

Utility of recombinant *Aspergillus fumigatus* antigens in the diagnosis of allergic bronchopulmonary aspergillosis: A systematic review and diagnostic test accuracy meta-analysis

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Summary

Background: The role of recombinant *A. fumigatus* (rAsp) antigens in the diagnosis of allergic bronchopulmonary aspergillosis (ABPA) has not been systematically evaluated. Herein, we evaluate the utility of recombinant *A. fumigatus* (rAsp) antigens in diagnosing ABPA.

Methods: We systematically reviewed the PubMed, EmBase and Scopus databases for studies evaluating rAsp antigens in ABPA. The QUADAS-2 tool and the GRADE approach were used to assess the risk of bias and the quality of evidence, respectively. The diagnostic performance of IgE or skin test against rAsp f1, f2, f3, f4, f6 and their combination was evaluated separately for ABPA complicating asthma or cystic fibrosis (CF), using an HSROC model. The reference standard for diagnosing ABPA was the composite (clinical, radiological, immunological) criteria.

Results: Our search yielded 26 studies ($n = 1694$) and 17 studies ($n = 1131$) for inclusion in the systematic review and meta-analysis, respectively. In asthmatics, the pooled sensitivity for diagnosing ABPA was best for IgE against a combination of rAsp f1 or f3 (96.7%; 95% confidence interval [CI], 87.6-99.2). The pooled specificity for diagnosing ABPA was highest (99.2%; 95% CI, 88.2-99.9) for IgE against a combination of f4 or f6. In CF patients, the pooled sensitivity of rAsp f1 or f3 was 93.3% (95% CI, 55.2-99.9) while the pooled specificity of rAsp f4 or f6 was 93.9% (95% CI, 68.8-99.9). The quality of evidence was low as per the GRADE approach.

Conclusions: A combination of IgE against rAsp antigens (f1, f2, f3, f4 and f6) is likely to be helpful in the diagnosis of ABPA.

KEYWORDS

abpm, allergic bronchopulmonary mycosis, allergy, *Candida*, component-resolved diagnostics, fungi

1 | INTRODUCTION

Aspergillus species can cause a myriad of illnesses depending on the interplay between the virulence of the fungi and the immune response of the host.¹ The pulmonary disorders caused by *Aspergillus fumigatus* are broadly classified as invasive, allergic and saprophytic.

Allergic bronchopulmonary aspergillosis (ABPA) is a respiratory illness caused by hypersensitivity reactions mounted against *A. fumigatus* colonizing the airways of patients with asthma and cystic fibrosis (CF).² The prevalence of *A. fumigatus* sensitization and ABPA in asthma ranges from 15%-48% and 2%-32%, respectively; in CF, these figures are 20%-65% and 3%-25%, respectively.^{3,4}

Currently, the diagnosis of ABPA is based on a composite criterion, which primarily rely on identifying immunological responses against *A. fumigatus*. Most of these involve demonstration of humoral responses (IgE, IgG, skin prick test) against crude extracts of *A. fumigatus*. With the advent of recombinant DNA technology, purified antigens have been isolated from *A. fumigatus*.^{5,6} Currently, 23 antigens of *A. fumigatus* have been identified.⁷ The commercially available recombinant *A. fumigatus* antigens (rAsp) may be useful tools in the diagnosis of ABPA. However, their utility has not been systematically evaluated. Herein, we conduct a systematic review of the literature to ascertain the diagnostic performance of rAsp antigens in the diagnosis of ABPA.

2 | METHODS

This review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis of diagnostic test accuracy (PRISMA-DTA) statement.⁸ An ethics committee approval was not required as it was a review of published data.

2.1 | Search strategy

We searched the PubMed, EmBase and Scopus databases (from inception till 5th December 2017) without any language restrictions, for articles describing the utility of rAsp antigens in ABPA. The following free text terms were used: ("aspergillus antigens" OR "recombinant antigen") AND ("ABPA" OR "ABPM" OR "asthma" OR "cystic fibrosis" OR "allergic bronchopulmonary aspergillosis" OR "allergic bronchopulmonary mycosis" OR "aspergillus sensitization" or "severe asthma with fungal sensitization" OR "fungal sensitization"). The reference list of all the included articles and relevant review articles were searched to identify any additional studies. We also sifted through our personal database.

2.2 | Inclusion and exclusion criteria

We identified studies describing the performance of rAsp antigens in the diagnosis of ABPA and its differentiation from asthma, cystic fibrosis (with or without *A. fumigatus* sensitization) or healthy controls. Studies were included if the reference standard to diagnose ABPA was a composite criterion (clinical, radiological, immunological). We noted the reference criteria (Nelson's criteria,⁹ Rosenberg-Patterson criteria,¹⁰ Cystic Fibrosis Consensus conference criteria¹¹ or the International society for human and animal mycology (ISHAM) ABPA working group criteria²) used for making a diagnosis of ABPA. The following were excluded: (a) case reports, correspondence, editorials, conference abstracts and review articles; (b) animal studies; (c) studies describing only cross-reactivity of antigens, but not describing the diagnostic performance in ABPA; and (d) studies that described the purification and isolation of rAsp antigens, but not their diagnostic efficacy.

2.3 | Initial review of studies

We created a database from the electronic searches using the reference manager Endnote (version X8.2; Clarivate Analytics, Philadelphia, PA, US) and discarded duplicate citations. Two authors (VM and RA) independently screened the database to identify relevant articles. Any discrepancy between the authors was resolved by consensus. After scrutinizing the records, we obtained the full text of the relevant articles and reviewed them in detail.

2.4 | Study selection and data abstraction

Two authors (VM and RA) independently assessed the studies and selected them for inclusion in the review. The following data were extracted: (a) details of publication (authors, country and year of publication); (b) number of patients under each category (asthma/CF [with or without *Aspergillus* sensitization], ABPA and healthy controls) and the criteria used to define them; (c) rAsp antigens and the crude *Aspergillus* antigens used; (d) the nature and methodology of the test under evaluation (skin prick test, intradermal test, serology against rAsp antigens [specific IgE, IgG]), the cut-offs at which the tests were interpreted; (e) prevalence of elevated IgE against the rAsp antigens in the various study groups (*Aspergillus*-sensitized asthma or CF, controls and ABPA); (f) allergic response and cross-reactivity to antigens other than *A. fumigatus* (if data provided); (g) diagnostic performance of the tests (sensitivity, specificity), whenever available.

2.5 | Risk of bias assessment

We used the Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 tool to assess the quality of the studies included in the meta-analysis. The QUADAS-2 consists of four key domains (patient selection, index test, reference standard, and the flow and timing of tests). Each domain is assessed in terms of risk of bias and the first three in terms of concerns regarding applicability. We then entered these details in the statistical package RevMan, and the methodological quality graph was constructed for the studies included in the meta-analysis. The quality of the individual studies was also independently assessed by two authors (VM and RA) using the QualSyst tool.¹² The QualSyst tool includes a set of 10 questions, with each having a possible score of 0, 1 and 2.

2.6 | Data analysis

The statistical software packages OpenMetaAnalyst (www.cebm.brown.edu/openmeta) and RevMan (Version 5.3; The Cochrane Collaboration) were used to perform all the statistical analyses.

2.7 | Determination of the pooled effect

The gold standard for evaluating the performance of the rAsp antigens was the diagnosis of ABPA established using composite criteria.

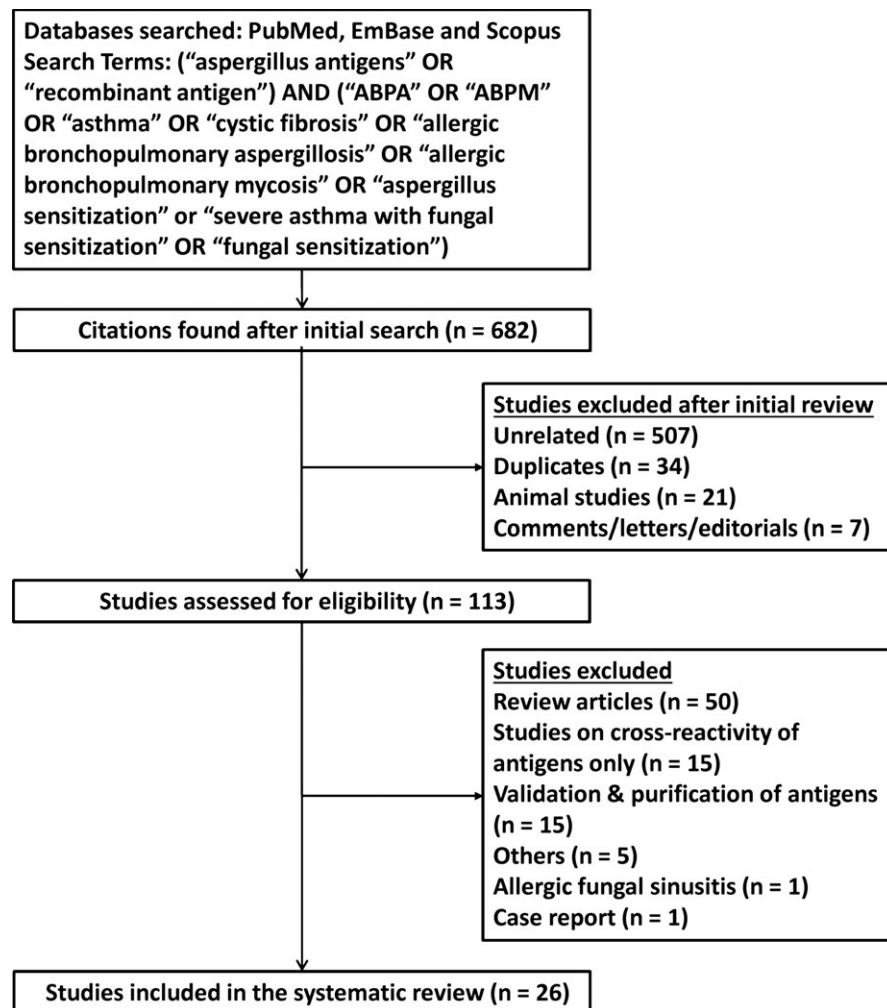


FIGURE 1 Flow Diagram Showing the Search Strategy and Study Selection Process for the Systematic Review and Meta-Analysis

The diagnostic performance of the test (sensitivity and specificity) was evaluated by its efficiency to distinguish individuals with ABPA from those without (*A. fumigatus* sensitization, asthma/CF without sensitization and healthy controls). Only those studies which allowed for calculating the above parameters were included in the meta-analysis. We calculated the sensitivity and specificity of each rAsp antigen from all the included studies and pooled the results using a hierarchical summary receiver operating characteristic (HSROC) model.¹³ The pooled sensitivity and specificity (along with 95% confidence interval [95% CI]) of each antigen were calculated separately for skin test or IgE against rAsp. The results for CF and asthma were pooled separately.

2.8 | Quality of evidence

The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach was used to evaluate the quality of evidence for sensitivity and specificity across studies.¹⁴ The risk of bias, inconsistency in the results, imprecision, indirectness of the evidence were assessed and summarized as per the GRADE working group recommendations.

3 | RESULTS

Our systematic review yielded 682 studies, of which 26 studies ($n = 1694$) were included in this analysis (Figure 1, Table 1).¹⁵⁻⁴⁰ Most of the included studies were of average quality, with a median (IQR) QualSyst score being 10.5 (10-11) (Table S1). Amongst the included participants, 32.1% ($n = 543$) had ABPA (asthma-ABPA, $n = 389$; CF-ABPA, $n = 154$). Fourteen studies were from the same group in Switzerland while six studies were from the United States.^{15-28,30,32,33,36,37} The study participants had asthma (14 studies),^{15,17-19,21,23,24,26-28,31,34,37,39} cystic fibrosis (10 studies)^{16,20,25,29,30,32,33,35,36,38} or both (2 studies).^{22,40} One study included atopic dermatitis with or without asthma in their cohort and evaluated the presence of cross-reactivity to several other allergens (*Alternaria alternata*, *Cladosporium herbarum*, *Trichophyton rubrum*, *Malassezia* spp.).³⁹ The utility of rAsp antigens was evaluated using either specific IgE (20 studies),^{16,17,19,21-23,25,27,28,30-40} skin test (4 studies),^{15,20,26,29} or both (two studies).^{18,24} Amongst the studies using rAsp antigens for skin testing, skin prick test (SPT) was performed in all except one, where intradermal testing was employed.²⁶

TABLE 1 Characteristics of the studies included in the systematic review describing the role of recombinant *A. fumigatus* antigens in the diagnosis of ABPA

References	Country	Study objective	Number of patients	Diagnosis of <i>Aspergillus</i> sensitization and ABPA	Details of the diagnostic test employed
Moser et al ¹⁵ (1994)	Switzerland	Compare commercially available crude antigens of <i>A. fumigatus</i> and rAsp f1 in asthmatics using serology, SPT and IDT	- Asthma without <i>A. fumigatus</i> sensitization (n = 6) - Asthma with <i>A. fumigatus</i> sensitization (n = 10) - ABPA (n = 15) - Healthy controls (n = 7)	- Asthma without <i>A. fumigatus</i> sensitization (allergic asthma): asthma with total IgE > 100 kU/L and skin prick test positive to any one of the allergens-house dust mite, cat allergen, dog dander, grass pollen, birch pollen - Asthma with <i>A. fumigatus</i> sensitization: patients had <i>A. fumigatus</i>-specific RAST class 2 or more during the study - ABPA: at least six of the seven Rosenberg-Patterson criteria	- Commercially available crude <i>A. fumigatus</i> antigen preparation for SPT, IDT (from Allergopharma, Hamburg Germany and Bencard, Smithkline Beecham, Neuss, Germany) - Total IgE by radioimmunoassay with anti-IgE Pharmacia CAP system (Uppsala, Sweden) <i>A. fumigatus</i>-specific IgE titre was determined by RAST with the Pharmacia allergen-specific CAP system - Anti rAsp f1 antibody binding was analysed by direct solid phase ELISA
Nikolaizik et al ¹⁶ (1995)	Switzerland	Diagnostic value of rAsp f1 in CF-ABPA and its utility in differentiating ABPA from <i>A. fumigatus</i> allergic patients and non-allergic CF patients	- CF patients with ABPA (n = 10) - CF patients with <i>A. fumigatus</i> allergy (n = 27) - CF control group with no allergy to <i>A. fumigatus</i> (n = 18)	- CF control had negative skin prick test with commercially available crude Bencard antigens (Smithkline Beecham, Neuss, Germany) <i>A. fumigatus</i> allergic CF patients had positive (≥ 3 mm wheal) skin prick test with the Bencard antigens (Smithkline Beecham, Neuss, Germany) - ABPA: at least six of the seven Nelson criteria	- Skin prick test for <i>A. fumigatus</i> and other allergens (Bencard, Smithkline Beecham, Neuss, Germany) was performed and read 15 min after administration - ELISA was used for measuring rAsp f1 antibodies
Banerjee et al ¹⁷ (1996)	United States	Molecular cloning of rAsp f2 antigen and assessing specific IgE binding in sera of ABPA patients	NA	NA	Solid phase ELISA was used to analyse anti- <i>A. fumigatus</i> antibody binding
Cramer et al ¹⁸ (1996)	Switzerland	Feasibility of using rAsp antigens in determining allergen-specific IgE by an automated serology system	- Asthma with <i>A. fumigatus</i> sensitization (n = 10) - Asthma without <i>A. fumigatus</i> sensitization (n = 9) - ABPA (n = 30) - Healthy controls (n = 10)	<i>A. fumigatus</i> sensitization was positive if the included subjects had a positive test to any one of the commercial crude <i>A. fumigatus</i> antigen preparations - ABPA: at least six of the seven Rosenberg-Patterson criteria¹⁰ - Healthy controls had no allergy or asthma and had a normal serum IgE level	- SPT was performed using commercially available crude <i>A. fumigatus</i> antigen preparation from Allergopharma (Hamburg, Germany) and Bencard (Smithkline Beecham, Neuss, Germany) - A wheal size of at least 9 mm² was considered positive - Radioimmunoassay with Pharmacia CAP was used to determine total IgE - Solid phase ELISA was used to determine anti rAsp f1 IgE binding

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TABLE 1 (Continued)

References	Country	Study objective	Number of patients	Diagnosis of <i>Aspergillus</i> sensitization and ABPA	Details of the diagnostic test employed
Cramer et al ¹⁹ (1996)	Switzerland	Evaluation of performance of rAsp f1 allergen using the Pharmacia CAP system, solid phase immunoassay for specific IgE in asthma patients	- Allergic asthmatics with sensitization to <i>A. fumigatus</i> ABPA (n = 30) - Healthy controls (n = 10)	- Asthmatics with <i>A. fumigatus</i> sensitization had positive reaction to intradermal skin test with commercially available crude <i>A. fumigatus</i> antigen preparation and RAST class 0 <i>A. fumigatus</i>-specific IgE, while those without sensitization did not have the above (specific IgE not detectable) - ABPA: at least six of the seven Rosenberg-Patterson criteria - Healthy controls had no allergy, asthma or atopy and normal total IgE levels	- IDT was performed using commercially available crude <i>A. fumigatus</i> antigen preparation from Allergopharma (Hamburg, Germany) and Bencard (Smithkline Beecham, Neuss, Germany) - Antigen-specific ELISA was used to determine anti rAsp f1 antibodies in sera - Sera was collected before skin tests and stored at -20°C
Nikolaizik et al ²⁰ (1996)	Switzerland	Evaluating the diagnostic value of rAsp f1 for skin testing in CF controls, CF with <i>A. fumigatus</i> sensitization and CF-ABPA	- CF controls (n = 13) - CF with <i>A. fumigatus</i> sensitization (n = 25) - CF-ABPA (n = 6)	- CF control group: routine skin prick tests with commercially available crude <i>A. fumigatus</i> antigen preparation (Bencard) negative for <i>A. fumigatus</i> (<3 mm) and a RAST of <2 - CF-ABPA: at least six of the seven Nelson criteria <i>A. fumigatus</i>-sensitized CF: CF patients demonstrating positive reaction with routine skin prick tests with a commercial allergen preparation (≥3 mm wheal to Bencard, Smithkline Beecham, Neuss, Germany) or RAST ≥2 for <i>A. fumigatus</i>	- IgG to <i>A. fumigatus</i> determined by solid phase ELISA using standardized DPC (Diagnostic Products Corporation, Los Angeles, California, USA) antigens - Skin prick tests to rAsp f1 antigen, DPC antigens LP-CF (late phase culture filtrate), three other commercially available <i>A. fumigatus</i> antigens (Bencard, ALK [Holshorn, Denmark], and Allergopharma [Hamburg, Germany]). rAsp f1 antigen was used at protein concentration of 0.1 mg/mL
Banerjee et al ²¹ (1997)	United States	Purification of rAsp f2 and their utility in diagnosing ABPA. Sensitivity and specificity of the purified protein derivative were evaluated using RAST and ELISA	- ABPA (n = 25) - Asthma with <i>A. fumigatus</i> sensitization (n = 10) - Healthy controls (n = 10)	NA	Sera from various subjects were analysed using ELISA for IgE against purified proteins as well as crude culture filtrate <i>A. fumigatus</i> antigens. RAST was also performed for IgE binding against purified and commercially available crude <i>A. fumigatus</i> antigen preparation
Banerjee et al ²² (1998)	United States	Complete nucleotide sequencing of Asp f2 antigen was performed. Demonstration of IgE binding to native and recombinant Asp f2 antigens from sera of ABPA patients	- CF-ABPA (n = 10) - ABPA (n = 10) <i>A. fumigatus</i>-sensitized asthma (n = 10)	NA	- IgE antibody binding to purified Asp f2 was analysed using ELISA - Antigen-specific ELISA was used to compare IgE binding of Asp f2 and ASPND1 (from <i>Aspergillus nidulans</i>) using sera from ABPA patients

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TABLE 1 (Continued)

References	Country	Study objective	Number of patients	Diagnosis of <i>Aspergillus</i> sensitization and ABPA	Details of the diagnostic test employed
Cramer et al ²³ (1998)	Switzerland	To evaluate the differential IgE antibody responses to different rAsp antigens and their potential role in differentiating ABPA from <i>A. fumigatus</i> sensitization	<i>A. fumigatus</i>-sensitized asthmatics with ABPA (n = 60) <i>A. fumigatus</i>-sensitized asthmatics without ABPA (n = 40) - Healthy controls (n = 20)	- Healthy controls had no atopy, allergy or asthma and normal total IgE levels <i>A. fumigatus</i>-sensitized individuals with asthma had positive skin prick test to commercially available <i>A. fumigatus</i> antigens and did not satisfy criteria for ABPA - ABPA: at least six of the seven Rosenberg-Patterson criteria satisfied	<i>A. fumigatus</i>-specific IgE titration was performed using Phadebas RAST (Pharmacia) - Commercially available crude <i>A. fumigatus</i> antigen preparations from Alleghropharma (Hamburg, Germany) and Bencard (Smithkline Beecham, Neuss, Germany) were used for skin prick tests - Intradermal skin tests with rAsp antigens were reported positive if the wheal was at least half the size of the skin reaction to histamine control and at least 5 mm or more at an allergen concentration of 1 µg/mL - Cut-off level used: 6, 10, 4 and 7 EU/mL for f1, f3, f4 and f6 respectively
Hemmann et al ²⁴ (1998)	Switzerland	Evaluate the role of rAsp f3 serology using ELISA and ImmunoCAP compared to skin test response in <i>A. fumigatus</i> -sensitized asthmatics	- Asthma with ABPA (n = 11) - Asthma with <i>A. fumigatus</i> sensitization without ABPA (n = 8) - Healthy controls (n = 4)	<i>A. fumigatus</i>-sensitized asthma: patients with stable bronchial asthma and <i>A. fumigatus</i> sensitization by RAST - ABPA: asthma patients with at least six Rosenberg-Patterson criteria - Healthy controls: no atopy, allergy, asthma and had normal serum IgE levels	- IDT was performed by injecting 100 µL solution containing 1 ng of rAsp f3. If no positive reaction by 20 min, a 10-fold higher amount of rAsp f3 was injected. Test was stopped if wheal was at least half the size of the wheal elicited by positive control or when there was no reaction to 10 µg of rAsp f3 - ImmunoCAP and ELISA were performed in sera collected and stored at -20°C before skin prick test. rAsp f3 specific IgA, IgG, IgG1, IgG2, IgG3, IgG4 and IgE were determined
Hemmann et al ²⁵ (1998)	Switzerland	Cloning and characterization of rAsp antigens and their role in differentiating CF controls, ABPA and <i>A. fumigatus</i> -sensitized individuals	- CF with ABPA (n = 20) - CF with <i>A. fumigatus</i> sensitization (n = 20) - CF controls (n = 10)	- CF patients with <i>A. fumigatus</i> sensitization: specific IgE by RAST was 1 or more and a wheal of more than 9 mm² when skin prick test was performed with commercial <i>A. fumigatus</i> extracts - ABPA- CF patients satisfying at least six of the seven criteria by Nelson et al - CF controls- No sensitization to <i>A. fumigatus</i>	- Solid phase ELISA was used for <i>A. fumigatus</i>-specific IgE and IgG levels - Pharmacia CAP system was used to determine <i>A. fumigatus</i>-specific IgE by RAST. Serum specific IgE levels three times the mean ELISA units per mL (EU/mL) of CF control group was taken as abnormal

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TABLE 1 (Continued)

References	Country	Study objective	Number of patients	Diagnosis of <i>Aspergillus</i> sensitization and ABPA	Details of the diagnostic test employed
Hermann et al ²⁶ (1999)	Switzerland	To evaluate the diagnostic role of <i>A. fumigatus</i> antigens f4 and f6 in the diagnosis of ABPA (asthma)	• Asthma with ABPA (n = 12) • <i>A. fumigatus</i>-sensitized asthma (n = 12) • Healthy individuals (n = 5)	• <i>A. fumigatus</i>-sensitized asthma: positive skin prick test to any one of the commercially available <i>A. fumigatus</i> antigen preparations • ABPA: diagnosed in <i>A. fumigatus</i> allergic individuals if they fulfilled seven of the eight Rosenberg-Patterson criteria • Healthy controls: no asthma or atopy and normal serum IgE levels	Skin prick test performed with commercially available crude <i>A. fumigatus</i> antigen preparation (Bencard, Smithkline Beecham, Neus, Germany and Allergopharma, Hamburg, Germany)
Kurup et al ²⁷ (2000)	United States and Switzerland	Evaluate the role of IgE antibody binding to rAsp antigens in the diagnosis of ABPA (asthma)	• ABPA (n = 24) • <i>A. fumigatus</i>-sensitized asthmatics (n = 16) • Healthy controls (n = 10)	• ABPA: majority of patients diagnosed with ABPA had satisfied at least 7 of the 8 Rosenberg-Patterson criteria • <i>A. fumigatus</i>-sensitized asthmatics: positive immediate skin test reactivity to <i>A. fumigatus</i> and not fulfilling ABPA criteria • Healthy controls: no asthma or allergy	• IgE binding to recombinant allergens was demonstrated by ELISA • Two different laboratories (US and Swiss) analysed the sera by ELISA
Banerjee et al ²⁸ (2001)	United States	Cloning, sequencing of Asp f16 antigen and assessing its utility in differentiating ABPA from <i>A. fumigatus</i> -sensitized individuals	• ABPA (n = 26) • Asthmatics with <i>A. fumigatus</i> allergy (n = 26) • Asthmatics without <i>A. fumigatus</i> allergy (n = 26) • Healthy controls (n = 26)	• ABPA: patients fulfilled six of the seven Rosenberg criteria • Asthmatics with <i>A. fumigatus</i> sensitization had skin test positivity to a mixture of different <i>Aspergillus</i> species extracts • Healthy controls had no clinical evidence of respiratory diseases	• cDNA was amplified using PCR and by gel electrophoresis pure Asp f16 was separated • Solid phase ELISA was used to assess IgE binding to rAsp f16 in sera of patients • PBMC from six ABPA and four <i>Aspergillus</i>-sensitized asthmatics were obtained and proliferation of PBMC was performed in the presence of rAsp f16 or <i>A. fumigatus</i> culture filtrate antigens

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TABLE 1 (Continued)

References	Country	Study objective	Number of patients	Diagnosis of <i>Aspergillus</i> sensitization and ABPA	Details of the diagnostic test employed
Nikolaizik et al ²⁹ (2002)	Switzerland	To assess the sensitivity and specificity of rAsp antigens in diagnosing ABPA in CF patients	- CF control (n = 17) - CF with <i>A. fumigatus</i> sensitization (n = 21) - CF-ABPA (n = 12)	- CF control: CF patients with negative skin prick test to commercially available crude <i>A. fumigatus</i> antigen preparation and specific IgE demonstrated by RAST <0.35 kUA/L <i>A. fumigatus</i>-sensitized CF patients: Routine skin prick test positive (wheal of 3 mm or more) with commercially available crude <i>A. fumigatus</i> antigen preparation or specific IgE to <i>A. fumigatus</i> by RAST more than or equal to 2 - ABPA: CF patients fulfilling at least six of the seven Nelson criteria	- rAsp antigens were produced from cDNA library and after production purity was assessed using SDS-PAGE and western blot analysis - Intracutaneous testing to recombinant <i>A. fumigatus</i> antigens was performed at protein concentrations of 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} µg/mL
Knutson et al ³⁰ (2004)	United States	- Retrospective study using banked sera - Comparing the serum IgE reactivity of commercially available crude <i>A. fumigatus</i> antigen preparation and recombinant purified antigens in CF patients with and without ABPA	- CF without <i>A. fumigatus</i> sensitization (n = 19) - CF with <i>A. fumigatus</i> sensitization (n = 23) - CF-ABPA (n = 28)	- ABPA: diagnosis was based on cystic fibrosis society recommendations <i>A. fumigatus</i>-sensitized CF patients: sensitization was defined by skin test positivity to commercially available <i>A. fumigatus</i> antigens and not satisfying ABPA criteria	- Recombinant allergens were produced from cDNA library constructed from <i>A. fumigatus</i>. Purity of antigen was studied by SDS-PAGE and recombinant antigens stored at -70°C - Total IgE and IgE to rAsp antigens were determined by ELISA
Bowyer et al ³¹ (2006)	United Kingdom	- Retrospective study - Evaluate the role of recombinant <i>A. fumigatus</i> antigens in the diagnosis of CCPA when compared to ABPA and SAFS	- ABPA (n = 16) - SAFS with <i>Aspergillus</i> sensitization (n = 5)	- ABPA: asthma, peripheral eosinophilia (>1000/mm³), immediate skin test reactivity to <i>A. fumigatus</i> antigens in 15 ± 5 min, IgG and IgE antibodies to <i>A. fumigatus</i>, elevated total IgE (>1000 kIU/L), history of pulmonary infiltrates - SAFS (only SAFS due to <i>Aspergillus</i> was included in this study): severe asthma (combination of long-acting bronchodilator, inhaled corticosteroid and occasional oral corticosteroid treatment) and either elevated specific IgE to <i>Aspergillus</i> or positive skin test to <i>Aspergillus</i> and total IgE <1000 kIU/L	Serum total and specific IgE were measured using the ImmunoCAP[™] system (Sweden diagnostics)

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TABLE 1 (Continued)

References	Country	Study objective	Number of patients	Diagnosis of <i>Aspergillus</i> sensitization and ABPA	Details of the diagnostic test employed
Kraemer et al ³² (2006)	Switzerland	- Retrospective study design - Onset of <i>A. fumigatus</i> sensitization and development of ABPA was evaluated	• CF control (n = 36) • CF with <i>A. fumigatus</i> sensitization (n = 70) • CF-ABPA (n = 16)	• ABPA: CF patients fulfilling at least six of the seven Nelson criteria • Sensitization to <i>A. fumigatus</i>: Specific IgE RAST class 2 or more and/or increased specific IgE to recombinant allergens and increased specific IgG to <i>A. fumigatus</i> • CF controls: no evidence of sensitization	• Allergen-specific IgE, specific IgG to rAsp antigens and specific IgG were determined by solid phase ELISA • ELISA cut-off values for the rAsp antigens were defined as three times the mean EU/mL values obtained by ELISA from the CF control groups. These were 9.6, 13.2, 8.4, 7.2 EU/mL for rAsp f1, f3, f4 and f6 respectively
Kurup et al ³³ (2006)	United States	To evaluate the role of rAsp antigens using the semi-automated ImmunoCAP and ELISA, in the diagnosis of CF-ABPA	• CF-ABPA (n = 17) • CF with <i>A. fumigatus</i> sensitization (n = 17) • CF without <i>A. fumigatus</i> sensitization (n = 22) • Healthy controls (n = 11)	• CF-ABPA: diagnosed as per the CF foundation consensus criteria¹¹ • CF with <i>A. fumigatus</i> sensitization: CF patients with positive skin test to <i>A. fumigatus</i> and not satisfying ABPA criteria • CF without <i>A. fumigatus</i> sensitization: negative skin prick test to <i>A. fumigatus</i>	• Solid phase ELISA was used to determine specific antibodies (IgE, IgG, IgG₁, IgG₂, IgG₃, IgA, IgA₁, IgA₂) to crude <i>A. fumigatus</i> extracts and recombinant <i>A. fumigatus</i> antigens • <i>A. fumigatus</i>-specific antibodies were also tested using UniCAP100 assay (CAP; Pharmacia Upjohn, Kalamazoo, MI) • ImmunoCAP class 1 or more (>0.35 kUA/L) was regarded positive

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TABLE 1 (Continued)

References	Country	Study objective	Number of patients	Diagnosis of <i>Aspergillus</i> sensitization and ABPA	Details of the diagnostic test employed
de Oliveira et al ³⁴ (2007)	Brazil	To evaluate the role of specific IgE to recombinant <i>A. fumigatus</i> antigens in the diagnosis of ABPA and <i>A. fumigatus</i> sensitization	- Asthma with ABPA (n = 6) - Asthma with <i>A. fumigatus</i> sensitization (n = 59)	- ABPA: positive precipitins, total IgE >1000 ng/mL, IgEm3 class II or more, IgGm3 >70 mg/mL, central bronchiectasis - Asthma with <i>A. fumigatus</i> sensitization: Immediate cutaneous reactivity to commercially available crude <i>A. fumigatus</i> antigen preparation	- Antigen extracts of <i>A. fumigatus</i> at 5% (Alk-Abello[®]) were used for immediate skin prick testing and if there was no reaction, <i>A. fumigatus</i> extract was intradermally administered at 1% (Alk-Abello[®]) - Total IgE measured by UniCAP[®] by employing the UniCAP 100 apparatus (Pharmacia diagnostics) - Gel diffusion was used to detect precipitins for <i>A. fumigatus</i>. The antigens used were obtained from Greer Laboratory (USA) and the Clementino Fraga Filho University Hospital, RJ, Brazil - UniCAP[®] method was employed to detecting serum specific IgE against <i>A. fumigatus</i> (IgEm3) and rAsp antigens (rAsp f1 IgERm218, rAsp f2 IgERm219, rAsp f3 IgERm220, rAsp f4 IgERm221, and rAsp f6 IgERm222) - Specific IgG for <i>A. fumigatus</i> (IgGm3) was also detected using UniCAP[®] method
Ringer et al ³⁵ (2007)	Germany	Role of cellular antigen stimulation test (CAST) and rAsp antigens in the diagnosis of ABPA in CF patients	- CF-ABPA (n = 8) - CF without ABPA (n = 19)	- CF: diagnosed as per the Cystic Fibrosis Foundation Consensus Panel criteria - CF-ABPA: CF patients with at least five of the seven Nelson's criteria	- UniCAP[®] fluoroenzyme immunoassay (Pharmacia, Uppsala, Sweden) was used to determine serum specific IgG, total serum IgE levels and antibodies to <i>A. fumigatus</i> extracts. CAP class >2 was considered positive - CAST: Leucocytes isolated from blood samples were primed with interleukin-3 and stimulated with <i>A. fumigatus</i> extracts. Sulfidoleukotrienes (sLT) produced from these leucocytes (particularly basophils) were measured using CAST[®]-2000 ELISA (Buehlmann, Scenenbuch, Switzerland) - Specific IgE antibodies to recombinant antigens (rAsp f1, f2, f3, f4, f6) were detected using UniCAP[®] system

(Continues)

TABLE 1 (Continued)

References	Country	Study objective	Number of patients	Diagnosis of <i>Aspergillus</i> sensitization and ABPA	Details of the diagnostic test employed
Latzin et al ³⁶ (2008)	Switzerland and Germany	Diagnostic value of Thymus- and activation- regulated chemokine (TARC) compared to serologic markers of ABPA in CF patients was assessed	- CF-ABPA (n = 12) - CF non-ABPA group (n = 36)	ABPA: six of the seven Nelson criteria for ABPA	- Total IgE, specific IgE to <i>A. fumigatus</i> by RAST, specific IgG to <i>A. fumigatus</i> by ELISA and specific IgE to recombinant antigens rAsp f1, f3, f4, f6 were measured - Thymus- and activation- regulated chemokine (TARC) levels were assayed in triplicate using sandwich ELISA (R & D systems, Minneapolis, MN, USA) - Cut-offs used for specific IgE to rAsp f1, f3, f4, f6 and T IgE were 75, 140, 10, 16 and 1624 IU/mL, respectively
Glaser et al ³⁷ (2009)	Switzerland	Cloning, production and immunological characterization of Asp f34 allergen of <i>A. fumigatus</i>	- Asthma with ABPA (n = 64) <i>A. fumigatus</i>-sensitized asthma (n = 24) - Asthma without <i>A. fumigatus</i> sensitization (n = 32) - Healthy controls (n = 32)	- ABPA: patients with asthma fulfilling six of the seven Patterson criteria <i>A. fumigatus</i>-sensitized asthma: positive skin prick test to <i>A. fumigatus</i> - Allergic asthma not sensitized to <i>A. fumigatus</i>: patients with negative skin prick test to <i>A. fumigatus</i>, but positive SPT to at least one allergens (house dust mite, cat, dog, grass or birch pollen) - Healthy controls: no allergy, atopy or asthma and normal IgE levels	<i>A. fumigatus</i> cDNA library was used and Asp f34 cDNA was amplified by standard PCR. Subsequent production of recombinant Asp f34 was performed in <i>E. coli</i> cells carrying the insert. Purity and size of the rAsp f34 was confirmed using SDS-PAGE (Nu Page, 12% Bis-Tris, Invitrogen limited, Paisley, UK) - Proliferation of peripheral blood mononuclear cells were assayed after stimulating with serial dilutions of Asp f34, starting from 1 μM for 6 days in humidified atmosphere with 5% CO₂. Measurement of tritiated thymidine incorporation was performed (Hartmann Analytics, Braunschweig, Germany) during the final 8 h of culture - Asp f34 specific IgE was measured using solid phase ELISA using Maxisorp[®] polystyrene microtitre plates (Nunc, Roskilde, Denmark) - Total serum IgE and specific IgE to <i>A. fumigatus</i> extract (m³) was determined by the ImmucAP system (Phadia, Uppsala, Sweden) - A solution containing 100 μg/mL was used for SPT with rAsp f34

(Continues)

TABLE 1 (Continued)

References	Country	Study objective	Number of patients	Diagnosis of <i>Aspergillus</i> sensitization and ABPA	Details of the diagnostic test employed
Fricker-Hidalgo et al ³⁸ (2010)	France	Retrospective study to evaluate the role of recombinant allergens (rAsp f4 and f6) along with <i>A. fumigatus</i> -specific IgG, precipitins, total serum IgE and sputum fungal culture in patients with ABPA	<i>A. fumigatus</i> associated diseases in CF (n = 20); definite ABPA seen in (n = 13), probable ABPA (n = 4), <i>Aspergillus</i> bronchitis (n = 2), <i>Aspergillus</i> rhinosinusitis (n = 1) - CF controls with no ABPA (n = 45)	CF foundation criteria used to diagnose ABPA. ¹¹ Further details not available	- Specific IgE to rAsp f4, f6 and serum total IgE were measured by Pharmacia UniCAP system (Phadia SAS, Saint Quentin en Yvelines, France) <i>A. fumigatus</i>-specific IgG was measured using homemade ELISA. Antigenic preparations were obtained from 2 <i>A. fumigatus</i> strains IHEM 9073 (isolated from an Aspergilloma) and IHEM 22 145 (isolated from a human cerebral biopsy sample)
Tanimoto et al ³⁸ (2015)	Japan	Retrospective evaluation of diagnostic utility of IgE and IgG antibodies to recombinant <i>A. fumigatus</i> antigens in differentiating ABPA from <i>A. fumigatus</i> -sensitized asthma	- ABPA (n = 53) - Asthma without AD (n = 142) - Asthma with AD (n = 111) - AD without asthma (n = 34)	- All asthma patients were sensitive to <i>A. fumigatus</i>, either with intradermal skin testing or positive serum IgE to <i>A. fumigatus</i>. They were further categorized as those with and without AD - ABPA: Asthma patients fulfilling six of the eight Rosenberg-Patterson criteria	- IgE and IgG antibodies to commercially available crude <i>A. fumigatus</i> antigen preparation and recombinant <i>A. fumigatus</i> antigens were determined using the ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden; allergen codes, m3, m218, m219, m220, m221 and m222). - Serum IgE antibodies to <i>Alternaria alternata</i> (m6), <i>Candida albicans</i> (m5), <i>Cladosporium herbarum</i> (m2), <i>Penicillium chrysogenum</i> (m1), <i>Trichophyton rubrum</i> (m205), <i>Malassezia</i> spp. (m227) to <i>A. flavus</i> (m228), <i>A. niger</i> (m207) and <i>A. terreus</i> (m228) were also measured - Antibody levels >0.35 kUA/L was considered positive - Ouchterlony double immunodiffusion test using crude <i>A. fumigatus</i> extract (HollisterStier, WA, USA) was used to assay precipitating antibodies

(Continues)

TABLE 1 (Continued)

References	Country	Study objective	Number of patients	Diagnosis of <i>Aspergillus</i> sensitization and ABPA	Details of the diagnostic test employed
Vitte et al ⁴⁰ (2016)	France	To evaluate the role of IgG4 and IgE antibodies against rAsp antigens in the diagnosis of ABPA and differentiating from <i>A. fumigatus</i> -sensitized CF patients	- CF with <i>A. fumigatus</i> sensitization (n = 55) - Asthma-ABPA (n = 7) - CF-ABPA (n = 2)	- ABPA diagnosed based on the following criteria: acute or subacute decline in pulmonary function in patients with asthma or CF, total IgE ≥ 500 kIU/L, elevated serum IgE and IgG to <i>A. fumigatus</i>, pulmonary infiltrates in chest radiograph or computed tomography, with high attenuation mucous plugging considered to be pathognomonic - CF with <i>A. fumigatus</i> sensitization: CF patients with detectable IgE antibodies to <i>A. fumigatus</i> crude antigen in serum	Serum IgG4 and IgE to recombinant antigens rAsp f1, f2, f3, f4 and f6 were measured with ThermoFisher ImmunoCAP (Uppsala, Sweden). Ratio of IgG4/IgE was calculated

ABPA, allergic bronchopulmonary Aspergillosis; *A. fumigatus*, *Aspergillus fumigatus*; AD, Atopic dermatitis; CCPA, chronic cavity pulmonary Aspergillosis; cDNA, complementary DNA; CF, cystic fibrosis; ELISA, enzyme linked immunosorbent assay; IDT, intra dermal test; Ig, immunoglobulin; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; rAsp, recombinant antigens of *A. fumigatus*; RAST, Radioallergosorbent test; SAFS, severe asthma with fungal sensitization; SDS-PAGE, sodium dodecyl sulphate- polyacrylamide gel electrophoresis; SPT, skin prick test.

The sensitivities and specificities of rAsp antigens against the composite reference standard varied widely amongst the included studies (Table 2), depending on the antigen employed (f1, f2, f3, f4 or f6), the nature of the test (specific IgE or skin test) and the patient population studied (asthma or cystic fibrosis).

We included 17 studies ($n = 1131$; of which 375 had ABPA) for the meta-analysis.^{15,18-21,23-27,29,30,32,35,36,38,39} The gold standard for diagnosing ABPA was the Rosenberg-Patterson criteria in eight studies,^{15,18,19,23,24,26,27,39} Nelson's criteria in six^{20,25,29,32,35,36} and the CF consensus conference criteria in two,^{30,38} whereas in one study, the criteria to diagnose ABPA was not explicitly mentioned.²¹ A high or intermediate risk of bias was evident in all except one study (Figure 2).³⁹ Of the included studies, 11 studies provided data on specific IgE^{19,21,23,25,27,30,32,35,36,38,39} (Table 3, Tables S2 and S3), four studies on skin tests^{15,20,26,29} and two studies provided both (Table 4, Tables S2-S5).^{18,24} In subjects with asthma, a pooled specificity of more than 90% each was seen with IgE against f4 and f6; however, their sensitivities were below 50% (Table 3). While utilizing IgE against rAsp antigens, f1 and f3 had the highest individual pooled sensitivities (80% [95% CI, 65.2%-89.5%] and 84.8% [95% CI, 56.5-99.9] respectively), but their specificities were below 75% (Table 3). In asthmatic patients, IgE against either f1 or f3 had the highest sensitivity (96.7% [95% CI, 86.7-99.2]); IgE to either f4 or f6 antigen had the highest specificity (99.2% [95% CI, 88.2-99.9]) (data from a single study); the latter however, had a sensitivity of only 89%. In CF patients, the highest pooled sensitivity was seen when IgE against a combination of f1 or f3 was used (93.3% [95% CI, 55.2-99.9]); whereas the pooled specificity was maximum for IgE against f6 (97.5% [95% CI, 87.3-99.9]) (Table 3). The number of studies providing data for pooled analysis of individual antigens varied from one to six (Tables S2-S5). The summary of findings as per the GRADE recommendations was tabulated (Tables 5 and 6). The strength of evidence for most of the analysis was very low to low, primarily because of the high risk of bias introduced by case selection (case-control study design), small sample sizes and significant heterogeneity of the included studies.

Pooled analysis of data for skin test in asthmatics showed that f3 antigen had the highest sensitivity (95.8% [95% CI, 57.5-99.7]) (Table 4). Skin test with a combination of either f4 or f6 had the highest specificity (97.2% [95% CI, 67.8-99.8]). In patients with CF, skin test using rAsp f4 antigen (data from a single study) was the most sensitive (96.2% [95% CI, 59.7-99.8]) and specific (88.5% [95% CI, 74.2-95.3]). Skin test using f3 and f6 also had a good sensitivity in subjects with CF (single study, 91.7% [95% CI, 58.7-98.8]).

3.1 | Heterogeneity

There was significant clinical heterogeneity in the studies included for pooled analysis. Different cut-offs have been used for interpreting the tests in the included studies, and this represents a major source for heterogeneity.

TABLE 2 Studies describing the role of recombinant *A. fumigatus* antigens to differentiate ABPA (diagnosed by composite reference criteria) from *A. fumigatus* sensitization and controls

Reference	Recombinant antigen	Disease	Test	Study population	Conclusion
Moser et al ¹⁵ (1994)	rAsp f1	Asthma	Skin prick test to commercially available crude <i>A. fumigatus</i> antigen preparation rAsp f1 skin prick test	Asthma with ABPA (n = 15) 15/15 (100%) 9/10 (90%) 0/13	- rAsp f1 skin test correlated with rAsp f1 specific IgE - ABPA patients had elevated IgG1 and IgG4 against rAsp f1
Nikolaizik et al ¹⁶ (1995)	rAsp f1	CF	rAsp f1 skin prick test	CF with ABPA (n = 10) 8/15 (53%) 5/10 (50%) 0/13	rAsp f1 serology is sensitive and specific for ABPA
Banerjee et al ¹⁷ (1996)	rAsp f2	Asthma	Specific IgG (IgG1, IgG4) and IgE to rAsp f1	As compared to those without ABPA and <i>A. fumigatus</i> sensitization, ABPA patients had 10-fold increase in rAsp f1 IgE; fivefold increase in rAsp f1 IgG1; fourfold increase in rAsp f1 IgG4 ABPA with CB ELISA OD of 0.076-1.44 compared to 0.04-0.08 in normal subjects Elevated in patients with ABPA-S, ABPA-CB, aspergilloma as compared to allergic asthma and healthy controls. Individuals with aspergilloma had relatively higher levels	rAsp f2 has immunologic activity comparable to native <i>A. fumigatus</i> antigens. Sensitive and specific for ABPA-CB
Cramer et al ¹⁸ (1996)	rAsp f1	Asthma	Skin prick test with commercially available crude <i>A. fumigatus</i> antigen preparation Skin test to rAsp f1 Specific IgE to rAsp f1	ABPA (n = 30) 30/30 (100%) 10/10 (100%) (one or another commercial test) 5/10 (50%) 5/10 (50%) 24/30 (80%) 24/30 (80%)	rAsp f1 skin test correlated with serologies. ImmunoCAP method and ELISA based IgE measurement of rAsp f1 IgE showed excellent (0.997) correlation

(Continues)

TABLE 2 (Continued)

Reference	Recombinant antigen	Disease	Test	Study population	Conclusion
Cramer et al ¹⁹ (1996)	rAsp f1	Asthma	Skin prick test with commercially available crude <i>A. fumigatus</i> antigen preparation <i>A. fumigatus</i>-specific IgE by RAST Specific IgE to rAsp f1	ABPA (n = 30) 30/30 (100%) 30/30 (100%) 30/40 (73%) (both by ImmunoCAP and ELISA based IgE)	Results obtained with rAsp f1 ImmunoCAP system had excellent correlation with ELISA IgE Asthma without <i>A. fumigatus</i> sensitization (n = 9) and controls (n = 10) 0/19 10/10 (100%) (at least one of the commercial tests) 10/10 (100%) 0/19 0/19
Nikolaizik et al ²⁰ (1996)	rAsp f1	CF	Skin prick test with crude antigens of <i>A. fumigatus</i> ALK pharma Allergopharma Bencard DPC pharma Late phase culture filtrate Skin prick test to rAsp f1	CF with ABPA (n = 6) 6/6 (100%) 5/6 (83.3%) 4/6 (66.7%) 4/6 (66.7%) 2/6 (33.3%) 3/6 (50%)	Reactions to rAsp f1 antigen 8.5-fold stronger in <i>A. fumigatus</i>-sensitized individuals and 3.3-fold stronger in ABPA compared to CF controls CF controls (n = 13) 3/13 (23%) 2/13 (15.4%) 0/13 0/13 0/13 1/13 (7.7%)
Banerjee et al ²¹ (1997)	rAsp f2	Asthma	Skin prick test with commercially available crude <i>A. fumigatus</i> antigen preparation Specific IgE to rAsp f2	ABPA (n = 25) 25/25 (100%) 24/25 (96%)	RAST and ELISA IgE binding had strong quantitative correlation in ABPA patients (r 0.89); asthma with <i>A. fumigatus</i> sensitization and healthy individuals did not show significant correlation Healthy controls (n = 10) 0/10
Banerjee et al ²² (1998)	rAsp f2	CF-ABPA and asthma	Specific IgE to rAsp f2	ABPA in asthma (n = 10) and CF (n = 10) 20/20 (100%)	rAsp f2 has a potential role in <i>A. fumigatus</i> related diseases and differentiating ABPA from sensitization Healthy control (n = 10) Significantly lower than ABPA patients (mean [SD] 0.606 [0.438] in CF/ABPA vs 0.048 [0.042] in controls, $P < 0.01$)

(Continues)

TABLE 2 (Continued)

Reference	Recombinant antigen	Disease	Test	Study population	A. fumigatus-sensitized asthma (n = 40)	Healthy controls (n = 20)	Conclusion
Cramer et al ²³ (1998)	rAsp f1, f3, f4 and f6	Asthma		ABPA (n = 60)			ImmunoCAP and ELISA had excellent correlation (0.996) for IgE against rAsp f6
			rAsp f1 IgE	50/60 (83%)	18/40 (45%)	Below cut-off	IgE to rAsp f1 or f3: PPV 65.9% and NPV 93.7% for ABPA
			rAsp f3 IgE	55/60 (88%)	21/40 (52.5%)	6 EU/mL and 10 EU/mL for f1 and f3 respectively) in all	IgE to rAsp f4 or f6: PPV 100% and NPV 90.9% for ABPA
			IgE to either rAsp f1 or f3	58/60 (96%)	30/40 (75%)		
			rAsp f4 IgE	48/60 (80%)	0/40	0/20	
			rAsp f6 IgE	33/60 (55%)	0/40	0/20	
Hemmann et al ²⁴ (1998)	rAsp f3	Asthma	IgE to either rAsp f4 or f6	54/60 (90%)	0/40	0/20	
				ABPA (n = 11)	Asthma with A. fumigatus sensitization (n = 8)	Healthy control (n = 4)	rAsp f3 specific IgG, IgG1, IgG4 and IgE elevated in ABPA
			Skin prick test to rAsp f3	11/11 (100%)	5/8 (62.5%)	0/4	rAsp f3 ELISA and ImmunoCAP correlate well
			Specific IgE to rAsp f3	11/11 (100%)	5/8 (62.5%)	0/4	Skin test was positive only in individuals with rAsp f3 ELISA level of >10 EU/mL
Hemmann et al ²⁵ (1998)	rAsp f1, f3, f4 and f6	CF		CF with ABPA (n = 20)	CF with A. fumigatus sensitization (n = 20)	CF controls (n = 10)	ABPA patients had a significantly higher mean IgE against all these recombinant antigens compared to allergic individuals as well as controls
			Skin prick test with crude antigens of A. fumigatus	20/20 (100%)	20/20 (100%)	0/10	rAsp f4 and/or f6 were almost exclusively bound by sera from ABPA patients (sensitivity of 100% [95% CI, 86.1-100] and specificity of 100% [95% CI, 86.1-100])
			Specific IgE to rAsp f1	20/20 (100%)	12/20 (60%)	0/10	
			Specific IgE to rAsp f3	20/20 (100%)	19/20 (95%)	0/10	
			Specific IgE to rAsp f4	16/20 (80%)	0/20	0/10	
			Specific IgE to rAsp f6	14/20 (70%)	0/20	0/10	
			Specific IgE to rAsp f1 or f3	20/20 (100%)	19/20 (95%)	0	
			Specific IgE to rAsp f4 or f6	20/20 (100%)	0	0	

(Continues)

TABLE 2 (Continued)

Reference	Recombinant antigen	Disease	Test	Study population	Healthy controls	Conclusion
Hemmann et al ²⁶ (1999)	rAsp f4 and f6	Asthma		Asthma with ABPA (n = 12)	Healthy controls (n = 5)	• Skin test positivity correlated with serum IgE levels against these antigens. Skin test to rAspf4 and f6 combined, had a sensitivity 92%, specificity 100%, NPV 94% and PPV 100%. IgE levels of 4 and 7 EU/mL would be required for eliciting a positive skin test to rAsp f4 and f6 respectively • Correlation between IgE by ELISA and ImmunoCAP was highly significant and the absolute ImmunoCAP cut-off for positive IDT were 0.9 kUA/L for rAsp f4 and 1.2 kUA/L for rAsp f6
			Skin prick test with crude antigens of <i>A. fumigatus</i>	12/12 (100%)	0/5 (0%)	
			Intradermal test with rAsp f4	10/12 (83%)	0/5 (0%)	
			Intradermal test with rAsp f6	5/12 (42%)	0/5 (0%)	
			Intradermal test with rAsp f4 and/or f6	11/12 (91.7%)	0/5 (0%)	

Kurup et al ²⁷ (2000)	rAsp f1, f2, f3, f4 and f6	Asthma	(Swiss lab)	Asthma with ABPA (n = 24)	Asthma with <i>A. fumigatus</i> sensitization (n = 16)	Healthy controls (n = 10)	• A combination of positive IgE against rAsp f2, f4 and f6 was positive in all ABPA patients and only 6% of the asthmatics in the Swiss lab and 45% of the asthmatics in the US lab
			Specific IgE to rAsp f1	20/24 (83%)	13/16 (81%)	1/10 (10%)	
			Specific IgE to rAsp f2	21/24 (87%)	1/16 (6%)	0/10	
			Specific IgE to rAsp f3	20/24 (83%)	6/16 (36%)	1/10 (10%)	
			Specific IgE to rAsp f4	22/24 (92%)	0/16	0/10	
			Specific IgE to rAsp f6	14/24 (58%)	0/16	0/10	
			(US Lab)	Asthma with ABPA (n = 24)	Asthma with <i>A. fumigatus</i> sensitization (n = 16)	Healthy controls (n = 10)	
			Specific IgE to rAsp f1	16/24 (68%)	0/16	0/10	
			Specific IgE to rAsp f2	21/24 (87%)	6/16 (36%)	0/10	
			Specific IgE to rAsp f3	19/24 (81%)	4/16 (27%)	1/10 (10%)	
			Specific IgE to rAsp f4	15/24 (62%)	3/16 (18%)	0/10	
			Specific IgE to rAsp f6	16/24 (68%)	4/16 (27%)	0/10	

(Continues)

TABLE 2 (Continued)

Reference	Recombinant antigen	Disease	Test	Study population		Conclusion
Banerjee et al ²⁸ (2001)	rAsp f16	Asthma		ABPA (n = 26)	Asthma with <i>A. fumigatus</i> sensitization (n = 26)	Healthy controls (n = 26)
			Specific IgE to rAsp f16	18/26 (70%)	0/26	0/26
						- Asp f16 may be useful in ABPA. No correlation of total IgE and <i>A. fumigatus</i>-specific IgE in ABPA. <i>A. fumigatus</i>-sensitized and non-sensitized asthma - Asp f16 was cloned, sequenced and found to share homology with Asp f9 - Of the 6 patients of ABPA and four sensitized asthmatics, significant PBMC stimulation with 5 µg/mL of rAsp f16 was noted in four and one patient, respectively
Nikolaizik et al ²⁹ (2002)	rAsp f1, f3, f4 and f6	CF		CF with ABPA (n = 12)	CF with <i>A. fumigatus</i> sensitization (n = 21)	CF controls (n = 17)
			Skin prick test with crude antigens of <i>A. fumigatus</i>	12/12 (100%)	21/21 (100%)	0/17
			Skin prick test to rAsp f1	8/12* (66.7%)	12/21* (57.1%)	0/17
			Skin prick test to rAsp f3	11/12* (91.7%)	12/21* (57.1%)	0/17*
			Skin prick test to rAsp f4	12/12* (100%)	4/21 (19%)	0/17*
			Skin prick test to rAsp f6	11/12* (91.7%)	12/21 (57.1%)	0/17*
						SPT positivity to Asp f4 and/or Asp f6 can be highly specific for ABPA: sensitivity 100% (95% CI 73.5-100) specificity of 100% (95% CI 83.9-100)
Knutsen et al ³⁰ (2004)	rAsp f1-f4, f6, f12 and f16	CF		CF-ABPA (n = 28)	CF with <i>A. fumigatus</i> sensitization (n = 23)	CF controls (n = 19)
			Specific IgE to rAsp f1	24/28 (86%)	12/23 (52%)	1/19 (5%)
			Specific IgE to rAsp f2	21/28 (75%)	10/23 (44%)	0/19
			Specific IgE to rAsp f3	22/28 (79%)	7/23 (30%)	0/19
			Specific IgE to rAsp f4	23/28 (82%)	8/23 (35%)	1/19 (5%)
			Specific IgE to rAsp f6	7/28 (25%)	0/23	0/19
			Specific IgE to rAsp f12	2/28 (7%)	0/23	0/19
			Specific IgE to rAsp f16	11/28 (41%)	0/23	0/19
			<i>A. fumigatus</i> -specific IgE (crude)	25/28 (89%)	14/23 (61%)	0/19
						- IgE against rAsp f3 and f4 had the best AUROC (better than crude extracts); 0.845 (f3) 0.773 (f4) - Levels of IgE against f1, f2, f3 and f6 were significantly elevated during flares than remission - Even though IgE against f4 and crude extracts were also elevated, the difference was not significant when compared to levels during remission

(Continues)

TABLE 2 (Continued)

Reference	Recombinant antigen	Disease	Test	Study population	Conclusion
Bowyer et al ³¹ (2006)	rAsp f1, f3, f4 and f6	ABPA, CCPA and SAFS	Specific IgE to crude antigens of <i>A. fumigatus</i> and rAsp antigens were performed	ABPA (n = 16) All three group of patients reacted to rAsp f1 and f2, while SAFS patients did not react to rAsp f4 (and reaction to rAsp f1, f2, f6 was very low). CCPA patients did not react to rAsp f6 IgE to any of the four rAsp antigens were not seen in 14 of 22 (64%) CCPA patients. Only one out of the five (20%) SAFS patients showed reactivity to any of the four recombinant allergens, whereas all 16 ABPA patients showed reactivity to one or more of the allergens	- IgE levels were highest in ABPA patients, lesser in CCPA patients (by 10-folds) and barely detectable in SAFS patient - IgE against recombinant antigens are unlikely to be useful in differentiating these three diseases
Kraemer et al ³² (2006)	rAsp f1, f3, f4 and f6	CF	Specific IgE to rAsp f1 Specific IgE to rAsp f3 Specific IgE to rAsp f4 Specific IgE to rAsp f6 Specific IgE to rAsp f1 or f3 Specific IgE to rAsp f4 or f6	CF-ABPA (n = 16) 16/16 (100%) 16/16 (100%) 14/16 (86%) 15/16 (92%) 16/16 (100%) 13/16 (79%)	- rAsp f4 plus f6 IgE was better in predicting ABPA in CF patients than rAsp f1 plus f3 (marker of sensitization) - rAsp f4 had the highest significance in differentiating ABPA from CF
Kurup et al ³³ (2006)	rAsp f1-f4, f6	CF	IgA, A1, A2, IgG G1, G2, G3, G4 and IgE against rAsp antigens	CF-ABPA (n = 17) CF with <i>A. fumigatus</i> sensitization (n = 17) All rAsp antibodies showed strong reactivity in CF-ABPA and CF with <i>A. fumigatus</i> sensitization patients, making differentiation between these two groups difficult	- IgG4 was elevated in ABPA CF and CF patients consistently - Controls could be differentiated from sensitized individuals and ABPA - Differentiation between CF-ABPA and CF with <i>A. fumigatus</i> sensitization was not possible using any of the antibodies
de Oliveira et al ³⁴ (2007)	rAsp f1-f4, f6	Asthma	rAsp IgE f1-f4, f6	ABPA (n = 6) 2/6 (33.3%) (one showed IgE to rAsp f3 and other to all rAsp antigens)	Asthma with <i>A. fumigatus</i> sensitization (n = 59) 17/59 (28.8%) (one or the other recombinant antigens)

(Continues)

TABLE 2 (Continued)

Reference	Recombinant antigen	Disease	Test	Study population	CF without ABPA	Conclusion
Ringer et al ³⁵ (2007)	rAsp f1-f4, f6	CF	CAST	CF with ABPA (n = 8)	CF without ABPA (n = 19)	Combination of CAST, total IgE >500 and IgE to rAsp f4 and/or f6 is 100% specific for ABPA
				8/8 (100%)	5/19 (26.3%)	
			Specific IgE to rAsp f1	5/8 (62.5%)	0/19	
			Specific IgE to rAsp f2	8/8 (100%)	1/19 (5.3%)	
			Specific IgE to rAsp f3	8/8 (100%)	2/19 (10.5%)	
			Specific IgE to rAsp f4	4/8 (50%)	0/19	
			Specific IgE to rAsp f6	3/8 (38%)	0/19	
			Specific IgE to rAsp f1 and/or f3	8/8 (100%)	3/19 (15.8%)	
Latzin et al ³⁶ (2008)	rAsp f1, f3, f4, f6	CF	Specific IgE to rAsp f4 and/or f6	6/8 (75%)	0/19	TARC had superior diagnostic efficacy than rAsp f4 or f6 and all other markers which were compared
			TARC	CF-ABPA (n = 12)	CF without ABPA (n = 36)	
				12/12 (100%)	1/36 (2.8%)	
			Total IgE (1624 cut-off)	10/12 (83.3%)	2/36 (5.6%)	
			Specific IgE to rAsp f1	8/12 (63.6%)	0/36	
			Specific IgE to rAsp f3	7/12 (54.6%)	0/36	
			Specific IgE to rAsp f4	7/12 (54.5%)	3/36 (9.7%)	
			Specific IgE to rAsp f6	9/12 (72.7%)	0/36	
			A. fumigatus-specific IgG (crude)	11/12 (90.9%)	5/36 (13.8%)	
Glaser et al ³⁷ (2009)	rAsp f34	Asthma		ABPA (n = 64)	Asthma with A. fumigatus sensitization (n = 24)	Asp f34 is relevant for diagnosis of A. fumigatus sensitization and ABPA. Skin prick test to rAsp f34 was tested in six ABPA, six sensitized asthma and 8 healthy controls; positive in 6/6 (100%) ABPA and 3/6 (50%) A. fumigatus-sensitized individuals, while negative in all control subjects
			Specific IgE to rAsp f34	60/64 (94%)	11/24 (45.8%)	

(Continues)

TABLE 2 (Continued)

Reference	Recombinant antigen	Disease	Test	Study population	CF controls (n = 45)	Conclusion
Fricker-Hidalgo et al ³⁸ (2010)	rAsp f4, f6	CF		ABPA (n = 13)		rAsp f4 IgE can improve diagnosis of ABPA when used in combination with other tests such as <i>A. fumigatus</i> -specific IgG, precipitins and total IgE
			Specific IgE to rAsp f4	12/13 (92.3%)	2/45 (4.4%)	
			Specific IgE to rAsp f6	7/13 (53.8%)	3/45 (6.7%)	
			Specific IgE to rAsp f4 and/or f6	12/13 (92.3%)	NA	
			<i>A. fumigatus</i> -specific IgG (crude)	12/13 (92.3%)	3/45 (6.7%)	
Tanimoto et al ³⁹ (2015)	rAsp f1-4, f6	Asthma	Total IgE	9/13 (69.2%)	5/45 (11.1%)	- ABPA can be differentiated from sensitized asthma with IgE to rAsp f1 and/or f2. AUROC for IgE to rAsp f1 and f2 were 0.75 and 0.78, respectively - Combination of rAsp f1 and/or f2 IgE had a better sensitivity to diagnose ABPA from <i>A. fumigatus</i>-sensitized asthma than either alone - rAsp f6 IgE interpretation should take into consideration comorbid AD
			<i>A. fumigatus</i> cultured in sputum	7/13 (53.8%)	7/45 (15.6%)	
				ABPA (n = 53)	BA with AD (n = 111)	
			<i>A. fumigatus</i> -specific IgE (crude)	53/53 (100%)	111/111 (100%)	
			Specific IgE to rAsp f1	32/53 (60%)	20/111 (18%)**	
			Specific IgE to rAsp f2	36/53 (68%)	25/111 (23%)**	
			Specific IgE to rAsp f3	30/53 (57%)	15/111 (14%)**	
			Specific IgE to rAsp f4	19/53 (36%)	22/111 (20%)**	
			Specific IgE to rAsp f6	20/53 (38%)	62/111 (56%)**	
			<i>Alternaria alternata</i> specific IgE	36/53 (68%)	79/111 (71%)	
			<i>Candida albicans</i> specific IgE	46/53 (87%)	104/111 (94%)	
			<i>Cladosporium herbarum</i> specific IgE	33/53 (62%)	82/111 (74%)	
			<i>Penicillium chrysogenum</i> specific IgE	52/53 (98%)	85/111 (77%)**	
			<i>Trichophyton rubrum</i> specific IgE	41/53 (77%)	90/111 (84%)	
			Specific IgE to <i>Malassezia</i> Spp.	31/53 (59%)	104/111 (94%)**	

(Continues)

TABLE 2 (Continued)

Reference	Recombinant antigen	Disease	Test	Study population	Conclusion
Vitte et al ⁴⁰ (2016)	rAsp f1, f2, f3, f4, f6	CF and Asthma		<i>A. fumigatus</i> -sensitized CF (n = 55)	- Neither the level nor the prevalence of sIgG4 to recombinant antigens differentiated <i>A. fumigatus</i> CF from ABPA - sIgE to recombinant antigens rAsp f3, f4, and f6 were able to differentiate <i>A. fumigatus</i> CF from ABPA
			Specific IgE to crude antigens <i>A. fumigatus</i> (Cut-off 29.8 kU/L)	1/55 (1.8%)	
			Specific IgE to rAsp f1 (Cut-off 15.6 kU/L)	0/55	
			Specific IgE to rAsp f2 (Cut-off 7.38 kU/L)	2/55 (3.6%)	
			Specific IgE to rAsp f3 (Cut-off 4.79 kU/L)	2/55 (3.6%)	
			Specific IgE to rAsp f4 (Cut-off 3.78 kU/L)	2/55 (3.6%)	
			Specific IgE to rAsp f6 (Cut-off 5.5 kU/L)	3/55 (5.5%)	

ABPA, Allergic Bronchopulmonary Aspergillosis; AD, atopic dermatitis; *A. f.* *Aspergillus fumigatus*; AUROC, area under the receiver operating characteristic curve; CB, central bronchiectasis; CF, cystic fibrosis; DPC, Diagnostic Products Corporation, Los Angeles; ELISA, enzyme linked immunosorbent assay; IDT, intradermal test; IgG (G1,G2,G3,G4), Immunoglobulin G and their subclasses (G1,G2,G3,G4); NPV, negative predictive value; PPV, positive predictive value; rAsp, recombinant *Aspergillus fumigatus* antigens; RAST, radioallergosorbent test; ROC, Receiver operating characteristic; TARC, thymus- and activation-regulated chemokine.

All values in the table are provided as, the number of patients with positive result (n)/total number of patients (N) expressed as percentages. That is, (n/N, [%]).

*Statistically significant difference between the groups.

**Significant difference as compared to ABPA group.

	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Banerjee 1997	—	?	?	?	+	+	+
Crameri (1) 1996	—	?	?	+	?	?	+
Crameri 1996	?	?	?	?	+	+	+
Crameri 1998	—	?	?	?	?	?	?
Fricker-Hidalgo 2010	—	?	—	—	?	?	?
Hemmann (1) 1998	—	—	?	?	?	+	?
Hemmann 1998	—	?	?	?	?	?	?
Hemmann 1999	—	?	?	?	?	?	?
Knutsen 2004	?	—	?	?	?	+	?
Kraemer 2006	?	?	+	?	?	+	?
Kurup 2000	—	?	?	?	?	?	?
Latzin 2009	—	?	?	?	?	?	?
Moser 1994	—	—	?	?	?	?	?
Nikolaizik 1996	—	?	?	?	?	+	?
Nikolaizik 2002	?	?	+	?	?	?	?
Ringer 2007	—	?	?	?	?	?	?
Tanimoto 2015	+	?	+	+	+	?	+

High
 Unclear
 Low

FIGURE 2 Risk of Bias Assessment of the Studies Included Only in the Meta-Analysis, Using the Quality Assessment of Diagnostic Accuracy Studies-2 Tool

4 | DISCUSSION

The results of this study suggest that IgE against either rAsp f1 or f3 had the highest sensitivity (96.7% and 93.3% in asthma and CF, respectively), in differentiating patients with and without ABPA. On the other hand, IgE against either rAsp f4 or f6 had the highest specificity (99%) in asthma, while rAsp f6 alone had the highest specificity (98%) in CF, respectively, to distinguish patients with and without ABPA.

The demonstration of immunological responses against *A. fumigatus* is an essential component in the diagnostic criteria for ABPA. The diagnostic performance of the immunological tests however, depends on the quality of the antigen used. The widely available *Aspergillus* antigens are crude proteins, highly variable in their quality and show significant cross-reactivity with other fungi.⁴¹ Also, the batch-to-batch variability in the antigenic components, and the variability introduced by the strain of *A. fumigatus* used for antigen preparation are other factors contributing to the false-negative and

TABLE 3 Diagnostic accuracy of IgE against rAsp antigens (pooled sensitivities^a and specificities^a) for diagnosing ABPA in asthma and CF using composite reference criteria as the gold standard

rAsp antigen	Asthma		Cystic fibrosis	
	Pooled sensitivity (95% CI), references	Pooled specificity (95% CI), references	Pooled sensitivity (95% CI), references	Pooled specificity (95% CI), references
f1	80% (65.2-89.5)* 76.3% (61.3-86.7)** ^{18,19,23,27,39}	70.1% (46.7-88.6)* 79.3% (55.4-97)** ^{18,19,23,27,39}	78.9% (41.2-99.9) ^{25,30,32,35,36}	81.9% (48.8-99.9) ^{25,30,32,35,36}
f2	71.3% (38.9-93.7)* 74.9% (43.7-99.3)** ^{21,27,39}	84.3% (64.9-99.9)* 79.7% (51.1-98.5)** ^{21,27,39}	75.4% (27.9-99.9) ^{30,35}	75.4% (33.3-99.9) ^{30,35}
f3	84.8% (54.5-99.9)* 83.3% (52.2-99.9)** ^{23,24,27,39}	68% (38.5-89.7)* 69.5% (38.9-92)** ^{23,24,27,39}	85.8% (50-99.9) ^{25,30,32,35,36}	75.2% (40.6-98.8) ^{25,30,32,35,36}
f4	50.2% (16.1-78.2)* 48.7% (11.6-75.4)** ^{23,27,39}	92% (73.2-99.9)* 94.2% (77.8-99.9)** ^{23,27,39}	67.4% (41.4-83.9) ^{25,30,32,35,36,38}	92.2% (80-99.9) ^{25,30,32,35,36,38}
f6	42.6% (8-75.7)* 49.7% (10.8-84.4)** ^{23,27,39}	94.9% (70.1-99.9)* 89% (54.1-99.9)** ^{23,27,39}	51.3% (20-75.7) ^{25,30,32,35,36,38}	97.5% (87.3-99.9) ^{25,30,32,35,36,38}
f1 or f3 ^b	96.7% (87.6-99.2) ²³	50% (37.6-62.4) ²³	93.3% (55.2-99.9) ²⁵	54.8% (15-93.8) ^{25,32,35}
f4 or f6 ^c	89.3% (78.8-95) ²³	99.2% (88.2-99.9) ²³	57% (17.6-88.3) ²⁵	93.9% (68.8-99.9) ^{25,32,35}

CF, cystic fibrosis; CI, confidence interval; rAsp antigens, recombinant *A. fumigatus* antigens; ref, references.

Samples from same patients processed in Swiss lab* and US lab** in the study by Kurup et al.²⁷

^aThe efficiency of the test to distinguish individuals with ABPA from those without (*A. fumigatus* sensitization, asthma/CF without sensitization and/or healthy controls).

^bIgE positive to either f1 or f3 rAsp antigens.

^cIgE positive to either f4 or f6 rAsp antigens.

TABLE 4 Diagnostic accuracy of skin test using rAsp antigens (pooled sensitivities^a and specificities^a) for diagnosing ABPA in asthma and CF employing composite reference criteria as the gold standard

rAsp antigen	Asthma		Cystic fibrosis	
	Pooled sensitivity (95% CI), references	Pooled specificity (95% CI), references	Pooled sensitivity (95% CI), references	Pooled specificity (95% CI), references
f1	66.1% (21.1-99.9) ^{15,18}	78.1% (39.3-99.9) ^{15,18}	55.9% (10.8-95.6) ^{20,29}	66.4% (28.3-99.8) ^{20,29}
f2	–	–	–	–
f3	95.8% (57.5-99.7) ²⁴	57.7% (31.2-80.4) ²⁴	91.7% (58.7-98.8) ²⁹	68.4% (52.2-81.1) ²⁹
f4	80.8% (51.4-94.3) ²⁶	97.2% (67.8-99.8) ²⁶	96.2% (59.7-99.8) ²⁹	88.5% (74.2-95.3) ²⁹
f6	42.3% (19.6-68.8) ²⁶	97.2% (67.8-99.8) ²⁶	91.7% (58.7-98.8) ²⁹	68.4% (52.2-81.1) ²⁹
f1 or f3 ^b	–	–	–	–
f4 or f6 ^c	88.5% (58.3-97.7) ²⁶	97.2% (67.8-99.8) ²⁶	–	–

CF, cystic fibrosis; CI, confidence interval; rAsp antigens, recombinant *A. fumigatus* antigens; ref, references.

^aThe efficiency of the test to distinguish individuals with ABPA from those without (*A. fumigatus* sensitization, asthma/CF without sensitization and/or healthy controls).

^bSkin test positive to either f1 or f3 rAsp antigens.

^cSkin test positive to either f4 or f6 rAsp antigens.

false-positive results.⁴¹ Recently, the availability of recombinant technology has enabled pure forms of *A. fumigatus* antigens to be isolated in large quantities.⁴² The ability of these recombinant allergens to bind antibodies has been found to be similar to that of their naturally existing counterparts.^{24,28}

Recombinant *A. fumigatus* antigens have been classified into two functional groups: (a) secreted allergens (rAsp f1 and rAsp f3), which are recognized by antibodies from patients with both ABPA and *Aspergillus*-sensitized asthma; and (b) non-secreted allergens (rAsp f4 and rAsp f6), which are detected primarily in ABPA patients.^{23,43} The

results of our study are also concordant with these observations. A combination of rAsp f4 or f6 specific IgE had the highest specificity to diagnose ABPA, whereas, a combination of rAsp f1 and/or f3 specific IgE had the highest sensitivity to detect *Aspergillus* sensitization. Contrary to our results, Tanimoto et al found that the combination of rAsp f1 or f2 had a better diagnostic performance in asthmatics. In this study, a high prevalence of specific IgE against several non-*Aspergillus* fungi (*Malassezia* spp., *Trichophyton rubrum*, *Penicillium chrysogenum*, *Alternaria alternata*, *Candida albicans*) was seen in asthmatics with or without atopic dermatitis. They

TABLE 5 Summary of findings of studies reporting the diagnostic accuracy of IgE against rAsp antigens for diagnosing ABPA in asthma using composite reference criteria as the gold standard

Outcome		No. of studies (No. of patients)	Study design	Factors that may decrease certainty of evidence					Effect per 1000 patients tested				Test accuracy CoE
				Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	Pre-test probability of 2%	Pre-test probability of 5%	Pre-test probability of 10%		
IgE against rAsp f1													
True positives	5 studies (594 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Serious ^c	None	16 (13-18)	40 (33-45)	64 (35-83)	⊕○○○	Very low	
False negative								4 (2-7)	10 (5-17)	20 (10-35)			
True negative	5 studies (594 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Serious ^c	None	687 (458-868)	666 (444-842)	631 (420-797)	⊕○○○	Very low	
False positives								293 (112-522)	284 (108-506)	269 (103-480)			
IgE against rAsp f2													
True positives	3 studies (401 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Serious ^c	None	14 (8-19)	36 (19-47)	71 (39-94)	⊕○○○	Very low	
False negative								6 (1-12)	14 (3-31)	29 (6-61)			
True negatives	3 studies (401 patients)	Case-control type accuracy study	Serious ^d	Not serious	Serious ^b	Serious ^e	None	826 (627-979)	801 (608-949)	759 (576-899)	⊕○○○	Very low	
False positives								154 (1-353)	149 (1-342)	141 (1-324)			
IgE against rAsp f3													
True positives	4 studies (499 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Serious ^c	None	17 (11-20)	42 (28-50)	85 (56-100)	⊕○○○	Very low	
False negatives								3 (0-9)	8 (0-22)	15 (0-44)			
True negatives	4 studies (499 patients)	Case-control type accuracy study	Serious ^d	Not serious	Serious ^b	Serious ^b	None	666 (377-879)	646 (366-852)	612 (347-807)	⊕○○○	Very low	
False positives								314 (101-603)	304 (98-584)	288 (93-553)			
IgE against rAsp f4													
True positives	3 studies (476 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Not serious	None	10 (3-16)	25 (8-39)	50 (16-78)	⊕⊕○○	Low	
False negatives								10 (4-17)	25 (11-42)	50 (22-84)			
True negatives	3 studies (476 patients)	Case-control type accuracy study	Serious ^c	Not serious	Not serious	Not serious	None	902 (717-979)	874 (695-949)	828 (659-899)	⊕⊕⊕○	Moderate	
False positives								78 (1-263)	76 (1-255)	72 (1-241)			
IgE against rAsp f6													
True positives	3 studies (476 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Not serious	None	9 (2-15)	21 (4-38)	43 (6-76)	⊕⊕○○	Low	
False negatives								11 (5-18)	29 (12-46)	57 (24-92)			
True negatives	3 studies (476 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Not serious	None	930 (687-979)	902 (666-949)	854 (631-899)	⊕⊕○○	Low	
False positives								50 (1-293)	48 (1-284)	46 (1-269)			
IgE against rAsp f1 or f3													
True positives	1 studies (120 patients)	Case-control type accuracy study	Serious ^f	Not serious	Serious ^f	Not serious	None	19 (18-20)	48 (44-50)	97 (88-99)	⊕⊕○○	Low	
False negatives								1 (0-2)	2 (0-6)	3 (1-12)			
True negatives	1 studies (120 patients)	Case-control type accuracy study	Serious ^f	Not serious	Serious ^f	Not serious	None	490 (368-612)	475 (357-593)	450 (338-562)	⊕⊕○○	Low	
False positives								490 (368-612)	475 (357-593)	450 (338-562)			

(Continues)

TABLE 5 (Continued)

Outcome	No. of studies (No. of patients)	Study design	Factors that may decrease certainty of evidence					Effect per 1000 patients tested				Test accuracy CoE	
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	Pre-test probability of 2%	Pre-test probability of 5%	Pre-test probability of 10%			
IgE against rAsp f4 or f6													
True positives	1 studies (120 patients)	Case-control type accuracy study	Serious ^f	Not serious	Serious ^f	Not serious	None	18 (16-19)	45 (39-48)	89 (79-95)	⊕⊕⊕⊕	Low	
False negatives								2 (1-4)	5 (2-11)	11 (5-21)			
True negatives	1 studies (120 patients)	Case-control type accuracy study	Serious ^f	Not serious	Serious ^f	Not serious	None	972 (864-979)	942 (838-949)	893 (794-899)	⊕⊕⊕⊕	Low	
False positives								8 (1-116)	8 (1-112)	7 (1-106)			

^aCase-control study (possibly non-consecutive patients).^bInconsistency with regards to patient selection and applicability concerns of the index test of all the included studies.^cSmaller sample size and wide confidence intervals for the estimate.^dOne study had a good sample size, while the others were small studies.^eWide confidence intervals for the estimate.^fOnly one study was included for these analyses and had a case-control design.

postulated that several fungal proteins with similar functions are highly conserved across different species of fungi, and these may result in cross-reactivity amongst various fungi.³⁹ Asthmatics with *Alternaria* allergy may cross-react with *Aspergillus*, due to homologous allergens including manganese superoxide dismutase (MnSODs; Asp f6 and Alt a14), enolases (Asp f22 and Alt a6) and heat shock proteins (Asp f12 and Alt a3). *Malassezia* spp., antigens Mal f2 and Mal f3 share sequence homology with *A. fumigatus* Asp f3 antigen.⁴⁴ Thus, the presence of other fungi could explain the discordant results seen in this study. However, this being the only study demonstrating discordance, the observations require confirmation in future studies.

What are the implications for a clinician from the current study? It is often difficult to differentiate asthma with *A. fumigatus* sensitization from ABPA, especially severe asthma with fungal sensitization (SAFS).⁴⁵ However, this distinction is important as glucocorticoid therapy is warranted only in ABPA to prevent irreversible lung damage. The presence of immediate cutaneous hypersensitivity to *Aspergillus* antigens, raised total IgE and specific IgE against *A. fumigatus* are features that are common to both ABPA and SAFS. Although the sensitivity of *A. fumigatus*-specific IgE in the diagnosis of ABPA is 100%, the specificity is only 69%.⁴⁶ Serum precipitins (and recently *A. fumigatus*-specific IgG) and a total eosinophil count >500 cells/μL are seen in 29% (88% for *A. fumigatus*-specific IgG) and 66% of ABPA patients, respectively.^{47,48} Adding to the diagnostic conundrum, patients with SAFS may also manifest bronchiectasis on computed tomography.⁴⁹ Thus, differentiating SAFS and ABPA based only on clinical, radiological and commonly available immunological tests may be occasionally difficult. The diagnosis of ABPA in CF is also fraught with difficulty as CF lung disease and ABPA share several clinical characteristics including the presence of pulmonary infiltrates and bronchiectasis.⁵⁰ Moreover, the immunological tests performed using crude antigens in CF patients tend to vary over time.⁵¹ In all the above situations, it is possible that rAsp antigens may enable a precise diagnosis of ABPA. The use of rAsp antigens may also help in distinguishing cross-reactivity to other fungal allergens from true *Aspergillus* sensitization.

Our study has a few limitations. The studies included in the current review have mainly used the Rosenberg-Patterson criteria or the Nelson criteria for diagnosing ABPA in asthma and CF patients, respectively.^{9,10} Whether the results would be different if the recommendations of the ISHAM-ABPA working group are used for diagnosing ABPA remains unclear.² Most of the included studies had a high risk of bias, and the number of studies providing data for pooling the results for each antigen was few (Tables S2-S5). Almost all the studies have been performed in centres specializing in allergic diseases; whether less specialized centres can reproduce these results also remains to be seen. Several of the included studies are from the same group of researchers; although not mentioned explicitly, a possibility of overlap of samples cannot be excluded. Further, the quantitative analysis in our study was obtained from 17 studies, of which only one was conducted outside of Europe or the United States.³⁹ The strength of evidence generated from our review was

TABLE 6 Summary of findings of studies reporting the diagnostic accuracy of IgE against rAsp antigens for diagnosing ABPA in cystic fibrosis using composite reference criteria as the gold standard

		Factors that may decrease certainty of evidence					Effect per 1000 patients tested				
		Study design	Risk of bias			Publication bias	Pre-test probability of 2%	Pre-test probability of 5%	Pre-test probability of 10%	Test accuracy CoE	
Outcome	No. of studies (No. of patients)		Study design	Serious ^a	Not serious						Serious ^b
IgE against rAsp f1											
True positives	5 studies (247 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Serious ^c	None	16 (8-20) 4 (0-12)	39 (21-50) 11 (0-29)	79 (41-100) 21 (0-59)	⊕○○○ Very low
False negatives											
True negatives	5 studies (247 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Serious ^c	None	803 (478-979) 177 (1-502)	778 (464-949) 172 (1-486)	737 (439-899) 163 (1-461)	⊕○○○ Very low
False positives											
IgE against rAsp f2											
True positives	2 studies (97 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Serious ^c	None	15 (6-20) 5 (0-14)	38 (14-50) 12 (0-36)	75 (28-100) 25 (0-72)	⊕○○○ Very low
False negatives											
True negatives	2 studies (97 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Serious ^c	None	739 (326-979) 241 (1-654)	716 (316-949) 234 (1-634)	679 (300-899) 221 (1-600)	⊕○○○ Very low
False positives											
IgE against rAsp f3											
True positives	5 studies (247 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Serious ^c	None	13 (8-17) 7 (3-12)	34 (21-42) 16 (8-29)	67 (41-84) 33 (16-59)	⊕○○○ Very low
False negatives											
True negatives	5 studies (247 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Serious ^c	None	737 (398-968) 243 (12-582)	714 (386-939) 236 (11-564)	677 (365-889) 223 (11-535)	⊕○○○ Very low
False positives											
IgE against rAsp f4											
True positives	6 studies (308 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Serious ^c	None	13 (8-17) 7 (3-12)	34 (21-42) 16 (8-29)	67 (41-84) 33 (16-59)	⊕○○○ Very low
False negatives											
True negatives	6 studies (308 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Not serious	None	904 (784-979) 76 (1-196)	876 (760-949) 74 (1-190)	830 (720-899) 70 (1-180)	⊕⊕○○ Low
False positives											
IgE against rAsp f6											
True positives	6 studies (308 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Serious ^c	None	10 (4-15) 10 (5-16)	26 (10-38) 24 (12-40)	51 (20-76) 49 (24-80)	⊕○○○ Very low
False negatives											
True negatives	6 studies (308 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Not serious	None	956 (856-979) 24 (1-124)	926 (829-949) 24 (1-121)	878 (786-899) 22 (1-114)	⊕⊕○○ Low
False positives											
IgE against rAsp f1 or f3											
True positives	3 studies (129 patients)	Case-control type accuracy study	Serious ^d	Not serious	Serious ^d	Serious ^c	None	19 (11-20) 1 (0-9)	47 (28-50) 3 (0-22)	93 (55-100) 7 (0-45)	⊕○○○ Very low
False negatives											
True negatives	3 studies (129 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Serious ^c	None	537 (147-919) 443 (61-833)	521 (143-891) 429 (59-807)	493 (135-844) 407 (56-765)	⊕○○○ Very low
False positives											

(Continues)

(Continues)

TABLE 6 (Continued)

Outcome	No. of studies (No. of patients)	Study design	Factors that may decrease certainty of evidence					Effect per 1000 patients tested			Test accuracy CoE	
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	Pre-test probability of 2%	Pre-test probability of 5%	Pre-test probability of 10%		
IgE against rAsp f4 or f6												
True positives	3 studies (129 patients)	Case-control type accuracy study	Serious ^d	Not serious	Serious ^d	Serious ^c	None	11 (4-18)	28 (9-44)	57 (18-88)	⊕○○○	Very low
False negatives								9 (2-16)	22 (6-41)	43 (12-82)		
True negatives	3 studies (129 patients)	Case-control type accuracy study	Serious ^a	Not serious	Serious ^b	Serious ^c	None	920 (674-979)	892 (654-949)	845 (619-899)	⊕○○○	Very low
False positives								60 (1-306)	58 (1-296)	55 (1-281)		

^aCase-control study (possibly non-consecutive patients).^bInconsistency with regards to patient selection and applicability concerns of the index test of all the included studies.^cSmaller sample size and wide confidence intervals for the estimate.^dOnly one study (Case-control design) was included in the analysis.

low as per the GRADE approach. Hence, the utility of rAsp antigens requires confirmation in larger prospective studies. Finally, whether genetic and geographic variations could influence the pattern of antibody response against rAsp antigens also remains to be explored.

In conclusion, rAsp antigens appear to have a promising role in the diagnosis of ABPA. A combination of rAsp antigens (rAsp f1, f2, f3, f4, f6) may provide better diagnostic performance than individual antigens. However, as the quality of currently available evidence is low, their exact utility in the diagnostic algorithm of ABPA needs to be elucidated on larger scale studies from different parts of the globe.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

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Supplemental table 1: Assessment of quality of the studies included in the systematic review using the QualSyst tool

	Criterion	Moser et al. 1994 (1)	Nikolaizik et al. 1995 (2)	Banerjee et al. 1996 (3)	Crameri et al. 1996 (4)	Crameri et al. 1996 (5)	Nikolaizik et al. 1996 (6)	Banerjee et al. 1997 (7)	Banerjee et al. 1998 (8)	Crameri et al. 1998 (9)
1	Question / objective sufficiently described?	2	1	1	1	1	1	1	1	1
2	Study design evident and appropriate?	1	2	2	1	1	1	1	1	1
3	Context for the study clear?	1	1	1	2	1	1	1	1	1
4	Connection to a theoretical framework /wider body of knowledge?	1	1	1	1	1	1	1	1	1
5	Sampling strategy described, relevant and justified?	1	1	0	1	1	1	0	1	1
6	Data collection methods clearly described and systematic?	1	1	0	1	1	1	1	0	1
7	Data analysis clearly described and systematic?	1	1	1	1	1	1	0	1	1
8	Use of verification procedure(s) to establish credibility?	0	1	1	1	1	1	1	1	1
9	Conclusions supported by the results?	1	1	1	1	1	1	1	1	1
10	Reflexivity of the account?	1	1	1	1	1	1	1	1	1
	Total	10	11	9	11	10	10	8	9	10

	Criterion	Hemmann et al. 1998 (10)	Hemmann et al. 1998 (11)	Hemmann et al. 1999 (12)	Kurup et al. 2000 (13)	Banerjee et al. 2001 (14)	Nikolaizik et al. 2002 (15)	Knutsen et al. 2004 (16)	Bowyer et al. 2006 (17)	Kraemer et al. 2006 (18)
1	Question / objective sufficiently described?	2	2	2	2	2	2	1	2	2
2	Study design evident and appropriate?	2	1	1	1	1	1	1	1	1
3	Context for the study clear?	1	1	1	2	1	1	2	1	1
4	Connection to a theoretical framework /wider body of knowledge?	1	1	1	1	1	1	1	1	1
5	Sampling strategy described, relevant and justified?	1	1	1	1	1	1	1	1	1
6	Data collection methods clearly described and systematic?	1	1	1	2	2	1	1	1	2
7	Data analysis clearly described and systematic?	1	1	1	2	1	2	1	1	1
8	Use of verification procedure(s) to establish credibility?	1	1	1	1	1	1	1	1	1
9	Conclusions supported by the results?	1	1	1	1	1	1	1	1	1
10	Reflexivity of the account?	1	1	1	1	1	1	1	1	1
	Total	12	11	11	14	12	12	11	11	12

	Criterion	Kurup et al. 2006 (19)	de Oliveira et al. 2007 (20)	Ringer et al. 2007 (21)	Latzin et al. 2008 (22)	Glaser et al. 2009 (23)	Fricker- Hidalgo et al et al. 2010 (24)	Tanimoto et al. 2015 (25)	Vitte et al. 2016 (26)
1	Question / objective sufficiently described?	1	1	1	1	1	1	2	1
2	Study design evident and appropriate?	1	1	1	1	1	1	1	1
3	Context for the study clear?	1	1	1	1	1	1	1	1
4	Connection to a theoretical framework /wider body of knowledge?	1	1	1	1	1	1	1	1
5	Sampling strategy described, relevant and justified?	1	1	1	1	1	1	2	1
6	Data collection methods clearly described and systematic?	1	1	1	1	1	1	2	1
7	Data analysis clearly described and systematic?	1	1	2	1	1	1	1	1
8	Use of verification procedure(s) to establish credibility?	1	1	1	1	1	1	1	1
9	Conclusions supported by the results?	0	1	1	1	1	1	1	1
10	Reflexivity of the account?	1	1	1	1	1	1	1	1
	Total	9	10	11	10	10	10	13	10

Supplemental table 2: Raw data used for calculating pooled sensitivity and specificity of IgE against rAsp antigens for diagnosing ABPA in asthma using composite reference standard as the gold standard

Antigen (n)*	Reference	TP	FN	FP	TN
f1 (5)	Crameri et al.(4) 1996	30	0	10	19
	Crameri et al.(9) 1998	50	10	18	42
	**Kurup et al.(13) 2000	20	4	14	12
		16	8	0	26
	Tanimoto et al.(25) 2015	32	21	45	208
f2 (3)	Crameri et al.(5) 1996	24	6	5	24
	Banerjee et al.(7) 1997	24	1	0	20
	**Kurup et al.(13) 2000	21	3	1	25
		21	3	6	20
	Tanimoto et al.(25) 2015	36	17	48	205
f3 (4)	Crameri et al.(9) 1998	55	5	21	39
	Hemmann et al.(10) 1998	11	0	5	7
	**Kurup et al.(13) 2000	20	4	7	19
		19	5	5	21
	Tanimoto et al.(25) 2015	30	23	53	200
f4 (3)	Crameri et al.(9) 1998	48	12	0	60
	**Kurup et al.(13) 2000	22	2	0	26
		15	9	3	23
	Tanimoto et al.(25) 2015	19	34	26	227
f6 (3)	Crameri et al.(9) 1998	33	27	0	60
	**Kurup et al.(13) 2000	14	10	0	26
		16	8	4	22
	Tanimoto et al.(25) 2015	20	33	75	178
f1 or f3 (1)	Crameri et al.(9)1998	58	2	30	30
f4 or f6 (1)	Crameri et al.(9) 1998	54	6	0	60

*The number of studies included for each analysis are provided in parentheses as (n)

**In this study same samples were tested at two different labs (Swiss and US labs). TP, TN, FN and FP are provided separately for both and were utilized separately for pooling the results

FN – false negative; FP – false positive; TN – true negative; TP – true positive

Supplemental table 3: Raw data used for calculating pooled sensitivity and specificity of IgE against rAsp antigens for diagnosing ABPA in cystic fibrosis using composite reference standard as the gold standard

Antigen (n)*	Reference	TP	FN	FP	TN
f1 (5)	Hemmann et al.(11) 1998	20	0	12	18
	Knutsen et al.(16) 2004	24	4	13	29
	Kraemer et al.(18) 2006	16	0	5	31
	Ringer et al.(21) 2007	5	3	0	19
	Latzin et al.(22) 2008	8	4	0	36
f2 (2)	Knutsen et al.(16) 2004	21	7	10	32
	Ringer et al.(21) 2007	8	0	1	18
f3 (5)	Hemmann et al.(11) 1998	20	0	19	11
	Knutsen et al.(16) 2004	22	6	7	35
	Kraemer et al.(18) 2006	16	0	5	31
	Ringer et al.(21) 2007	8	0	2	17
	Latzin et al.(22). 2008	7	5	0	36
f4 (6)	Hemmann et al.(11) 1998	16	4	0	30
	Knutsen et al.(16) 2004	23	5	9	33
	Kraemer et al.(18) 2006	14	2	0	36
	Ringer et al.(21). 2007	4	4	0	19
	Latzin et al. 2008	7	5	3	33
	Fricker-Hidalgo et al.(24) 2010	12	1	3	45
f6 (6)	Hemmann et al.(11) 1998	14	6	0	30
	Knutsen et al.(16) 2004	7	21	0	42
	Kraemer et al.(18) 2006	15	1	5	31
	Ringer et al.(21) 2007	3	5	0	19
	Latzin et al.(22) 2008	9	3	0	36
	Fricker-Hidalgo et al.(24) 2010	7	6	4	44
f1 or f3 (3)	Hemmann et al.(11) 1998	20	0	19	11
	Kraemer et al.(18) 2006	16	0	0	36
	Ringer et al.(21) 2007	8	0	3	16
f4 or f6 (3)	Hemmann et al.(11) 1998	20	0	0	30
	Kraemer et al.(18) 2006	13	3	0	36
	Ringer et al.(21) 2007	6	2	0	19

*The number of studies included for each analysis are provided in parentheses as (n)

FN – false negative; FP – false positive; TN – true negative; TP – true positive

Supplemental table 4: Raw data used for calculating pooled sensitivity and specificity of skin prick test using rAsp antigens for diagnosing ABPA in asthma using composite reference standard as the gold standard

Pooled analysis (n)*	Reference	TP	FN	FP	TN
f1 (2)	Moser et al.(1) 1994	8	7	5	18
	Crameri et al.(4) 1996	24	6	5	24
f3 (1)	Hemmann et al.(10) 1998	11	0	5	7
f4 (1)	Hemmann et al.(12) 1999	10	20	0	17
f6 (1)	Hemmann et al.(12) 1999	5	7	0	17
f4 or f6 (1)	Hemmann et al.(12) 1999	11	1	0	17

*The number of studies included for each analysis are provided in parentheses as (n)

FN – false negative; FP – false positive; TN – true negative; TP – true positive

Supplemental table 5: Raw data used for calculating pooled sensitivity and specificity of skin prick test using rAsp antigens for diagnosing ABPA in cystic fibrosis using composite reference standard as the gold standard

Pooled analysis (n)*	Reference	TP	FN	FP	TN
f1 (2)	Nikolaizik et al.(6) 1996	3	3	13	25
	Nikolaizik et al.(15) 2002	8	4	12	26
f3 (1)	Nikolaizik et al.(15) 2002	11	1	12	26
f4 (1)	Nikolaizik et al.(15) 2002	12	0	4	34
f6 (1)	Nikolaizik et al.(15) 2002	11	1	12	26

*The number of studies included for each analysis are provided in parentheses as (n)

FN – false negative; FP – false positive; TN – true negative; TP – true positive

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