ISHAM INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2017, 55, 48–55
doi: 10.1093/mmy/myw116
Advance Access Publication Date: 5 November 2016
Review Article

Review Article

Aspergillus serology: Have we arrived yet?

Malcolm D. Richardson[1,2,3,*] and Iain D. Page[2,3]

[1]Mycology Reference Centre Manchester, University Hospital of South Manchester, Manchester, UK, [2]National Aspergillosis Center, University Hospital of South Manchester and [3]Division of Infection, Immunity and Respiratory Medicine, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, The University of Manchester, UK

*To whom correspondence should be addressed. Professor Malcolm Richardson, Mycology Reference Centre Manchester, Education and Research Centre, Wythenshawe Hospital, Manchester, M23 9LT, UK.
E-mail: malcolm.richardson@manchester.ac.uk

Received 7 August 2016; Revised 9 October 2016; Accepted 12 October 2016; Editorial Decision 9 October 2016

Abstract

Aspergillosis presents in various clinical forms, among them chronic pulmonary aspergillosis, which is a spectrum of disease entities including aspergilloma, chronic cavitary pulmonary aspergillosis, and chronic fibrosing pulmonary aspergillosis. *Aspergillus* also contributes to fungal allergy and sensitization. Analysis of the immune response to *Aspergillus* and its antigens is an integral part of the diagnosis of these diseases. Over the past half century, the techniques used to determine antibody titers have evolved from testing for precipitating and agglutinating antibodies by agar gel double diffusion and immunolectrophoresis to enzyme-linked immunosorbent assays using recombinant proteins as capture antigens. A resurgence of interest in the detection of immunoglobulins, primarily *Aspergillus*-specific IgG, has hinted at the possibility of distinguishing between colonization and invasion in immunocompromised patients with invasive aspergillosis. Even though there appears to be a greater degree of discrimination between the clinical forms of aspergillosis there is still a long way to travel. This review presents illustrative examples of where new diagnostic platforms and technologies have been applied to this intriguing spectrum of diseases.

Key words: Aspergillus serology, Aspergillus antigens, Aspergillus antibodies.

Introduction

Some *Aspergillus* species, most commonly *Aspergillus fumigatus*, may lead to a variety of allergic reactions and life-threatening systemic infections. Invasive aspergillosis occurs primarily in patients with severe immunodeficiency, and has dramatically increased in recent years.

Measuring *Aspergillus*-specific antibodies is an important part of the diagnostic pathway for allergic bronchopulmonary aspergillosis (ABPA) and chronic pulmonary aspergillosis (CPA). These infections are likely to represent a major public health issue on a global scale as 20–35% of patients develop *Aspergillus*-specific antibodies following tuberculosis treatment, and 63% of these develop pulmonary aspergillosis within 3 years.[1] The global 5-year period prevalence of (CPA) secondary to tuberculosis has been estimated to be between 0.8 and 1.37 million cases, with for example, 43 cases per 100,000 population estimated to occur in Congo, which has a high prevalence of tuberculosis.

Detection of specific antibodies provides key diagnostic evidence in chronic forms of aspergillosis. The evidence

to support the use of *Aspergillus* serology in diagnosing invasive aspergillosis is limited. There are numerous techniques for quantifying antibodies but most of these are not suitable for use in developing countries with limited laboratory resources and trained personnel, reviewed by Page and colleagues.[2]

The practice of *Aspergillus* serology: old and new

All serological tests have their limitations and the reliability of individual serodiagnostic tests as indicators of disease varies appreciably. Causes for this variation include the large numbers of antigens produced by the fungal cell, the widespread occurrence of cross-reactions between related and at times, unrelated pathogenic species and the paucity of accepted reference reagents and standard procedures. Moreover, antibody formation may be delayed, reduced or absent in patients with defective immune systems. In fulminant or early stages of infection, soluble antigens rather than antibodies maybe present in serum and other body fluids, and tests for circulating antigen can therefore provide valuable supportive evidence for infections with opportunistic mycoses. This present review focuses on serology, that is, the expression of the immune system to *Aspergillus*.

Precipitin tests were first described in the 1950s and 1960s following the introduction of the Ouchterlony double diffusion method.[3,4] It was demonstrated that the numerous variations of this test could detect elevated levels (titres) of *Aspergillus* antibody in various manifestations of aspergillosis. Crude antigen extracts, typically prepared from conidia and hyphae were used.

Indirect hemagglutination as a method for detecting *Aspergillus*-specific agglutinins was first described in 1974.[5] Hemagglutination tests involve coating erythrocytes with antigens. Erythrocytes clump together when antibodies cross-react with antigens on more than one cell and become visible to the human eye. The method also detects all *Aspergillus* antibody types. Its simplicity and speed (~2 hours) and commercial production make it highly suited for epidemiological and prevalence studies in low and middle resource countries. A study described by Tönder and Rodsaether showed that when 441 sera were analyzed there was good correlation between high titres and presence of aspergillosis.[5] Titers above 128 were highly indicative of active disease. However, based on our personal experience the results from this assay can be highly subjective.

Enzyme-linked immunosorbent assay (ELISA) platforms for the detection of *Aspergillus* immunoglobulin classes were first developed in the 1980s. Many commercial products are currently available and have provided a wealth of information on host antibody responses to *Aspergillus*.

Aspergillus antigens old and new

Aspergillus has a complex cytoplasmic organization, an impressive array of morphological expression, and a remarkable diverse and adaptable metabolism. The fungus is able invade tissue following inhalation. *Aspergillus* is angiotropic. Hyphal fragments adhere to endothelial cells and secrete a variety of enzymes that assist in further tissue invasion and acquisition of nutrients. During germination, colonization, and invasion, *Aspergillus* must respond and adapt to diverse types of stressors, nitrogen and oxygen deprivation, and effector cells.[6] Many cellular and extracellular products are immunogenic, and antibodies are readily formed by immunocompetent patients infected with *Aspergillus*.

The quality of crude extracts for diagnosis and therapy is very unsatisfactory in case of fungal extracts. Currently, the quality of mould extracts varies dramatically between commercial suppliers in Europe and the USA since no standardized extracts are available, reviewed in.[7] The reasons for the insufficient quality are manifold. On the one hand, crude extracts from different fungi have been shown to vary considerably in their protein composition. These problems are caused by variations between strains and batch-to-batch variations. Additionally, mould extracts may be produced from mycelial cells and/or spores, which may vary in their protein pattern. On the other hand, growth conditions, protein extraction methods and storage conditions are critical with respect to the quantity and even existence of individual antigens. Finally, degradation of the extracted proteins may occur.

To some extent, the problems with fungal extracts may be overcome by the use of recombinant antigens. The major advantages of recombinant proteins over crude fungal extracts are threefold. First, the protein preparations are reproducible. Second, the production of large quantities of pure proteins is possible. Third, using recombinant allergens, it is possible to differentiate among co-exposure, co-sensitization, and cross-reactivity.

Over the years, diagnostic studies have compared recombinant fungal proteins and crude mould extracts with respect to their negative and positive predictability of mould sensitization and disease. For *A. fumigatus*, a large number of antigens have been published. Since *A. fumigatus* is particularly known for its broad spectrum of human disorders, many researchers have aimed to find a link between a given disease and the patients' reactivity pattern to individual recombinant allergens. Individual recombinant allergens can be used to discriminate between ABPA (Asp f 2, Asp f 4, and Asp f 6) and fungal allergy (Asp f 1 and Asp f 3).[8,9] However, attempts to reproduce this have not been particularly successful.[2]

Mitogillin as a candidate antigen for antibody detection

During infection, *A. fumigatus* secretes an 18-kDa protein that can be detected as an immunodominant antigen in the urine of infected patients. In 2001 Weig and colleagues showed that this protein was mitogillin, a ribotoxin that cleaves a single phosphodiester bond of the 29S rRNA of eukaryotic ribosomes.[10] The immunogenic capacity of mitogillin in a rabbit animal model was demonstrated, indicating its usefulness as an antigen for serological diagnosis of invasive aspergillosis. An ELISA for detection of immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA) antibodies to recombinant mitogillin was developed. In serum samples of patients with an aspergilloma (n = 32), invasive pulmonary aspergillosis (IPA) (n = 42), or invasive disseminated aspergillosis (n = 40), a good correlation between production of IgG antibody against mitogillin and clinical disease was observed (for patients with aspergilloma, 100% were positive; for patients with IPA, 64% were positive; for patients with invasive disseminated aspergillosis, 60% were positive). In contrast, positive titers for serum IgG and IgM antibodies against mitogillin were found in only 1.3% of the serum samples of healthy volunteers and positive titres for IgA antibody were found in only 1.0% of the serum samples of healthy volunteers (n = 307; specificity = 95.4%). These results suggested that recombinant mitogillin could be used for the serodiagnosis of *A. fumigatus*-associated diseases.

Recombinant proteins

A comprehensive evaluation of putative diagnostic antigens of *A. fumigatus* was performed by Sarfati and colleagues.[11] Eight recombinant proteins and purified galactomannan were tested by ELISA to quantify the anti-*Aspergillus* antibodies in sera of patients with aspergilloma, ABPA, and invasive aspergillosis. In spite of the variability observed in the immune responses of individual patients, quantification of the antibody titers against the 18-kDa ribonuclease (RNU), the 360-kDa catalase (CAT), and the 88-kDa dipeptidylpeptidase V (DPPV) was useful for the diagnosis of aspergilloma and ABPA. Differential diagnosis of ABPA was even possible among cystic fibrosis as well as non–cystic fibrosis patients. In the group of immunocompromised patients with IA, no antibody response was mounted in response to the *Aspergillus* infection in any of the patients. Interestingly, about half of the patients with proven IA, when tested on admission to hospital, had high titers of *Aspergillus*-specific antibodies, suggesting that they were previously colonized or infected. The authors suggest that recombinant RNU, CAT, and DPPV have potential in the serodiagnosis of all forms of aspergillosis in the immunocompromised and immunocompetent patient.

Chitosanase CsnB

Another *Aspergillus* secreted protein that has attracted attention as a potential antigen for antibody-based diagnostics is chitosanase CsnB. To analyze the biological function of CsnB Beck and colleagues deleted the *csnB* gene and generated CsnB-specific antibodies.[12] It was found that *A. fumigatus* was able to grow on chitosan in a CsnB-dependent manner. During growth on chitosan, elevated levels of CsnB were found in the supernatants indicating that chitosan triggers enhanced CsnB production. Using recombinant proteins, antibody responses in patients at risk to develop invasive aspergillosis were analyzed. The study focused on two antigens: CsnB and for comparison, mitogillin. IgG responses were found to both proteins, but elevated antibody levels to CsnB and/or mitogillin showed no correlation to the results of the galactomannan antigen assay or clinical signs that are characteristic for *Aspergillus* infections.

Antibody detection in different diseases

The clinical manifestations of aspergillosis vary and can be divided into three main categories, according to the location and extent of colonization and invasion (both of which are influenced by the fungal virulence and immune response of the host); these are (1) allergic reactions, (2) chronic pulmonary aspergillosis, and (3) invasive aspergillosis.[13] Serological tests can be of great value as diagnostic aids in many of the clinical manifestations of aspergillosis.[14] All serological tests have their limitations. The most recent developments in designing assays for *Aspergillus* antibodies, or new studies using established techniques, in chronic and invasive disease are presented below.

Chronic pulmonary aspergillosis and allergic bronchopulmonary aspergillosis

Chronic pulmonary aspergillosis (CPA) is estimated to affect 3 million persons worldwide.[13] *Aspergillus*-specific IgG is a key component in CPA diagnosis. CPA usually occurs in patients with underlying pulmonary disease, and the lesion usually progresses latently. Therefore, it is not uncommon for patients to present with hemoptysis and/or respiratory failure. CPA is considered to be one of the most refractory pulmonary infectious diseases; the estimated 5-year survival for CPA ranges from 50–85%, which is similar to that of idiopathic pulmonary fibrosis. Long-term clinical stability can, however be achieved with azole therapy[15] and surgical cure is possible in selected cases. [16]

As *Aspergillus* is the causative agent, isolation of the responsible *Aspergillus* species from the airway tract has a role in diagnosis and is essential to identify drug resistance, but unfortunately the rate of isolation on sputum culture

Table 1. Evaluation of ELISA platforms compared with agar gel double diffusion (precipitin test) in the serological diagnosis of chronic pulmonary aspergillosis (based on reference[18]).

Assay	ROC/AUC*	Optimal diagnostic cut-offs	Sensitivity (%)	Specificity (%)
ThermoScientific ImmunoCAP	0.996	20	96	98
Siemens Immunolite	0.991	10	96	98
Virion\Serion	0.973	35	90	98
Dynamiker	0.918	65 AU/ml	77	97
Genesis	0.902	20 AU/ml	75	99
'Classic' precipitin test	NA	NA	59	100

Receiver operator curve—the accuracy of the test depends on how well the test separates the patient group being tested into those with and without disease. The accuracy of each assay is shown by the area under the ROC curve (the AUC). An area of 1 represents a perfect test; an area of 0.5 represents a worthless test.

examination is relatively low with current standard culture techniques. At present, serum detection of IgG antibodies to *Aspergillus* is considered to be the most reliable method for diagnosing CPA.[17,18]

Allergic bronchopulmonary aspergillosis (ABPA) is a rare disease characterized by an allergic inflammatory response to the colonization by aspergillus or other fungi in the airways. It is estimated that ABPA complicates around 7–14% of cases of asthma.[12] ABPA should be ruled out in every case of uncontrolled asthma. Diagnostic criteria for ABPA include the presence of bronchial asthma, immediate skin test reactivity to *A. fumigatus*, elevated serum IgE levels, total and *A. fumigatus*-specific, pulmonary infiltrates (transient or fixed), central bronchiectasis, peripheral blood eosinophilia, and elevated levels of total and *Aspergillus*-specific IgE.[15]

Diffusion of antibodies and antigens in an agarose gel is widely used to detect *Aspergillus* precipitins; however, this technique can take up to one week to obtain results and lacks sensitivity. A study designed to establish the optimal diagnostic cut offs for CPA by comparing the performance of six serological assays, including precipitin testing, has been reported by Page and colleagues.[18] Sera from 241 patients with CPA and 100 healthy blood donors were tested using five *Aspergillus*-specific IgG assays plus precipitin testing using cytoplasmic and culture filtrate *Aspergillus* antigens. Receiver operating characteristic (ROC) curve area under the curve (AUC) results comparing British CPA cases to healthy young adult Ugandan controls are shown in Table 1. The best performance was seen with the ImmunoCAP and Immulite platforms.

In a retrospective study Fujiuchi and colleagues evaluated the clinical utility of the ImmunoCAP assay for diagnosing CPA. *Aspergillus*-specific IgG levels in patients who met the following criteria were included: (1) chronic (duration of > 3 months) pulmonary or systemic symptoms, (2) radiological evidence of a progressive (over months or years) pulmonary lesion with surrounding inflammation, and (3) no major discernible immunocompromising factors.[19] Anti-*Aspergillus* IgG serum levels were retrospectively analyzed according to defined classifications. Mean *Aspergillus* IgG levels were significantly higher in the proven group than those in the possible and control groups ($P < .01$). Receiver operating characteristic curve analysis revealed that the *Aspergillus* IgG cutoff value for diagnosing proven cases was 50 mg of antigen-specific antibodies/liter. The sensitivity and specificity for diagnosing proven cases using this cutoff were 0.77 and 0.78, respectively. The positive rates of *Aspergillus* IgG in the proven and possible groups were 97.9% and 39.2%, respectively, whereas that of the control group was 6.6%. These two studies confirms that the ImmunoCAP and Immulite assays for *Aspergillus* IgG offer reliable sensitivity and specificity for diagnosing chronic pulmonary aspergillosis and are superior to the conventional precipitin test in this context.

A number of studies have compared a number of difference serological techniques by analyzing sera from both chronic pulmonary aspergillosis and allergic disease caused by *Aspergillosis*. Baxter and colleagues assessed the performance of two commercial EIAs compared with counterimmunoelectrophoresis (CIE).[20] In a prospective cohort study of 175 adult patients with chronic or allergic pulmonary aspergillosis, *Aspergillus* IgG antibodies were detected using CIE, Phadia ImmunoCap *Aspergillus* IgG and Bio-Rad Platelia *Aspergillus* IgG. Inter-assay reproducibility was determined for each method and 25 patients had two serum samples analyzed within a 6-month interval. When compared with CIE, both ImmunoCap and Platelia *Aspergillus* IgG had good sensitivity for detection of *Aspergillus* IgG antibodies. The level of agreement between the two EIAs for positive results was good, but the concentration of antibodies was not correlated between the tests or with CIE titer. The direction of CIE titer change over six months was mirrored by ImmunoCap IgG levels in 92% of patients, and by Platelia IgG in 72% of patients. The authors concluded that both ImmunoCap and Platelia Aspergillus IgG EIAs are sensitive measures of *Aspergillus* IgG antibodies compared with CIE. However, ImmunoCap appears to have better

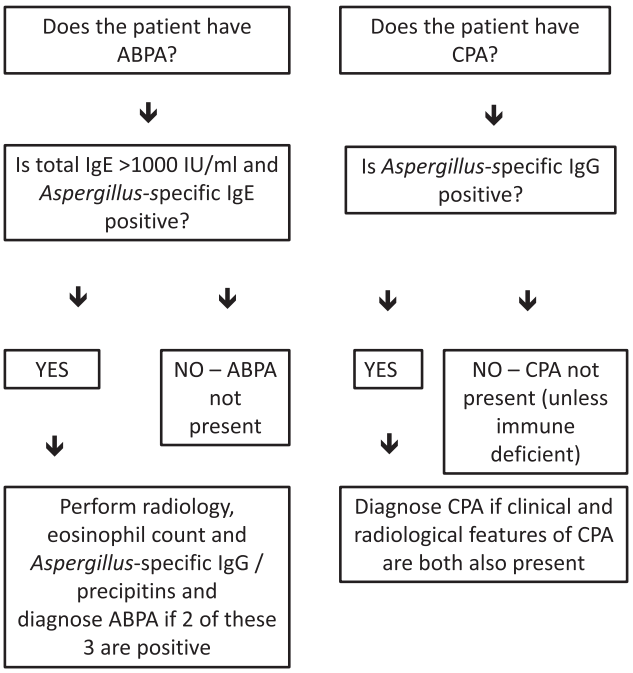

Figure 1. The combination of serological and clinical parameters in the diagnosis of allergic bronchopulmonary aspergillosis and chronic pulmonary aspergillosis

reproducibility and may be more suitable for monitoring patient disease. This concurs with previous studies.

The performance of a new commercial enzyme immunoassay for *Aspergillus* IgG (Bordier Affinity Products) compared with that of the Platelia *Aspergillus* IgG (Bio-Rad Laboratories, France) and Virion\Serion *Aspergillus fumigatus* IgG assays has recently been reported by Dumollard and colleagues.[21] This assay utilizes two recombinant antigens that share features with traditional somatic and metabolic antigens of *Aspergillus fumigatus*. The study showed that the use of recombinant, somatic, and metabolic antigens in a single EIA improved the balance of sensitivity and specificity, resulting in an assay highly suitable for use in the diagnosis of chronic and allergic aspergillosis.

Notwithstanding the promising development of new serological tests the diagnosis of allergic and chronic forms of pulmonary aspergillosis currently relies on well-established assays for *Aspergillus* IgG and IgE, in conjunction with clinical parameters (Fig. 1).

Serological diagnosis of allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis

Allergic bronchopulmonary aspergillosis (ABPA) is seen in approximately 10% of patients with cystic fibrosis (CF) and can be difficult to diagnose.[22] Consensus criteria require the presence of multiple elevated immunologic markers such as total immunoglobulin E (IgE), *Aspergillus* IgE and *Aspergillus* IgG, or precipitins for a definitive diagnosis.[22] There is some degree of standardization of total IgE and *Aspergillus* IgE levels, but there is no standardization in the measurement of IgG antibodies or precipitins to *Aspergillus*. The interpretation of results may, therefore, be confusing. Barton and colleagues conducted a two-stage analysis to explore these issues further.[22] Eighty-seven patients with CF were categorized as having ABPA or as controls and an in-house enzyme immunoassay was used to measure IgG levels to *Aspergillus*. All sera from these patients were then analyzed using the Phadia/ThermoScientific ImmunoCap assay for the quantitative detection of anti-*Aspergillus* IgG. ImmunoCap results were analyzed against the consensus conference minimum diagnostic criteria to ascertain a cutoff point, which could predict a diagnosis of ABPA in CF. Eighty patients with CF and with no or incomplete evidence of ABPA had a mean ImmunoCap score of 51.1 mg/l, whereas seven CF patients with ABPA had a mean ImmunoCap score of 132.5 mg/l. This analysis provisionally suggested that a score of 90 mg/L may be used as a cutoff point, which would give a sensitivity of 91% and specificity of 88.0% for the diagnosis of ABPA, though this requires further validation.

Serological differentiation between ABPA and allergic bronchopulmonary mycoses

Allergic bronchopulmonary mycosis (ABPM) is a hypersensitivity-mediated disease of the lower airways caused by environmental fungi, the most common being *Aspergillus fumigatus*, reviewed.[23] Other etiologic agents include *Candida albicans, Schizophyllum commune*, species of *Alternaria, Bipolaris, Cladosporium, Curvularia, Fusarium, Penicillium, Pseudallescheria, Rhizopus, Saccharomyces, Stemphylium,* and *Trichosporon*. While ABPA has been extensively studied worldwide, little is known about ABPM and how this differs from ABPA.

A recent publication by Fukutomi and colleagues addresses the specific issue that the presence of IgE to *A. fumigatus* does not always indicate genuine sensitization to *A. fumigatus* because of cross-reactivity between crude extracts from different fungal sources.[24] The authors found that the specificity of testing could be greatly improved by measuring IgE to Asp f 1 and f 2, specific allergen components to detect genuine *A. fumigatus* allergy. It is also recognized that the problem of cross-reactivity between crude fungal extracts is also true for the identification of genuine causal fungi in individual ABPM patients. Some patients with ABPM induced by fungi other than *Aspergillus* may be consistent with ABPA diagnostic criteria because current criteria depend on IgE/IgG reactivity to crude extracts. Accurate identification of genuine causal fungi for ABPM is of clinical importance, considering that clinical presentation, anti-fungal treatment strategies and disease prognosis can be influenced by different causal fungi. The diagnosis of causal fungi can be robustly validated by the confirma-

tion of genuine sensitization to fungi after measuring IgE to specific allergen components, as well as repeated microbiological isolation of the fungi from their airway.

Evaluation of new assays for the serological diagnosis of chronic pulmonary and allergic bronchopulmonary aspergillosis

There are numerous EIA formats for quantifying antibodies, but these are not suitable for use in developing countries with limited laboratory resources.

A recent study compared the efficacy of a commercially available indirect hemagglutination assay designed to detect *Aspergillus* agglutinating antibodies (ELI.H.A. *Aspergillus* indirect hemagglutination, ELITech MICROBIO, Signes, France), with an agar double diffusion system used for the detection of *Aspergillus* precipitins in patients with CPA (Richardson MD, Page ID, Rautemaa-Richardson RMK, et al. Detection of *Aspergillus* antibodies by a new indirect hemagglutination assay. 6th Advances Against Aspergillosis 2014, Madrid, 27/02/14 – 01/03/14, abstract number 97). The antigens used in these assays were a combination of cytoplasmic (somatic) and culture filtration extracts. In the indirect hemagglutination assay sera with a titre < 1:320 were considered to be a nonsignificant reaction. Sera with a titer equal to 1:320 were considered to be an indeterminant reaction. Titres ≥1:640 indicated a significant reaction in favour of aspergillosis. A positive precipitin reaction in the immunodiffusion test signified the presence of *Aspergillus* precipitating antibodies. The performance of positive control sera in both assays was excellent and reproducible. All sera positive in one test were positive in the other. The concordance between titers determined by either the indirect hemagglutination assay or the precipitin test were varied. The total performance time of the indirect haemagglutination test is 3 hours. The EliTech indirect hemagglutination assay for the detection of *Aspergillus* antibody in patients with chronic manifestations of pulmonary demonstrated has many advantages compared with precipitin tests: it was rapid and very user friendly. However, it is not easy to read. The ideal point-of-care test for field studies and for community clinics would be a lateral flow device which could be used for screening patients and support the efforts to understand the global epidemiology of chronic pulmonary aspergillosis. We await developments in this area.

Oliva and colleagues evaluated the performance of the *Aspergillus* Western blot IgG kit (LDBio Diagnostics, Lyon, France), a commercially available immunoblot assay for the diagnosis of various clinical presentations of chronic aspergillosis.[24] Three hundred and eight serum samples from 158 patients with aspergillosis were analyzed. More specifically, 267 serum samples were derived from patients with *Aspergillus* disease, including 89 cases of chronic pulmonary aspergillosis, 10 of aspergilloma, and 32 of allergic bronchopulmonary aspergillosis. For blood donor controls, the Western blot kit specificity was 94%, while the kit displayed a sensitivity for *Aspergillus* disease of 88.6%. The study demonstrated that the *Aspergillus* Western blot kit performed well for the diagnosis of various clinical presentations of aspergillosis in nonimmunocompromised patients, with an enhanced standardization and a higher sensitivity compared with precipitin testing. Whether the Western blot IgG kit is suitable for resource limited environments remains to be seen.

Invasive aspergillosis

The clinical significance of *Aspergillus* antibody assays for the diagnosis of invasive of aspergillosis (IA) is still unclear even though numerous studies have addressed this issue over the past decades.[25] Some of the more recent studies are reviewed here.

A study reported from two Lisbon hospitals enrolled a total of 89 patients at risk for, or with invasive aspergillosis (IA) and followed for two and half years to monitor their immune response.[26] Of these patients, six developed probable IA; five died. The presence of serum IgG or IgA antibodies against seven *Aspergillus* recombinant antigens was assessed in patients with IA by an ELISA. In parallel, the serum levels of galactomannan (GM) were also monitored, Superoxide dismutase and a 94 kDa antigen were the most immunogenic for IgA, while the IgG pattern of recognition changed from patient to patient. It was concluded that although follow-up of antibodies against these antigens should not be used as a diagnostic method, patients with IA do produce an immune response that may influence disease outcome.

Du and colleagues explored further the utility of *Aspergillus*-specific antibody detection in IA by measuring baseline serum immunoglobulin responses against six purified recombinant *A. fumigatus* proteins (Enolase, Ahp1, Hsp90, Crf1, Cdc37, Pep2) before hematopoietic stem cell transplantation (HSCT) or chemotherapy in 73 subjects, including 19 patients who subsequently developed proven or probable IA and 54 uninfected controls.[27] Immunoglobulin levels were assessed at the time of diagnosis of IA and four weeks later (acute and convalescent sera, respectively). Baseline IgG responses against the majority of these antigens were significantly higher in the patients with IA compared with controls. Positive IgG responses against Hsp90, Pep2, Crf1, and Cdc37 were specifically associated with early-onset IA (<40 days) rather than late-onset IA. Increased IgG concentrations against Hsp90, Pep2, and Crf1 in convalescent sera versus baseline sera were more likely in the patients with IA who survived. IgG responses in acute

sera were not correlated with outcomes, and IgM and IgA responses did not differ in baseline, acute, or convalescent sera between the patients and controls. The study suggests that baseline IgG responses against *Aspergillus* proteins may be useful screening tests for patients at low risk for IA. Immunoglobilin responses indicate that some patients with IA have significant colonization or ongoing *Aspergillus* infections before immunosuppression.

Erdmann and colleagues used the Platelia *Aspergillus* IgG ELISA for detection of anti-*Aspergillus* immunoglobulin G in serum in allo-HSCT recipients.[29] In previous evaluations this kit had demonstrated high sensitivity and specificity. In a cohort of 104 allogeneic-HSCT recipients, *Aspergillus* IgG was measured before and weekly during the patients' hospital stay. Overall prevalence of possible, probable, and proven IA during hospital stay was 10%, 6%, and 0%. There was no correlation between *Aspergillus* IgG levels before allogeneic-HSCT, or after allogeneic-HSCT, and the prevalence of IA during hospital stay. Furthermore, the median *Aspergillus* IgG levels did not differ between patients with history of probable or proven IA, as compared to patients without history of IA. The authors concluded that measuring *Aspergillus* IgG levels is of no value in diagnosing or predicting IA in patients undergoing allogeneic-HSCT.

Conclusion

It is evident that there is a substantial body of work that describes the application of serology in the diagnosis of *Aspergillus* infection. Monitoring immune responses to chronic forms of pulmonary diseases caused by *Aspergillus* is central to the diagnosis of these conditions. Many methods exist to detect *Aspergillus*-specific antibodies. The background and evolution of these techniques has been reviewed elsewhere.[2] Many of the evaluations described in the present update have been conducted in well resourced environments and healthcare settings. The majority of cases of chronic diseases caused by *Aspergillus* occur in resource-poor or limited settings. Further research, building on a wealth of existing knowledge will hopefully provide greater insights into how well characterised antigens and the immune responses can be harnessed to produce develop point of care tests that do not require a sophisticated laboratory. Interestingly enough, although many of these technologies have been conceived and developed over many years very few of these approaches have been applied to fungal diseases.

Acknowledgments

Some of the summary data presented here is based on studies carried out in the authors' institution, made possible by the generous gift of antibody detection kits and other consumables by a number of diagnostic companies.

Declaration of interest

The authors received free serological kits from the suppliers specified in the text. No other conflicts of interest are declared. The authors alone are responsible for the content and the writing of the paper.

References

1. British Tuberculosis Association. Aspergilloma and residual tuberculous cavities: the results of a resurvey. *Tubercle*. 1970; **51**: 227–245.
2. Page ID, Richardson M, Denning DW. Antibody testing in aspergillosis—quo vadis? *Med. Mycol.* 2015; **53**: 417–439.
3. Stallybrass FC. The precipitin test in human invasive aspergillosis. *Mycopathot et Mycol Appl*. 1963; **21**: 272–278.
4. Longbotom JL, Pepys J, Clive FT. Diagnostic precipitin test in *Aspergillus* pulmonary mycetoma. *Lancet*. 1964; **1**: 588–589.
5. Tönder O, Rodsaether M. Indirect haemagglutination for demonstration of antibodies for *Aspergillus fumigatus*. *Acta Path Microbiol Scand Sect B*. 1974; **82**: 871–878.
6. Paulussen C, Hallsworth JE, Álvarez-Pérez S et al. Ecology of aspergillosis: insights into the pathogenic potency of *Aspergillus fumigatus* and some other *Aspergillus* species. *Microb. Biotechnol.* 2016; doi: 10.1111/1751-7915.12367.
7. Simon-Nobbe B, Denk U, Pöll V et al. The spectrum of fungal allergy. *Int. Arch. Allergy Immunol.* 2008; **145**: 58–86.
8. Hemmann S, Menz G, Ismail C et al. Skin test reactivity to 2 recombinant *Aspergillus fumigatus* allergens in *A. fumigatus*-sensitized asthmatic subjects allows diagnostic separation of allergic bronchopulmonary aspergillosis from fungal sensitization. *J. Allergy. Clin. Immunol.* 1999; **104**(3 Pt 1): 601–607.
9. Kurup VP, Banerjee B, Hemmann S et al. Selected recombinant *Aspergillus fumigatus* allergens bind specifically to IgE in ABPA. *Clin. Exp. Allergy*. 2000; **30**: 988–993.
10. Weig M, Frosch M, Tintelnot K et al. Use of recombinant mitogillin for improved serodiagnosis of *Aspergillus fumigatus*-associated diseases. *J. Clin. Microbiol.* 2001; **39**: 1721–1730.
11. Sarfati J, Monod M, Recco P et al. Recombinant antigens as diagnostic markers for aspergillosis. *Diagn. Microbiol. Infect. Dis*. 2006; **55**: 279–291.
12. Beck J, Broniszewska M, Schwienbacher M. Characterization of the *Aspergillus fumigatus* chitosanase CsnB and evaluation of its potential use in serological diagnostics. *Int. J. Med. Microbiol.* 2014; **304**: 696–702.
13. Kosmidis C, Denning DW. The clinical spectrum of pulmonary aspergillosis. *Thorax*. 2015; **70**: 270–277.
14. Persat F. Sérologie aspergillaire, d'hier à aujourd'hui pour demain (*Aspergillus* serology, from yesterday to today for tomorrow). *J Mycol Médicale*. 2012; **22**: 72–82.
15. Agarwal R, Chakrabarti A, Shah A et al. Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. ABPA complicating asthma ISHAM working group. *Clin. Exp. Allergy*. 2013; **43**: 850–873.

16. Chen QK, Jiang GN, Ding JA. Surgical treatment for pulmonary aspergilloma: a 35-year experience in the Chinese population. *Interact Cardiovasc Thorac Surg*. 2012; **15**: 77–80.
17. Denning DW, Cadranel J, Beigelman-Aubry C et al. Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management. European Society for Clinical Microbiology and Infectious Diseases and European Respiratory Society. *Eur Respir J*. 2016; **47**: 45–68.
18. 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.
19. Fujiuchi S, Fujita Y, Suzuki H et al. Evaluation of a quantitative serological assay for diagnosing chronic pulmonary aspergillosis. *J. Clin. Microbiol*. 2016; **54**: 1496–1499.
20. Baxter CG, Denning DW, Jones AM et al. Performance of two *Aspergillus* IgG EIA assays compared with the precipitin test in chronic and allergic aspergillosis. *Clin. Microbiol. Infect*. 2013; **19**: E197–204.
21. Dumollard C, Bailly S, Perriot S et al. Prospective evaluation of a new *Aspergillus* IgG enzyme immunoassay kit for diagnosis of chronic and allergic pulmonary aspergillosis. *J. Clin. Microbiol*. 2016; **54**: 1236–1242.
22. Barton RC, Hobson RP, Denton M et al. Serologic diagnosis of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis through the detection of immunoglobulin G to *Aspergillus fumigatus*. *Diagn. Microbiol. Infect. Dis*. 2008; **62**: 287–291.
23. Chowdhary A, Agarwal K, Kathuria S et al. Allergic bronchopulmonary mycosis due to fungi other than *Aspergillus*: a global overview. *Crit. Rev. Microbiol*. 2014; **40**: 30–48.
24. Fukutomi Y, Tanimoto H, Yasueda H et al. Serological diagnosis of allergic bronchopulmonary mycosis: Progress and challenges. *Allergol Int*. 2016; **65**: 30–36.
25. 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.
26. Kappe R, Rimek D. Antibody detection in patients with invasive aspergillosis. *Mycoses*. 2004; **47** (Suppl 1): 55–59.
27. Centeno-Lima S, de Lacerda JM, do Carmo JA et al. Follow-up of anti-*Aspergillus* IgG and IgA antibodies in bone marrow transplanted patients with invasive aspergillosis. *J. Clin. Lab. Anal*. 2002; **16**: 156–162.
28. Du C, Wingard JR, Cheng S et al. Serum IgG responses against *Aspergillus* proteins before hematopoietic stem cell transplantation or chemotherapy identify patients who develop invasive aspergillosis. *Biol. Blood. Marrow. Transplant*. 2012; **18**: 1927–1934.
29. Erdmann JH, Graf B, Blau IW, Anti-*Aspergillus* immunoglobulin-G testing in serum of hematopoietic stem cell transplant recipients. *Transpl. Infect. Dis*. 2016; **18**: 354–360.