

Allergic bronchopulmonary aspergillosis: the hunt for a diagnostic serological marker in cystic fibrosis patients

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The diagnosis of allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis patients remains challenging, mainly owing to overlapping symptoms of the underlying lung disease with clinical symptoms of ABPA. In addition, a varying mixture of diagnostic criteria, including clinical status, radiological findings and immunological measurements, has led to confusion and differing recommendations. In order to help simplify as well as standardize the diagnostic criteria for ABPA, different serological markers have been evaluated in the last 20 years and their usefulness has been assessed in many clinical studies. This review presents current diagnostic criteria of ABPA, with a special focus on serum markers supporting the diagnosis and explains why the hunt for a serological marker for ABPA is still ongoing.

KEYWORDS: allergic bronchopulmonary aspergillosis • cystic fibrosis • IgE • recombinant IgE • serum marker
• thymus- and activation-regulated chemokine

Allergic bronchopulmonary aspergillosis (ABPA) is an allergic disease against *Aspergillus fumigatus* occurring mainly in patients with cystic fibrosis (CF) but also in asthmatics. Chronic colonization of the airways with *A. fumigatus* itself leads to a neutrophilic inflammatory response and bronchiectasis [1]. In ABPA, sensitization against *A. fumigatus* occurs, resulting in a Th2-mediated allergic reaction and bronchial obstruction with wheezing [2]. *Aspergillus* species are ubiquitous fungi in nature and are commonly found as saprophytes in soil, plants and decomposing organic matter. Their spores measure 2–3.5 µm in diameter and are, therefore, able to reach the terminal airways and alveoli where they can grow at human body temperature. ABPA occurs in approximately 10% of CF patients and may lead to both acute worsening of respiratory status and long-term decline in lung function [3]. Without appropriate treatment, ABPA can progress to a chronic state, even resulting in lung fibrosis [4]. The current treatment of choice for ABPA is anti-inflammatory therapy with oral steroids [5]. In addition, oral antifungal agents, such as itraconazole, are used widely. However, there remains limited evidence of their usefulness in the control of

exacerbations or for steroid reduction [6]. This article will focus on the diagnosis of ABPA in CF patients and review the ongoing hunt for a serological marker for ABPA diagnosis.

Pathophysiological background

In order to understand why the hunt for a serological marker for ABPA is still ongoing, one has to appreciate the complexity of the underlying immunological mechanisms linking chronic colonization with *A. fumigatus* and Th2-driven immune response, as well as of the regulation of inflammatory and immune cells in allergic airways disease in general. Recent excellent articles have reviewed in great detail the pathogenesis of ABPA in CF [7] and immunological mechanisms linking CF lung disease with ABPA [8]; thus, we will only summarize current knowledge here (FIGURE 1).

Immune sensitization

One of the necessary requirements for the occurrence of ABPA is the sensitization against *A. fumigatus*. Upon colonization of the airways with *A. fumigatus*, local pulmonary dendritic cells (DCs) recognize *A. fumigatus* moieties via MyD88-dependent Toll-like receptors (TLRs)

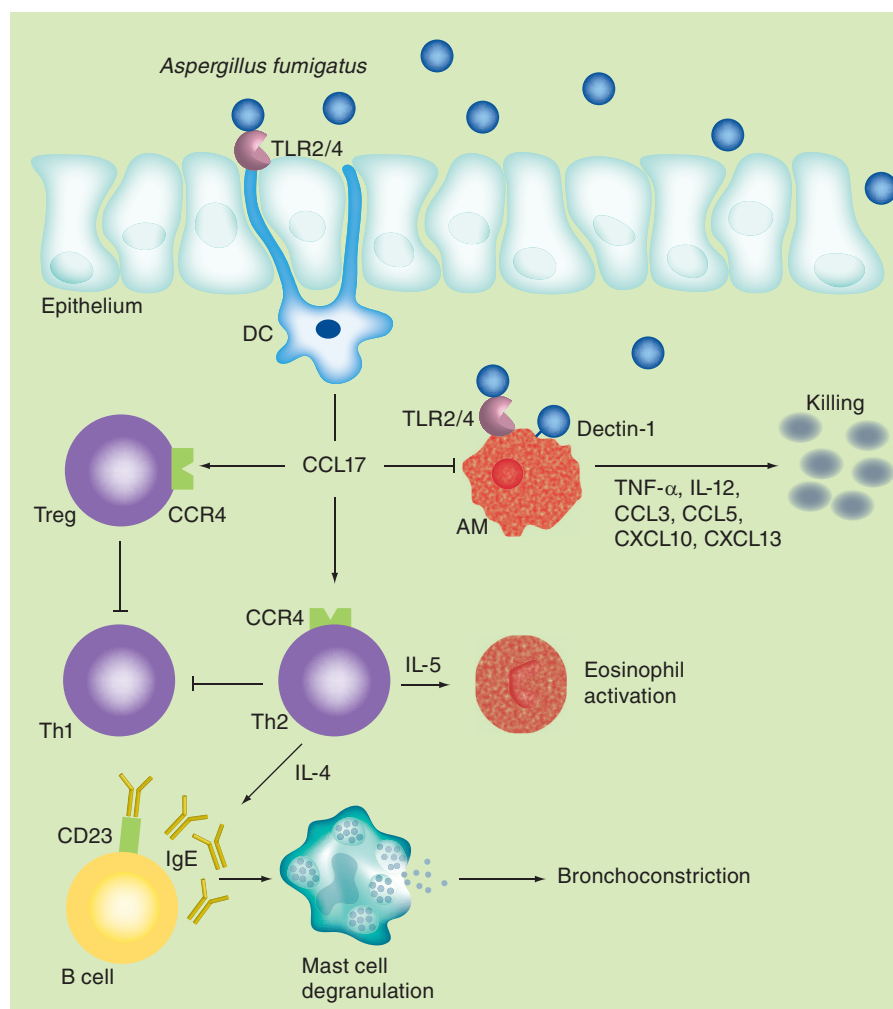


Figure 1. Pathophysiological background of immune mechanisms in ABPA, indicating a central role of TARC/CCL17 in orchestrating the immune response to *Aspergillus fumigatus*. *A. fumigatus* components are recognized via TLR2 and TLR4 on dendritic cells and alveolar macrophages. Dendritic cells present allergen peptides to naive T cells and attract both Th2 and regulatory T cells into the lung by secretion of CCL17 via CCR4. Both types of T cells impair an effective, antifungal Th1 response. The recruited Th2 cells secrete IL-4 leading to IgE production by B cells. The secreted IgE binds to mast cells, finally leading to clinical symptoms of ABPA, such as bronchoconstriction.

ABPA: Allergic bronchopulmonary aspergillosis; DC: Dendritic cells.

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and/or β -glucan dectin-1 receptors [9–11]. Among TLRs, TLR2 in particular seems to play a major role [12,13]. After antigen recognition and uptake, *A. fumigatus* allergens are processed and DCs present allergen peptides to resident naive T cells in the draining pulmonary lymph nodes. Th2-cell responses are initiated via MHCII/HLA and costimulatory molecules (i.e., B7–1, B7–2, CD40, Ox40L, LFA, CD54 and DC-Sign), as well as via the secretion of Th2-associated cytokines at the immunological DC/T-cell synapse. DCs are conditioned by thymic stromal lymphopoietin (TSLP) secreted by epithelial cells and mast cells, and release the chemokines CC-chemokine ligand (CCL) 17 or thymus and activation-regulated chemokine [TARC] and CCL22 (or

macrophage-derived chemokine [MDC]), which both act on CC-chemokine receptor (CCR) 4 to attract Th2 cells to the pulmonary site of inflammation [14]. The recruited Th2 cells themselves release IL-4, -5, -9 and -13, which leads to activation of B cells (IL-4), activation and recruitment of eosinophils from the bone marrow (IL-5 and granulocyte monocyte-colony stimulating factor), production of antigen-specific IgE and IgG1 antibodies (IL-4), mast cell proliferation and stimulation of mucus production (IL-9), and other tissue effects resulting in chronic airway remodeling [15]. Based on this molecular interplay, all cellular and soluble components described in the processes above are thus possible serological markers indicating a sensitization against *A. fumigatus* allergens.

Immune activation & maintenance of the immune response

In allergic processes with occasional inhalation of allergens (e.g., extrinsic allergic asthma), the processes of sensitization and activation occur only intermittently, as the immune system does not need to continuously deal with the allergens. In CF patients who are chronically colonized with *A. fumigatus*, however, the human immune system is continuously exposed to *Aspergillus* antigens. This persistent antigen exposure leads to continuous DC activation, Th2-cell priming and the resulting downstream cascade of Th2 airway inflammation. Antibody-specific IgE-sensitized mast cells are activated by *A. fumigatus* allergens by cross-linking surface-bound IgE molecules and releasing various mediators, such as histamine and leukotrienes, leading to bronchoconstriction, airway inflammation and hyper-responsiveness [7]. Th2 cells have a central

role in orchestrating this inflammatory response by activating B cells, mast cells and eosinophils, as described earlier. It is, however, increasingly realized that other cells than Th2 cells (e.g., DCs and epithelial cells) can activate mast cells and/or DCs and thus directly lead to an allergic response without activation of Th2 cells [15].

Owing to the overlap of the inflammatory pathways involved in the stages of immune sensitization, activation and maintenance of the immune response, mediators involved in any step (and thus possible serological markers) are neither specific for ABPA in general, nor for particular phases of the immunological response to *A. fumigatus* colonization.

Clinical diagnosis

The diagnosis of ABPA is complicated and therefore the definition of clinical ABPA is spongy. Since the late 1970s, the diagnosis of ABPA is based on the so-called Nelson criteria, which include wheezing, new pulmonary infiltrates, elevated total IgE serum levels, elevated specific IgE and IgG serum levels against *A. fumigatus*, positive skin prick test reactivity against *A. fumigatus*, and the detection of *A. fumigatus* in sputum [16]. Although these criteria underwent some minor modifications over time, they are still considered to be the gold standard for ABPA diagnosis and are used by many CF clinicians in daily practice. If related to the pathophysiological picture described earlier, the Nelson criteria combine the stages of *A. fumigatus*-specific sensitization, activation of Th2 response and maintenance of the allergic process. It therefore becomes clear that for the diagnosis of ABPA, all steps are required in addition to the clinical picture reflecting downstream pathophysiological consequences of these processes.

Although the Nelson criteria built the basis for current recommendations regarding diagnostic criteria for ABPA, these vary considerably among countries and centers. This is probably one of the explanations for the different estimates regarding prevalence of ABPA [17]. Such differences are illustrated by the use of different definitions in North America and Europe. For the Epidemiologic Study of CF, conducted in the USA and Canada, at least two features out of wheezing, peripheral blood eosinophilia, history of pulmonary infiltrates, elevated specific IgE against *A. fumigatus*, clinical response to steroids or *A. fumigatus* in sputum; and two of the following three criteria were required for diagnosis of ABPA: immediate cutaneous reaction to *A. fumigatus*, serum precipitating antibodies to *A. fumigatus* and total serum IgE of more than 1000 IU/ml [18]. For the European CF Registry, the following four criteria were required for diagnosis of ABPA: physician's suspicion of ABPA based on at least one clinical feature out of wheezing, pulmonary infiltrates, peripheral eosinophilia, response to steroids or *A. fumigatus* in sputum; immediate cutaneous reaction to *A. fumigatus*; serum precipitating antibodies to *A. fumigatus*; and total serum IgE of over 1000 IU/ml [19]. The difficulty in defining clear criteria for diagnosis of ABPA especially in CF is reflected by the latest recommendations from a consensus conference on ABPA in CF published in 2003 [6]. Herein, an attempt was made to define different criteria for different clinical subgroups of ABPA, namely the classical case of new-onset ABPA, minimal criteria for diagnosis as well as screening criteria [6].

Despite these efforts, the examples of significant differences between societies illustrate that the diagnosis of ABPA remains challenging and that there is no clear consensus as to which criteria are the most helpful for diagnosing ABPA. These examples also suggest that positive serological responses may be required more strictly for an accurate diagnosis of ABPA, especially in CF. In fact, many clinical diagnostic criteria for ABPA in CF overlap with common manifestations of the CF lung disease: pulmonary infiltrates, bronchial obstruction and bronchiectasis are common features of CF lung disease and thus are not specific for ABPA [20]. Furthermore, lung colonization with *A. fumigatus* occurs in a large proportion of

CF patients [19,21,22]. Owing to this overlap of clinical symptoms common in CF patients and characteristic for ABPA, a diagnosis based only on clinical features is very difficult and may lead to both underestimation as well as to delayed diagnosis of the disease. For this reason, serological markers indicating active immune processes towards *A. fumigatus* have been studied for approximately three decades to help in diagnosing ABPA [16]. This effort has been recognized and is reflected by the following statement in the recent consensus document: "Serological findings should contribute strongly to the confirmation or exclusion of clinically suspected ABPA" [6]. Many studies on possible serum markers for ABPA have been conducted and a substantial body of literature examining their usefulness for ABPA diagnosis exists. In the following section, these markers will be introduced and their clinical value reviewed.

Serological markers for the diagnosis of ABPA

Serological markers have emerged from animal studies where knocking out certain pathways have provided insight into the pathophysiology of the disease, from the increased knowledge regarding allergic diseases in general and from clinical studies in CF patients. Unfortunately, the latter are mostly small, cross-sectional studies including patients from a single center only, thus limiting the significance and generalization of the results.

IgE antibodies to *A. fumigatus*

Specific IgE antibodies to *A. fumigatus*

Detecting specific IgE antibodies to *A. fumigatus* in serum has been advocated in establishing the diagnosis of ABPA for a long time [23]. The outcome of the test, however, directly depends on the quality of the fungal extract used [24]. Because there is no standardization of such tests and because most of the time uncharacterized extracts from the mycelium of the fungus or from secreted antigenic materials present in culture media have been used with huge variations of allergen content of fungal extracts [25], such tests are not thought to be suitable for inclusion in diagnostic algorithms for ABPA, especially not for discrimination between CF patients sensitized against *A. fumigatus* and those with clinically active ABPA [26]. Nevertheless, besides skin prick test reactivity to *A. fumigatus*, the detection of specific IgE antibodies to *A. fumigatus* is still recommended for screening purposes [6], possibly explaining parts of the discrepancy reported between studies.

Specific IgE against recombinant *A. fumigatus* allergens

Because of these drawbacks regarding specific IgE antibodies to *A. fumigatus*, new biological methods have been applied in order to standardize the detection of *A. fumigatus*-specific antigens. The production of recombinant *A. fumigatus* (r Asp f) antigens has identified fungal products involved in ABPA immune mechanisms, with r Asp f1, r Asp f2, r Asp f3, r Asp f4 and r Asp f6 believed to be especially important [27,28]. It was thought that by differentiating between antibodies against secreted and cytoplasmic antigens, representing immune responses against different parts of *A. fumigatus*, it would be possible to discriminate patients with *A. fumigatus* sensitization from those with clinically active ABPA; that is to say, those with maintenance of an immune reaction against *A. fumigatus* [29].

The clinical usefulness of these allergens was first described more than 10 years ago in ABPA patients with CF [30] and asthma [31], with several groups being able to show an elevation of specific serum IgE antibodies in ABPA patients [32]. These and other studies [33] led to the increased use of serological measurements of recombinant *A. fumigatus* antibodies for diagnosis of ABPA. The inclusion of recombinant *A. fumigatus* allergens into a commercially available measurement system (ImmunoCAP; UniCAP®) provided for the first time a *de facto* standardization of the IgE response to *A. fumigatus*, enabling comparison between centers and studies. This has since then led to even more widespread use of this measurement technique [34].

In a study by Knutsen *et al.* comparing CF–ABPA patients with CF patients showing a positive *A. fumigatus* skin test and CF patients with negative *A. fumigatus* skin test, particularly IgE to rAsp f3 and f4 (measured by ELISA) showed a high sensitivity for ABPA diagnosis (82% for rAsp f3 and f4) but a low specificity of 30% for rAsp f3 and 35% for rAsp f4 based on the gold-standard ABPA diagnosis, as recommended by the CF society [35]. By contrast, Casaulta *et al.* showed that only the combined use of rAsp f4 and/or rAsp f6 (measured by ELISA) with increased total serum IgE (>1000 IU/l) is best to diagnose ABPA [36]. In this study, classic ABPA (full clinical and serological picture of ABPA) was recognized with 100% specificity and 64% sensitivity, with a high positive (100%) and negative (94%) predictive value [36].

The use of commercial CAP systems for detection of antibodies against these recombinant *A. fumigatus* antigens has also been evaluated [34,37]. In a recent study, the results obtained by ELISA were compared with those from ImmunoCAP [38]. The authors compared CF patients with ABPA, CF patients with asthma, CF patients with neither ABPA nor asthma and age-matched controls. Regarding the tests used, they found that the specificity of ImmunoCAP was inferior to that of the one obtained by ELISA [38]. In agreement with Casaulta *et al.* [36], they also found that only a combination of several antigens (r Asp f3, f4 and f6) increases the value for diagnosis of ABPA [38]. They further concluded that specific IgE against recombinant *A. fumigatus* antigens show less usefulness in the diagnosis of ABPA in CF patients than in asthma patients [38]. However, it has to be mentioned that the extract used to produce ImmunoCAPs is not available as skin prick test solution. This further contributes to noncomparable serological outcomes, making comparison between different study centers impossible. The controversy regarding the clinical usefulness of recombinant IgE allergens is thus ongoing, especially when they are applied to other patient populations. For example, a study from Brazil found huge discrepancies of ABPA diagnoses based on the use of recombinant *A. fumigatus*-specific IgE and the recent consensus guidelines [39].

Of note is the discrepancy in ABPA diagnosis based on recombinant allergens between asthmatic and CF patients, with the serological diagnosis being more reliable in asthmatics [31]. Possible reasons for this difference are better characterization of ABPA in asthmatics (without the overlap of diagnostic criteria with the underlying lung disease, as occurring in CF) and the immunological mechanisms unique for ABPA when occurring in CF patients [8]. Whether this ABPA–CF link is due to bacterial

infections [40], a general Th1/Th2 imbalance or other immunological factors remains to be determined [8]. Despite the current belief that a serologic diagnosis of ABPA based on recombinant allergens is more reliable in asthmatic patients, there are also reports of authors concluding that “the determination of serum IgE against recombinant *A. fumigatus* allergens was not helpful to make the diagnosis of ABPA, neither to detect sensitization to fungus” in asthmatic patients [41].

Taken together, for several years now, IgE antibodies against different *A. fumigatus* allergens have been evaluated for diagnosis of ABPA without entirely convincing evidence of their clinical usefulness. Whether the recent identification of novel *A. fumigatus* allergens by new laboratory approaches [42,43], the two cross-reacting *A. fumigatus* thioredoxins Asp f28 and Asp f29 [44] or the cross-reacting *A. fumigatus* cyclophilin Asp f27 [45] will help for clinical diagnosis in the future, remains to be determined.

IgG antibodies to *A. fumigatus*

Even though the existence of IgG antibodies to *A. fumigatus* is said to be one of the main criteria for ABPA diagnosis in CF [6], there is no standardized specific test to measure a possible serum IgG antibody response. A widely used method is the immunoprecipitation technique, where, via double immunodiffusion, nonisotype-specific precipitating IgG antibodies (precipitins) to *A. fumigatus* are detected. The results can then be qualitatively administered as positive and negative, or semiquantitatively as number of precipitation lines [46] or titer [37]. Hutcheson *et al.*, for example, found in a 12-year longitudinal study that positive precipitating IgG antibodies to *A. fumigatus* were highly sensitive (100%) but less specific for diagnosis of ABPA with additional fluctuation over time [47]. Skov *et al.* showed significantly higher IgG of all subclasses except for IgG3 in ABPA patients compared with CF patients colonized with *A. fumigatus* [48]. Specificity for the different IgG subclass antibodies for diagnosis of ABPA were as high as 88% for IgG4, with the sensitivity between 65 and 73% being rather moderate [48]. In another study of this group, positive precipitating antibodies to *A. fumigatus* had a positive predictive value of 80% and a negative predictive value of 50% for the diagnosis of ABPA [37]. Skov *et al.* also demonstrated in 2004 that the IgG immune response may be individually different based on the genetic background of the patient [49].

The immunoprecipitation technique has several drawbacks: quantitative results cannot be obtained, thus sensitivity is limited; due to the lack of standardized tests reproducibility is poor; and the method is time-consuming and difficult to perform [50]. The development of a commercially available CAP IgG assay (ImmunoCAP) has made the measurement of exact antibody concentrations possible. It was first applied to the serum of ABPA patients in 2006, when van Hoeyveld *et al.* compared 11 ABPA patients with 112 CF patients without ABPA. They showed that a cut-off of 35 mg/l yields a sensitivity of 90% and a specificity of 65%, whereas using a higher cut-off of 70 mg/l led to a sensitivity and specificity of 70 and 85%, respectively [46]. In a recent paper from the CF Centre of Leeds, UK [51], comparing seven ABPA patients with 80 CF patients without ABPA, receiver operating

characteristic (ROC) curve analysis suggested that an even higher cut-off value of 90 mg/l results in the best diagnostic accuracy, with a sensitivity of 91% and a specificity of 88% in comparison to the minimal consensus conference diagnostic criteria [51]. These discrepant results illustrate that more validation and comparison studies are needed before the CAP IgG assay can be recommended for general diagnostic use.

Cellular allergen stimulation test

The cellular allergen stimulation test (CAST) is based on the determination of sulfidoleukotrienes. Isolated leukocytes are primed with IL-3 and stimulated with allergens (e.g., *A. fumigatus* extract) *in vitro*. In particular basophilic cells produce allergic mediators as sulfidoleukotrienes LTC₄ and its metabolites LTD₄ and LTE₄.

There is so far just one study in 27 CF patients including eight subjects with positive clinical and serological signs of ABPA. CAST results showed a sensitivity of 100% but only a specificity of 74%: it was positive in all eight ABPA patients, but positive as well in five controls lacking signs of ABPA except positive specific IgE against *A. fumigatus* as indication for sensitization to *A. fumigatus*. Combining the CAST results with rAsp f 4 and/or rAsp f 6 increased the sensitivity to 75% with a specificity of 100% [52].

Serum total IgE

Besides *A. fumigatus*-specific IgE and IgG, the allergic reaction against *A. fumigatus* is characterized by an increase of total IgE. In a comparative study of different serum markers, Latzin *et al.* determined an optimal cut-off level of 514 IU/ml total IgE for diagnosis of ABPA according to the Nelson criteria with a sensitivity and specificity of 65 and 81%, respectively [53]. In an earlier study by Casaulta *et al.*, an even higher specificity of 92% was reported with a comparable sensitivity of 64% [36]. The drawback of using total IgE as serological marker for ABPA is the fact that it is heavily influenced by steroid use and that a number of patients with ABPA without elevated total serum IgE levels have been described [22,54–57]. Furthermore, total IgE is known to vary markedly with age and atopic background [6].

Thymus- & activation-regulated chemokine/CCL17

In animal and human studies, ABPA is associated with a Th2 immune response to *A. fumigatus* antigens [2,58,59]. Chemokines attract leukocytes through their corresponding receptors and are classified as Th1- and Th2-associated chemokines and chemokine receptors [60]. Th2 cells preferentially express the chemokine receptor CCR4 and are attracted by TARC (FIGURE 1) [61,62].

The clinical relevance of TARC as a marker for ABPA in CF patients has been examined in two separate clinical study cohorts in patients with asthma and CF [53,63]. The first study showed that TARC serum levels differentiated CF patients with clinically active ABPA from several diseased and healthy control groups [63]. In a short longitudinal substudy, TARC serum levels peaked with ABPA exacerbations and indicated these exacerbations in the clinical course of disease more clearly than total IgE serum levels did. Notably, TARC serum levels

were lower in ABPA patients treated with corticosteroids. A TARC-lowering role of steroids cannot be proven using this cross-sectional data; whether corticosteroid treatment may be able to control increased TARC levels remains to be tested. The second study, published by Latzin *et al.*, systematically evaluated the diagnostic value of several potential ABPA serum markers and compared TARC with previous putative serum markers for ABPA in CF patients [53]. This latter study reproduced the finding from the first study, showing that TARC serum levels were significantly increased in CF ABPA patients compared with CF patients sensitized to *A. fumigatus* without clinically active ABPA. Most importantly, ROC curves revealed that TARC-levels (measured by ELISA technique) could discriminate ABPA patients from non-ABPA patients with a sensitivity of 100% and a specificity of 97.2%, and are clearly superior to other serum markers for diagnosis of ABPA [53]. In addition, in a nested case-control study, it was shown that TARC levels were not just an epiphenomenon of increased IgE levels, suggesting that TARC levels may indicate ABPA Th2 responses more specifically than just reflecting general Th2 system activation.

In summary, these studies suggest TARC as a putative biomarker for ABPA in CF patients. Despite these promising results, both studies also revealed that TARC serum level analysis is influenced by methodological issues, as suggested by the relatively high interassay variability observed in the second study. Thus, further clinical studies involving a representative number of CF patients are necessary to evaluate the practical value of TARC serum levels in the diagnosis and follow-up of ABPA in CF patients.

Macrophage-derived chemokine/CCL22

There is so far just one clinical study evaluating MDC serum levels in CF patients with ABPA [63]. Hartl *et al.* showed a significant increase in MDC serum levels at peaks of IgE; however, there was no association of serum MDC levels with IgE levels within an ABPA episode [63].

Expert commentary

Despite considerable progress over the last few years, the diagnosis of ABPA remains challenging for all clinicians caring for CF patients owing to the overlapping clinical symptoms between 'normal' CF lung disease and ABPA, complicated by the side effects of treatment. From a diagnostic point of view, ABPA is a clinical syndrome, with multiple criteria, including clinical status, radiological findings and reactions of the immune system. One way to assess these possible immunological reactions is to perform serological measurements. As pointed out in this review, many different serum markers have been evaluated for their potential use in the diagnosis of ABPA, without finding one perfect serological marker in CF patients. There are two major reasons. First, the immunological mechanisms in ABPA on the background of the underlying CF lung disease are very complex, with different stages occurring during the typical course of ABPA, making the situation even more complex. Second, despite the progress in understanding the underlying immune mechanisms and thus continuously detecting new serum markers or developing more

standardized tests, ABPA remains a clinical diagnosis – no clinician would diagnose ABPA in a CF patient based on serological measurements without the appropriate clinical signs. Thus, instead of finding the one and only serum marker for ABPA, the huge potential of serology lies in improving the diagnosis. From our current understanding, it seems rather realistic that some markers are best to indicate sensitization; others are best for early diagnosis; others best indicate the occurrence of exacerbations; and again, other markers indicate response to treatment.

After decades of research based on IgE-related mechanisms without groundbreaking results, recent studies evaluating TARC seem to be very promising from a practical clinical point of view. Nevertheless, before TARC can be used routinely or even included in diagnostic algorithms for ABPA in CF patients, its contribution to clinical decision-making needs to be evaluated in further studies and the results need to be confirmed in different CF cohorts as correctly requested recently [64]. However, the results regarding TARC are also interesting from an innovative point of view: very shortly after the detection of TARC and after only few reports from animal studies, the clinical usefulness of this chemokine was already assessed in human serum samples. Whether or not TARC will in the end make it into diagnostic algorithms, the process itself may help to promote the whole field of serological diagnosis of ABPA. With the ongoing detection of new mechanisms in allergic airway disease in general and ongoing identification of novel *A. fumigatus* allergens owing to new methodological approaches [42], it seems realistic that possible new serological markers are already on the horizon and can be tested in clinical studies soon.

Five-year view

As outlined earlier, in 5 years time, it may be realistic to have different serological markers for various purposes, for example: specific IgE against recombinant *A. fumigatus* antigens in order to determine sensitization; TARC as screening parameter for early

diagnosis or to detect exacerbations; and total IgE to indicate response to treatment. In order to achieve this, however, prospective multicenter trials involving large numbers of patients and clearly defined end points are essential to establish the usefulness of these markers in daily clinical practice.

Measuring existing serum markers in sputum or broncho-alveolar lavage – where the immune processes are thought to be most active – could not only help understand ABPA disease process but could perhaps even add additional information from a clinical point of view. Maybe in 5 years time we will measure r Asp f4 and f6 as well as TARC in the sputum for early detection of sensitization or active ABPA.

In addition, in a recent study from Australia, it was shown that colonization with *A. fumigatus* and prevalence of ABPA in this region are comparable to other parts of the world despite a high prevalence of atopy and very humid climate [22]. Thus, it seems likely that other factors, such as the genetic background, contribute to the immunological switch between tolerance and allergy.

Within the next few years, research will hopefully lead to identification of these factors, will help to clarify the role of well-known markers and assess the exact contribution as well as the possible clinical usefulness of newly-identified mediators as mentioned previously or involved in general in immune processes, such as Th17 cells, regulatory T cells or thymic stromal lymphopoietin [7].

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Key issues

- Diagnosis of allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis (CF) patients remains a challenging task for clinicians.
- Various serological markers have been suggested in recent years to support clinical diagnosis of ABPA.
- Most data are available on the use of serum IgE to recombinant *Aspergillus fumigatus* allergens.
- Inconclusive results have hindered the widespread clinical use of IgE.
- The newly described chemokine TARC may be an alternative marker.
- Replication of the first promising studies needs to be established before TARC can be recommended for routine practice.
- Progress in the understanding of immune processes may in the future lead to new serological markers of ABPA.

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