focusing on chemokines in ABPA show the high sensitivity and specificity of TARC detection and suggest that TARC could be a new and early marker for ABPA diagnosis in CF [8,11,29]. However, before TARC can be included in ABPA criteria of CF patients, it needs to be evaluated in further studies.

In conclusion, the diagnosis of CF-ABPA is known to be a more difficult task than diagnosing ABPA in patients without cystic fibrosis [1,3,7,12]. For this reason, it may be useful to combine clinical criteria with serological findings that seem to be able to confirm or exclude ABPA in CF patient [10,12–14,22,23]. Specific IgE towards recombinant antigens, i.e., rAsp f4 and rAsp f6 (that have been described as specific markers for clinically active ABPA), and TARC (that appears to be clinically useful to identify ABPA episodes in CF patients already sensitized to *A. fumigatus*) are currently the most promising biomarkers. Furthermore, higher IgG levels in ABPA-CF patients

suggest quantitative approaches could be useful to differentiate sensitized and ABPA CF patients, especially using an increased cut-off point [9].

If the recent studies in the area offer new horizons, further research targeting large groups of CF patients (*Aspergillus* sensitization and ABPA-CF patients) as well as their prospective follow-up is currently needed to improve our knowledge and to assess the exact contribution of these biomarkers in the daily clinical management of ABPA in CF patients. Since IgE reactivity to individual purified recombinant allergens (especially to rAsp f4 and to rAsp f6) as well as TARC production seem to change with the different stages of ABPA [8,13], we need to analyze their ability to differentiate ABPA stages and to clarify their clinical relevance, as the clinical significance of decline in serum IgE levels in ABPA has been described in asthma [86]. Lastly, some studies suggest that these new biomarkers, in particular TARC, may have added value for monitoring therapy by antifungal and/or corticoid [38], which also needs to be more documented. Moreover, new advances in technology such as protein microarrays could offer significant advantages in terms of time and costs compared with traditional test formats, since it was recently described to measure a multitude of allergen-specific IgE antibodies in the same test [87].

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