

REVIEW

Prevalence of *Aspergillus* sensitization and allergic bronchopulmonary aspergillosis in cystic fibrosis: systematic review and meta-analysis

V. N. Maturu and R. Agarwal

Department of Pulmonary Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Clinical & Experimental Allergy

Summary

Background The prevalence of *Aspergillus* sensitization (AS) and allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis (CF) has been varying reported. The aim of this systematic review was to estimate the overall prevalence of AS/ABPA in CF.

Methods We searched the PubMed and EmBase databases for studies reporting the prevalence of AS/ABPA in CF. We calculated the proportion with 95% confidence interval (CI) to assess the prevalence of AS and ABPA in the individual studies and then pooled the results using a random effects model. Statistical heterogeneity was assessed using the I^2 test while publication bias was assessed using both graphical and statistical methods.

Results Our search yielded 64 eligible studies. The pooled prevalence of AS was 39.1% (95% CI: 33.3–45.1) and was higher with skin test compared to specific IgE (43.8% vs. 32.8%, $P = 0.002$); however, the prevalence did not vary with the type of skin test used (intradermal or percutaneous). The prevalence of ABPA was 8.9% (95% CI: 7.4–10.7) and was higher in adults as compared to children (10.1% vs. 8.9%, $P < 0.0001$). There was a wide variation in the criteria used for diagnosing ABPA. Almost 50% (12/23) of the publications after 2004 used criteria other than the CF foundation criteria for diagnosing ABPA. There was significant statistical heterogeneity and evidence of publication bias.

Conclusions There is a high prevalence of AS and ABPA in patients with CF. Despite six decades of research, there is still a need to adopt uniform methodology and criteria for the diagnosis of AS/ABPA.

Keywords allergic bronchopulmonary aspergillosis, *Aspergillus*, fungal sensitization, meta-analysis, prevalence

Submitted 5 May 2015; revised 8 June 2015; accepted 3 July 2015

Correspondence: Ritesh Agarwal, Department of Pulmonary Medicine, Postgraduate Institute of Medical Education and Research, Sector-12, Chandigarh-160012, India.

E-mail: agarwal.ritesh@outlook.in

Cite this as: V. N. Maturu and R.

Agarwal. *Clinical & Experimental Allergy*, 2015 (45) 1765–1778.

Introduction

Allergic bronchopulmonary aspergillosis (ABPA) is an allergic pulmonary manifestation of *Aspergillus fumigatus* characterized by the presence of elevated total and *A. fumigatus*-specific IgE levels, and radiologic infiltrates, with or without bronchiectasis [1, 2]. It usually complicates the course of patients with asthma and cystic fibrosis (CF). *Aspergillus* sensitization (AS) is defined by the presence of immediate skin test positivity to *Aspergillus* antigens or elevated serum *A. fumigatus*-specific IgE levels. The prevalence of AS in CF has been variably reported from as low as 20% to as high as 65%. This variation could be due to the differences in the methods used for diagnosing AS (cutaneous tests vs. specific IgE level or both), type of skin test used

(percutaneous vs. intradermal or both), varying cut-offs used for defining elevated *A. fumigatus*-specific IgE levels, diversity in the age group and ethnicity of the population screened. Similarly, the quoted prevalence of ABPA in CF varies widely from 3% to 25%. This again could be due to the different diagnostic criteria used, differences in the age group of population and varying cut-offs used for defining elevated IgE and specific IgE levels. Although a recent study did estimate the burden of different manifestations of aspergillosis in CF, it was based on mathematical assumptions and universalized prevalence rates generated at a single centre using novel methods, not yet validated externally [3]. To the best of our knowledge, no study has systematically reviewed the prevalence rates of AS/ABPA in CF, and the factors that might influence the prevalence rates.

We have previously performed a systematic review and meta-analysis and calculated the pooled prevalence of AS and ABPA in patients with bronchial asthma [4]. A similar study that systematically measures the prevalence of AS/ABPA in CF will provide the clinicians the magnitude of problem, further strengthening the management practices for AS/ABPA. These prevalence values can be further used for the estimation of disease burden and may also help in estimating the sample size required in conducting future epidemiological studies. The aim of this study was to generate a large database, thereby enhancing the sample size for determination of the prevalence of AS/ABPA in patients with CF, using a systematic review methodology.

Methods

Search strategy

We searched the PubMed and EmBase databases (till January 2015) using the following free text terms: ('ABPA' OR 'allergic bronchopulmonary aspergillosis' OR 'allergic bronchopulmonary mycosis') AND ('CF' OR 'cystic fibrosis'). We reviewed the reference lists of reviews and editorials on this topic. We also reviewed the references from the primary studies identified during the search. In addition, we searched our personal libraries.

Inclusion and exclusion criteria

We included studies reporting the prevalence of AS and/or ABPA. *Aspergillus* sensitization was defined as the presence of either an immediate (type 1) cutaneous reaction to *A. fumigatus* antigen (commercial or locally prepared) or the presence of an elevated IgE against *A. fumigatus* in the serum. For articles that described the prevalence of AS using both skin testing and specific IgE, the prevalence of *A. fumigatus*-specific IgE was taken as the reference for AS, in calculating the overall prevalence. We excluded the following studies: (a) abstracts, editorials, case reports and studies that included less than 10 patients; (b) studies describing the prevalence of AS/ABPA in diseases other than CF; (c) studies in which the number of patients with CF screened (i.e. the denominator) was not reported; and (d) studies not in English language. Any disagreement between the authors was resolved by consensus.

We also performed a sensitivity analysis decided *a priori* based on (a) age group of the study population included (children: mean/median age of the study population ≤ 16 years vs. adults); (b) type of study (registry vs. non-registry data); and (c) decade of publication.

Data extraction

The database created from the electronic searches was compiled in a reference manager package, and all duplicate citations were eliminated. The citations were first screened by the authors to capture the relevant studies. The full text of each citation was obtained and reviewed in detail. Data were recorded on a standard data extraction sheet. The following items were extracted: (a) publication details (title, authors and other citation details including geographic area); (b) type of study (prospective or retrospective); (c) age group of the study population; (d) details of the criteria used for diagnosis of ABPA; (e) method used for skin test (skin prick test or intradermal injection); and (f) prevalence of AS or ABPA in patients with CF.

Meta-analysis

The statistical software package (StatsDirect, version 2.7.9 for MS-Windows, StatsDirect Ltd. (www.statsdirect.com), England, 2013) was used to perform all the statistical analysis. We calculated the prevalence by calculating proportion with 95% confidence intervals (CIs) for each study and then pooled the data to derive a summary proportion with 95% CI, using DerSimonian weights for the random effects model in the presence of significant heterogeneity [5]. The impact of heterogeneity on the pooled estimates of the individual outcomes of the meta-analysis was assessed using the I^2 test. An I^2 value of more than 50% indicates significant heterogeneity. Publication bias was assessed using the funnel plot and three statistical methods (Egger's test, Harbord's test and Begg-Mazumdar's test).

As this was a meta-analysis of published studies, institutional review board clearance was not required for the study.

Results

Our initial database search retrieved a total of 667 citations, of which 64 were finally included in the systematic review (Fig. 1) [6–69]. Most studies were from the developed countries with only three studies from the developing world (Table 1). Forty-four studies included children, and 24 studies included adults. The mean/median age was unknown in eleven studies. Forty-one studies (3362 patients) described the prevalence of AS in patients with CF [6–23, 25–27, 29, 30, 32–37, 40, 42, 46, 48, 49, 52, 53, 62, 64–66, 69], while 45 studies (35 308 patients) reported the prevalence of ABPA in CF [15, 16, 19, 22, 24–26, 28, 29, 31–36, 38, 39, 41–64, 66–69]. Although the prevalence of ABPA and AS varied over the last six decades, neither the prevalence of AS nor that of ABPA could be shown to be consistently

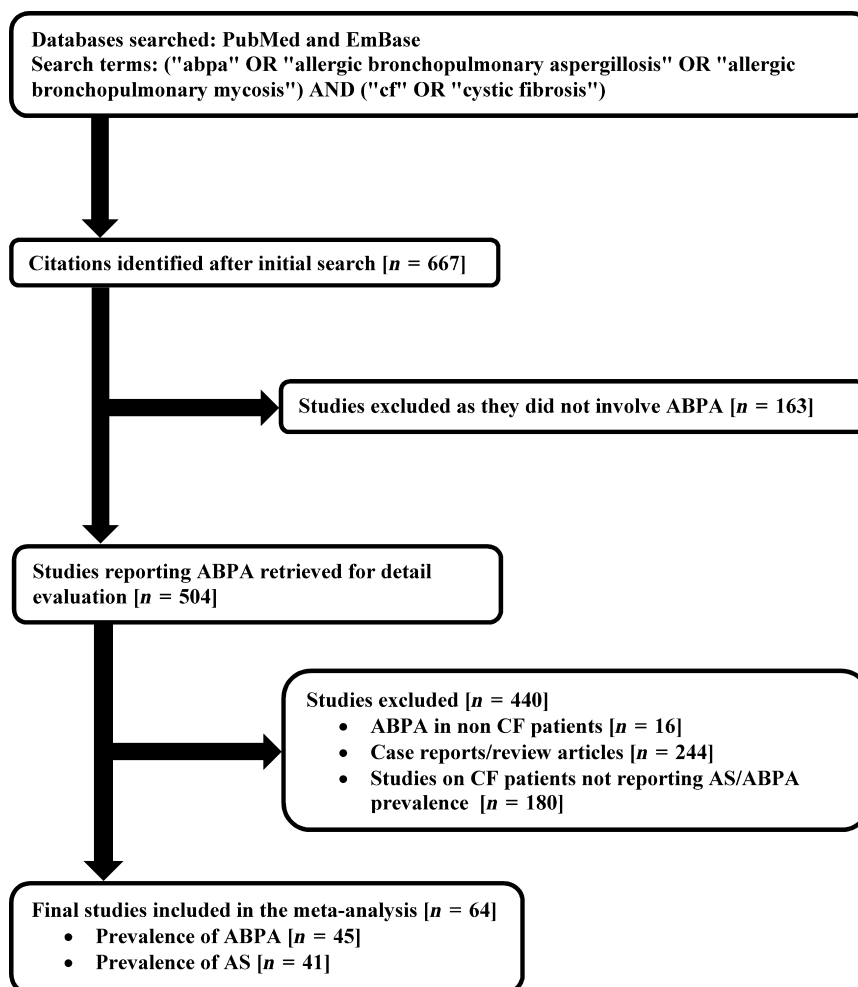


Fig. 1. Citation selection process for the systematic review.

increasing or decreasing over the last half a century (Tables 2 and 3).

Prevalence of AS in cystic fibrosis

Of the 41 studies reporting AS, 34 studies reported the results of skin testing and 15 studies reported the results of *A. fumigatus*-specific IgE levels. The skin test was performed by the percutaneous (prick/scratch/epicutaneous) technique in 22 studies, by the intradermal method in five and both (percutaneous followed by intradermal) in five studies. The method was not mentioned in two studies.

The prevalence of AS in patients with CF varied from 20% to 65%, with a pooled prevalence of 39.1% (95% CI: 33.3–45.1) (Fig. 2). The prevalence of AS using cutaneous testing was higher when compared with *A. fumigatus*-specific IgE (42.8% vs. 32.8%, $P = 0.0002$). The pooled prevalence of AS was similar when skin test was carried out with an intradermal method or the percutaneous method. However, the prevalence of AS was

significantly higher when both methods were used compared to single method (percutaneous/intradermal) alone (46.5% vs. 44.3%, $P = 0.021$). The prevalence of AS was not different between children and adults (41.6% vs. 36.1%) (Table 2).

Significant clinical heterogeneity was reflected in the antigen used for skin testing (locally prepared vs. commercially available), the method used for skin testing (prick vs. intradermal) and the cut-offs used for *A. fumigatus*-specific IgE (Table 1). There was also significant statistical heterogeneity (I^2 92%, 95% CI: 90.4–93.2). There was evidence of publication bias on visual examination of the funnel plot (Fig. 3) and on the statistical tests (Egger bias = 6.73, $P < 0.0001$; Harbord bias = 4.29, $P = 0.0076$; Begg-Mazumdar Kendall's tau = 0.17, $P = 0.1136$).

Prevalence of ABPA in CF

The prevalence of ABPA in patients with CF varied from 3% to 25%, with a pooled prevalence of 8.9%

Table 1. Studies describing the prevalence of *Aspergillus* sensitization and/or ABPA in patients with cystic fibrosis

Study (year) reference	Country, type of study	Age group	Type of skin test	Criteria used for diagnosis of ABPA	Prevalence of positive AST	Prevalence of elevated Af IgE	Prevalence of ABPA
Kulczycki et al. (1961) [6]	USA, Prospective	Children	Scratch		22/266		
Mearns et al. (1967) [7]	UK, Prospective	Children	NA		33/86		
Allan et al. (1975) [8]	UK, Prospective	Children	Prick		11/30		
Bardana et al. (1975) [9]	USA, Prospective	NA	Intradermal		11/18		
Counahan et al. (1975) [10]	UK, Prospective	Children	Prick		8/23		
Warren et al. (1975) [11]	UK, Prospective	NA	Prick		25/43		
Warner et al. (1976) [12]	UK, Prospective	Children	Prick		41/123		
Silverman et al. (1978) [13]	UK, Prospective	Children	Prick		17/48		
Carswell et al. (1979) [14]	UK, Prospective	Children	Prick		39/44		
Nelson et al. (1979) [15]	USA, Prospective	Children	Scratch and intradermal	Major (R/W/C/T/I/S/P)	18/46		5/46
Ormerod et al. (1980) [16]	UK, Prospective	Children	Prick	NA	30/48		7/48
Van Asperen et al. (1980) [17]	Australia, Prospective	Children	Prick		22/78		
Clarke et al. (1981) [18]	Australia, Retrospective	Children	Prick		13/22		
Laufer et al. (1984) [19]	USA, Prospective	Children	Prick and Intradermal	Major (R/W/E/T/P/I/B)	53/100		10/100
Schonheyder et al. (1985) [20]	Denmark, Prospective	Children	Prick test		26/59		
Wonne et al. (1985) [21]	Germany, Prospective	Children	Intradermal		13/31		
Feanny et al. (1988) [22]	Canada, Prospective	Adults	Prick and intradermal	Major (T/S/P/E/I/W)	36/117		12/117
Forsyth et al. (1988) [23]	Australia, Prospective	NA				9/47	
Schonheyder et al. (1988) [24]	Denmark, Retrospective	Children		Major (R/W/E/T/I/S/P/C)			10/200
Zeaske et al. (1988) [25]	USA, Prospective	Children	Prick and Intradermal	Laufer's criteria	44/75	38/75	10/75
Knutsen et al. (1990) [26]	USA, Prospective	Children	Intradermal	Laufer's criteria	35/73		9/73
Nicolai et al. (1990) [27]	Germany, Prospective	Children				68/148	
Simmonds et al. (1990) [28]	USA, Prospective	Children		Major (R/I/S/P/E/C)			8/137
Hutcheson et al. (1991) [29]	USA, Prospective	NA	Epicutaneous	Laufer's criteria	24/75	16/75	4/79
El-Dahr et al. (1994) [30]	USA, Prospective	Children	Prick		15/50	30/147	
Marchant et al. (1994) [31]	UK, Retrospective	Children		R-P criteria			11/160
Mroueh et al. (1994) [32]	USA, Retrospective	Children	Intradermal	Major (W/R/I/E/T)	38/87		15/236
Nikolaizik et al. (1995) [33]	UK, Prospective	Adults	Prick	Nelson criteria	37/55		10/55
Becker et al. (1996) [34]	USA, Prospective	Adults	Intradermal	Greenberger criteria	15/51	12/53	1/53
Hutcheson et al. (1996) [35]	USA, Prospective	NA	Epicutaneous	Laufer's criteria	47/112	60/112	6/118
Nikolaizik et al. (1996) [36]	Switzerland, Prospective	Adults	Prick	Nelson criteria	27/44		6/44
Wojnarowski et al. (1997) [37]	Austria, Prospective	Children	Prick			31/118	
Geller et al. (1999) [38]	USA & Canada, Retrospective	Adults		Major (T/P/I) Minor (W/E/R/S/ C/SR/G)			281/1421

Table 1 (continued)

Study (year) reference	Country, type of study	Age group	Type of skin test	Criteria used for diagnosis of ABPA	Prevalence of positive AST	Prevalence of elevated Af IgE	Prevalence of ABPA
Nepomuceno et al. (1999) [39]	USA Retrospective	Adults		Major (T/E/I/P/SR) Minor (W/B/R/C/F)			16/172
Torres-Lozano et al. (1999) [40]	Mexico, Prospective	Children	Prick		7/30		
Cimion et al. (2000) [41]	France, Prospective	NA		R-P Criteria			5/128
Henry et al. (2000) [42]	Ireland, Retrospective	Adults	Prick	Greenberger criteria	9/21		1/21
Mastella et al. (2000) [43]	Europe (9 countries), Retrospective	Children		Major (T/P/I) Minor (W/R/E/C/S/G)			967/12447
Skov et al. (2000) [44]	Denmark, Retrospective	Adults		Nelson criteria			26/238
Taccetti et al. (2000) [45]	Italy, Retrospective	NA		R-P criteria			171/3089
Maiz et al. (2002) [46]	Spain, Prospective	Children	Prick and intradermal	Nelson Criteria	34/66	49/76	10/76
Casaulta et al. (2005) [47]	Switzerland, Prospective	Children		Nelson criteria			11/74
Ritz et al. (2005) [48]	Switzerland, Retrospective	Children		Major (W/I/S/G/C/rAspf1, 4 and 6)		20/160	11/160
Skov et al. (2005) [49]	Australia, Prospective	Children	Prick	CF foundation criteria	30/88		13/277
Almeida et al. (2006) [50]	Brazil, Prospective	Children		CF foundation criteria			3/32
Kraemer et al. (2006) [51]	Switzerland, Retrospective	Children		Nelson criteria			16/122
Barton et al. (2008) [52]	UK, Prospective	Adults		Major (W/I/S) Minor (R/G/M/A)		28/87	7/87
Carneiro et al. (2008) [53]	Brazil, Prospective	Adults	NA	Major (T/I/P) Minor (W/E/S/G/R/C/SR)	19/74	2/74	2/74
Chotirmall et al. (2008) [54]	Ireland, Prospective	Adults		CF foundation criteria			6/50
Latzin et al. (2008) [55]	Switzerland, Prospective	Children		Nelson criteria			12/48
Rapaka et al. (2008) [56]	USA, Prospective	NA		CF foundation criteria			31/440
Simmonds et al. (2009) [57]	UK, Retrospective	Adults		NA			11/112
Amin et al. (2010) [58]	USA, Retrospective	Children		W/I/SR			6/230
Jubin et al. (2010) [59]	France, Retrospective	Children		CF foundation criteria			8/85
Paugam et al. (2010) [60]	France, Retrospective	Adults		CF foundation criteria			18/201
Mortensen et al. (2011) [61]	Denmark, Prospective	Adults		CF foundation criteria			24/287
Fillaux et al. (2012) [62]	France, Prospective	Children		Major (I/S/P)		102/251	34/251
Sequeiros et al. (2012) [63]	UK, Prospective	Adults		Major (W/I/S/P)			9/58
Baxter et al. (2013) [64]	UK, Prospective	Adults	Prick	NA	75/115	42/146	23/146
Baxter et al. (2013) [65]	UK, Prospective	Adults	Prick	NA	33/55	29/55	
Fillaux et al. (2014) [66]	France, Prospective	Children		CF Foundation criteria		50/117	4/117
Noni et al. (2014) [67]	Greece, Retrospective	Children		CF Foundation criteria			18/121

Table 1 (continued)

Study (year) reference	Country, type of study	Age group	Type of skin test	Criteria used for diagnosis of ABPA	Prevalence of positive AST	Prevalence of elevated <i>Af</i> IgE	Prevalence of ABPA
Parize et al. (2014) [68]	France, Retrospective	Children		NA			28/373
Sharma et al. (2014) [69]	India, Prospective	Children	Prick	CF Foundation criteria	14/41		6/41

A, elevated *A. fumigatus*-specific IgA level; ABPA, allergic bronchopulmonary aspergillosis; B, bronchiectasis; CF, cystic fibrosis; E, elevated eosinophils; F, low FEV1; G, elevated *A. fumigatus*-specific IgG level; I, elevated total IgE level; M, elevated *A. fumigatus*-specific IgM level; NA, not available; R, radiologic infiltrates; R-P, Rosenberg–Patterson criteria; S, elevated *A. fumigatus*-specific IgE level; SR, steroid responsiveness; T, immediate skin test positivity; UK, United Kingdom; USA, United states of America; W, wheezing.

Table 2. Pooled prevalence of *Aspergillus* sensitization (AS) stratified according to subgroups (random effects model)

Subgroup	Number of studies	<i>n/N</i>	Pooled prevalence (%) (95% CI)	<i>P</i> value
Method used for determining AS				
Skin testing	32	921/2294	43.8 (37.1–50.6)	0.0002
Specific IgE levels	15	555/1623	32.8 (24.2–42.0)	
Method used for skin testing				
Percutaneous (prick, scratch)	22	572/1470	44.3 (34.5–54.3)	0.205
Intradermal	5	112/260	43.1 (37.2–49.1)*	
Method used for skin testing				
Single method	27	684/1730	44.3 (36.0–52.7)	0.021
Both methods	5	185/404	46.5 (35.7–57.5)	
Age				
Children	27	849/2273	41.6 (34.6–48.7)	0.123
Adults	9	222/652	36.1 (22.9–50.4)	
Decade of study				
< 1980	12	277/853	43.0 (28.5–58.2)	< 0.0001
1981–1990	9	291/672	43.1 (35.8–50.6)	
1991–2000	10	267/742	37.7 (27.0–49.0)	
2001–2010	5	129/485	26.5 (9.2–48.9)	
2011–2015	5	237/610	39.4 (32.2–46.8)	

*Pooled prevalence determined by fixed effects model as the I^2 value was < 50%.

P value determined by two proportion analysis or chi-square test.

(95% CI: 7.4–10.7) (Fig. 4). The prevalence of ABPA was significantly higher in adults when compared to children (10.1% vs. 8.9%, $P < 0.0001$) (Table 3). Of the 45 studies reporting the prevalence of ABPA, two presented data from the CF registries namely the Epidemiologic Study of Cystic Fibrosis (ESCF) registry from the United States and Canada (ABPA prevalence of 2%), and the Epidemiologic Registry of Cystic Fibrosis (ERCF) from Europe (ABPA prevalence of 7.8%). The prevalence of ABPA calculated after excluding data from these CF registries was similar to the overall

Table 3. Pooled prevalence of ABPA stratified according to subgroups (random effects model)

Subgroup	Number of studies	<i>n/N</i>	Pooled prevalence (%) (95% CI)	<i>P</i> value
Age				
Children	27	1523/29936	8.9 (6.7–11.3)	< 0.0001
Adults	15	171/1715	10.1 (8.7–11.6)*	
Type of study				
Registry based	2	1248/26657	4.4 (5.4–11.8)	< 0.0001
Not registry based	43	654/8644	9.1 (7.8–10.4)	
Decade of study				
< 1990	8	71/796	9.0 (7.1–11.1)*	< 0.0001
1991–2000	15	1520/31050	7.2 (4.8–9.9)	
2001–2010	15	165/2068	9.0 (6.8–11.6)	
2011–2015	7	146/1394	10.6 (7.4–14.3)	
Criteria used for diagnosed				
CFF consensus criteria	10	131/1651	8.5 (6.5–10.8)	< 0.0001
Nelson criteria	8	96/703	13.9 (11.4–16.5)*	
R-P criteria	8	226/3822	7.2 (5.3–9.3)	
Other criteria	19	1449/29132	7.6 (5.3–10.4)	

*Pooled prevalence determined by fixed effects model as the I^2 value was < 50%.

P value determined by two proportion analysis or chi-square test.

ABPA, allergic bronchopulmonary aspergillosis; CFF, cystic fibrosis foundation; R-P, Rosenberg–Peterson or the Laufer's modification.

prevalence (9.1%, 95% CI: 7.82–10.4%). There was a wide variation in the criteria used for diagnosing ABPA (Table 1). There was also significant difference in the prevalence of ABPA when the studies were stratified according to the criteria used for diagnosis (Table 3).

There was significant statistical heterogeneity, with an I^2 value of 94.5% (93.6–95.1%). There was evidence of publication bias on visual examination of the funnel

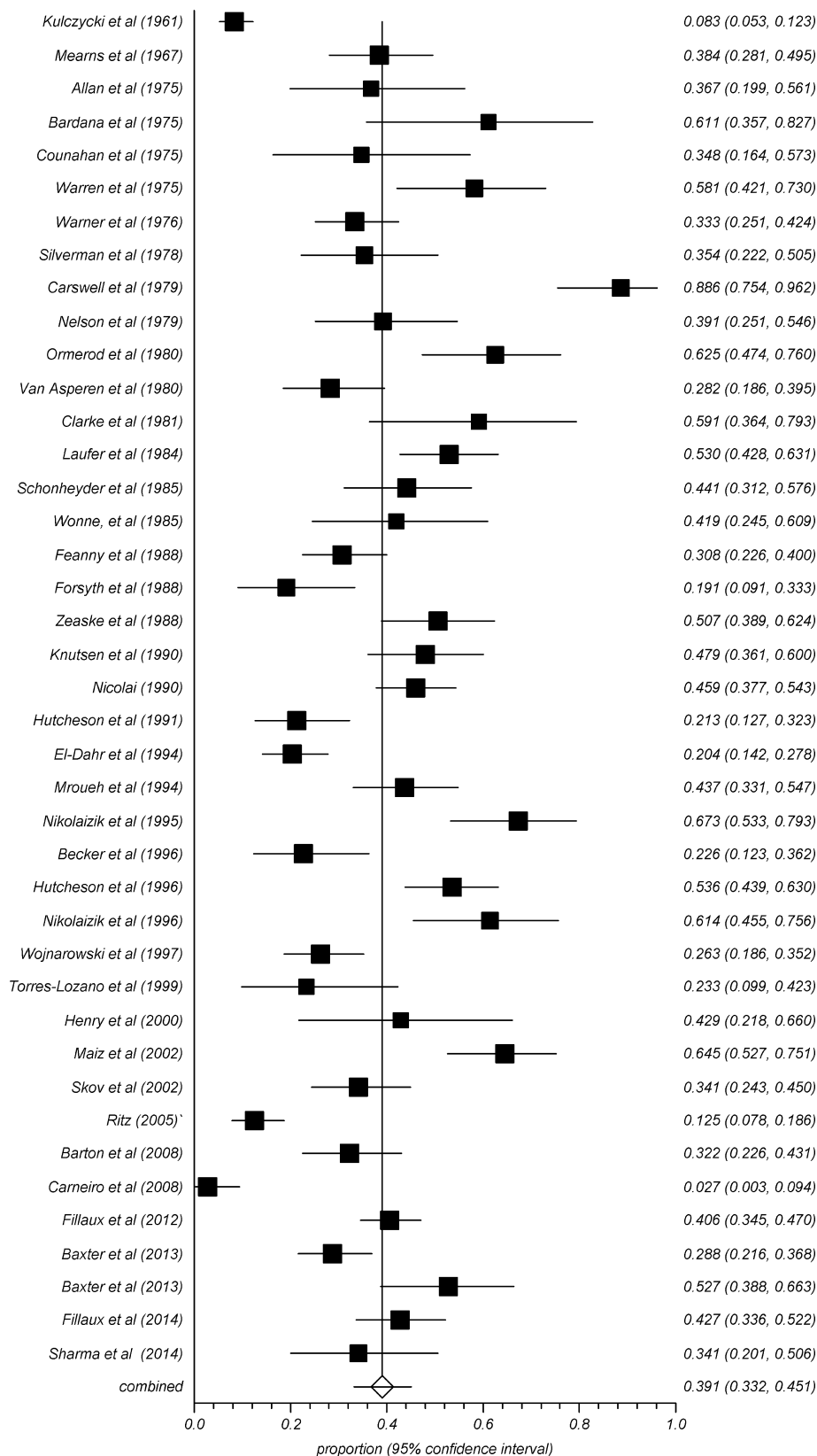


Fig. 2. Prevalence of *Aspergillus* sensitization (AS) in patients with cystic fibrosis (random effects model). The prevalence of AS in the individual studies is represented by a square (percentage) which runs a horizontal line (95% confidence intervals [CI]). The diamond at the bottom represents the pooled prevalence from the studies.

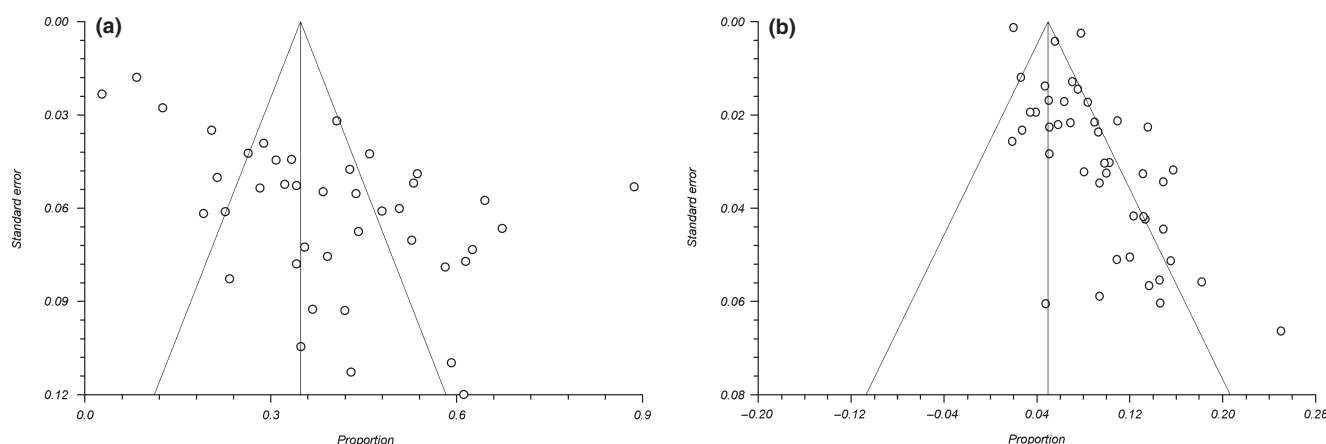


Fig. 3. Funnel plots comparing the proportion vs. the standard error of the proportion for the prevalence of *Aspergillus* sensitization (a) and allergic bronchopulmonary aspergillosis (b) in patients with cystic fibrosis. The circles represent the trials included in the meta-analysis. The line in the centre indicates the summary proportion. The other lines represent the 95% confidence intervals. There was evidence of publication bias for *Aspergillus* sensitization and allergic bronchopulmonary aspergillosis.

plot (Fig. 3) and on the statistical tests (Egger bias = 2.37, $P = 0.0001$; Harbord bias = 2.52, $P = 0.0009$; Begg-Mazumdar Kendall's tau = 0.047, $P = 0.653$).

Discussion

The results of this meta-analysis suggest the prevalence of *Aspergillus* sensitization and ABPA of approximately 39.1% and 8.9%, respectively, in patients with CF. The prevalence of AS was similar in children and adults, but the prevalence of ABPA was significantly higher in adults. This intuitively implies that sensitization to *Aspergilli* occurs early in the course of the illness, while ABPA occurs later in the disease course. As most reports of ABPA in CF are from specialized CF clinics, a possibility of referral bias cannot be excluded.

The classification of *Aspergillus*-related pulmonary disorders is constantly changing [70]. Inhalation of *A. fumigatus* spores leads to a spectrum of pulmonary manifestations in patients with CF. In general, after initial colonization by *A. fumigatus*, the patient may (a) harbour the fungus for a short duration and later clear it, the transient carrier; (b) become sensitized to the *A. fumigatus*, that is *Aspergillus* sensitization; (c) harbour the strain for long durations, which constitutes *Aspergillus* colonization; or (d) develop the syndrome of ABPA. In addition to the above, less common pulmonary manifestations of *A. fumigatus* in CF include the development of aspergilloma [71], invasive pulmonary aspergillosis [72] and the syndrome of *Aspergillus* bronchitis [73]. These manifestations are not discrete, and there is significant overlap amongst them. In CF, it is often difficult to differentiate AS from ABPA as several patients demonstrate spontaneous normaliza-

tion in many immunologic parameters including IgE levels [29]. Recently, attempts have been made to differentiate between AS and ABPA in CF. The basophil CD203c levels upon stimulation by *A. fumigatus* allergen have good predictive value in discriminating ABPA from AS [74]. In another study, a galactomannan index of > 0.5 and *A. fumigatus*-specific IgG value of > 75 mg/L offered a sensitivity and specificity of 96% and 100%, and 96% and 90%, respectively, in differentiating ABPA from AS [64].

Allergic bronchopulmonary aspergillosis

The diagnosis of ABPA in CF is challenging as the clinical features of ABPA such as cough, expectoration, wheezing and hemoptysis can occur in CF *per se* without ABPA [75]. Bronchiectasis, a manifestation of CF, can also be encountered in ABPA. The earliest criteria for diagnosing ABPA in patients with CF were proposed by Nelson et al. [15] in the year 1979. Following this, several criteria have been proposed for the diagnosis of ABPA (Table 4). All the criteria rely on several of the following parameters for diagnosis of ABPA: wheezing, immediate skin test reactivity, elevated *A. fumigatus*-specific IgE/IgG, elevated IgE levels, radiologic infiltrates, central bronchiectasis, presence of serum precipitins, peripheral eosinophilia, presence of *Aspergillus* in sputum and a steroid responsive disease. None of the aforementioned parameter alone specific for diagnosing ABPA. Also, there is no consensus on the number of criteria needed for the diagnosis of ABPA. The most specific criteria for diagnosing ABPA are high-attenuation mucus (defined as impacted mucus within airways being visually denser than paraspinal skeletal muscle) [76–79]; however, this

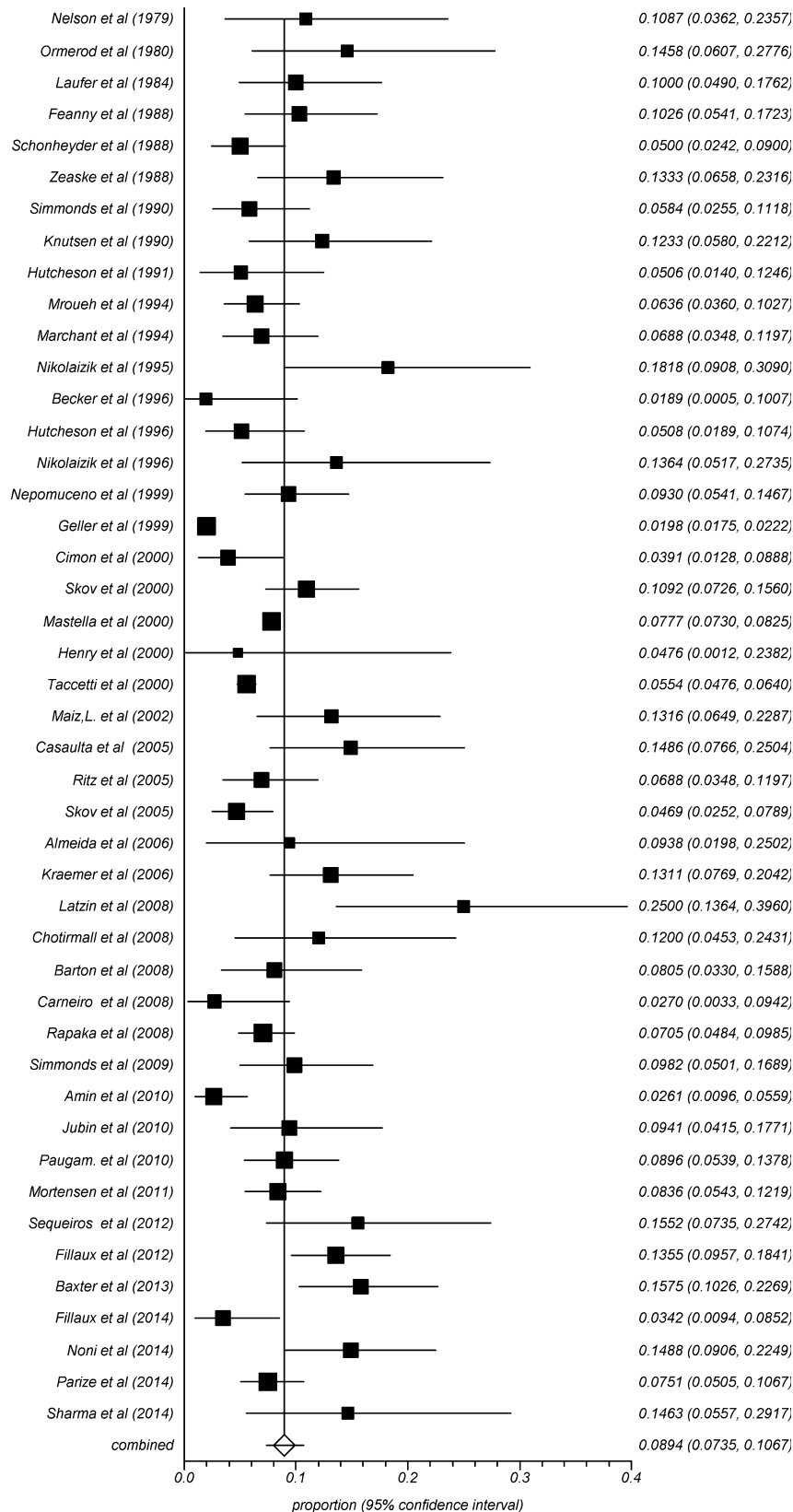


Fig. 4. Prevalence of allergic bronchopulmonary aspergillosis (ABPA) in patients with cystic fibrosis (random effects model). The prevalence of ABPA in the individual studies is represented by a square (percentage) which runs a horizontal line (95% confidence intervals [CI]). The diamond at the bottom represents the pooled prevalence from the studies.

Table 4. Comparison of various criteria used for diagnosing allergic bronchopulmonary aspergillosis (ABPA) in patients with cystic fibrosis

Criterion used for diagnosis of ABPA in patients with cystic fibrosis	Rosenberg & Patterson criteria (1977)	Laufer's modification of R-P criteria (1984)	Nelsons criteria (1979)	Greenberger & Patterson criteria (1986)	ESCF criteria (1999)	ERCF Criteria (2000)	Cystic fibrosis foundation consensus criteria (2003)
Episodic bronchial obstruction/wheezing	1 (M)	1 (M)	1 (M)	1 (M)	1 (m)	1 (m)	1 (M)*
AST (Type 1 reaction)	2 (M)	2 (M)	2 (M)	2 (M)	1 (M)	1 (M)	2 (M)
Elevated serum total IgE	3 (M)	3 (M)	3 (M)	3 (M)	2 (M)	2 (M)	3 (M)
Serum AsplgE (> 0.35 kUA/L)		4 (M)*	4 (M)*	4 (M)*	2 (m)	2 (m)	2 (M)
Elevated serum AsplgG		4 (M)	4 (M)	4 (M)	2 (m)	3 (m)	4 (M)
Serum precipitins against <i>A. fumigatus</i>	4 (M)	5 (M)	5 (M)	4 (M)	3 (M)	3 (M)	4 (M)
Eosinophilia (> 1000/ μ L)	5 (M)	6 (M)		5 (M)	3 (m)	4 (m)	
Pulmonary infiltrates	6 (M)	7 (M)	6 (M)	6 (M)	4 (m)	5 (m)	5 (M)
Central bronchiectasis	7 (M)	8 (M)		7 (M)			5 (M)
Sputum culture for <i>A. fumigatus</i>	1 (m)		7 (M)		5 (m)	6 (m)	
Clinical response to steroids					6 (m)	7 (m)	
Expectoration of brown/blackish sputum	2 (m)						
Delayed skin test positivity	3 (m)						
No. of criteria required for diagnosis of ABPA	Exact number not specified	Definite ABPA: Any five of criteria 1, 2, 3, 5, 6, 7, 8 and any one of the criteria 4 Probable ABPA: Any five of criteria 1, 2, 3, 5, 6, 7 and 8.	All seven criteria	ABPA-S: Criteria 1, 2, 3, 5, 6, and any one of criteria 4. ABPA-CB: Criteria for ABPA-S plus criteria 7	Major: Any two of three major criteria (1, 2 and 3) Minor: Any two of six minor criteria	Major: All three major criteria (1, 2, and 3) Minor: One or more of the seven minor criteria	ABPA classic: Criteria 1, 3 (> 1000 U/L) and any one each of criteria 2, 4 & 5 ABPA minimal: Criteria 1, 3 (> 500 U/L), any one of criteria 2 and either of criteria 4 or 5.

ABPA-S, Serologic ABPA; ABPA-CB, ABPA with central bronchiectasis; AsplgE, *A. fumigatus*-specific IgE; AsplgG, *A. fumigatus*-specific IgG; AST, *Aspergillus* skin test; ERCF, Epidemiologic Registry of Cystic Fibrosis; ESCF, Epidemiologic Study of Cystic Fibrosis; m, minor criterion; M, Major criterion; R-P, Rosenberg and Patterson.

*Serum *A. fumigatus*-specific IgE used was a specialized assay and is not commercially available.

has not been systematically evaluated in patients with CF [80].

Recognizing the lack of uniform diagnostic criteria, a workshop was conducted by the international cystic fibrosis foundation (CFF), and criteria were proposed for the diagnosis of ABPA in CF [81]. Yet, 50% of the publications after 2005 used a different criteria for diagnosing ABPA (Table 1). Thus, significant clinical heterogeneity still exists in the diagnosis of ABPA. To make the diagnosis more accurate, several novel markers are also being evaluated. Some of these include the recombinant *Aspergillus* allergens [50, 82], the use of sputum RT-PCR and sputum galactomannan [64]. However, it would be ideal if a single criteria are used as it would allow comparison of patients and will thus benefit clinical research particularly studies planning to evaluate aetiology and/or newer therapies.

Aspergillus sensitization

The pooled prevalence of AS was estimated to be 39.1% and was higher when determined using skin tests compared to specific IgE levels (43.8% vs. 32.8%). Older studies in asthmatic patients also have shown skin tests to have higher sensitivity compared to specific IgE levels in determining fungal sensitization [83, 84]. The reason for this is not clear but could be related to the presence of IgE antibodies in the serum which react to non-specific antigens used in skin testing but are not detected by the RAST assay [85]. Up to 20% of the patients can have discordant results of skin tests and specific IgE levels [85, 86]. The better of the two methods is not clear due to the absence of a reference standard. Whether both tests should be performed to detect fungal sensitization also remains unknown [87]. In a recent study, we found *A. fumigatus*-specific IgE to demonstrate 100% sensitivity in the diagnosis of ABPA complicating asthma [79]. Similar studies are also warranted in ABPA complicating CF. In the current meta-analysis, the prevalence of skin test positivity was similar with the intradermal technique as well as the percutaneous technique. However, when both the tests were used sequentially, the rates of positive skin tests were higher. This is in contrast to the results of an earlier meta-analysis in asthma, where intradermal tests were found to be more sensitive than skin prick tests [4]. The clinical significance of *Aspergillus* sensitization

in patients with CF is well established, and several reports suggest that CF patients with AS have poorer lung function and a greater decline in lung function as compared to those without [27, 37, 51, 64, 65, 88, 89].

Finally, our study is not without limitations. The presence of significant clinical and statistical heterogeneity limits the confidence in the estimates of prevalence. However, this clinical heterogeneity in the studies was inevitable as the understanding, diagnosis and management of *Aspergillus*-induced respiratory diseases in CF population has evolved over the last half a century. The statistical heterogeneity was partially compensated by adopting the DerSimonian and Laird's random effects model for pooling the individual studies. A majority of the studies came from the developed world (USA and Europe). This limits the reproducibility of the estimated prevalence rates to populations from other areas. While calculating the pooled prevalence of AS, for studies reporting both the results of skin test as well as specific IgE levels, we used the values of specific IgE levels. This might have led to a slight underestimation of the true prevalence as skin testing has been shown to be more sensitive than specific IgE levels. Finally, we used an arbitrary cut-off of 16 years to differentiate children from adults.

In conclusion, we have systematically analysed the prevalence of *Aspergillus* sensitization and ABPA in cystic fibrosis. Future studies should adopt uniform testing procedure for detecting sensitization and employ uniform diagnostic criteria for defining AS and ABPA.

Conflict of interests

None.

Financial disclosures

None.

Author contributions

VNM performed systematic review and meta-analysis, and drafted revised the manuscript. RA conceived the idea, performed systematic review and meta-analysis, and revised the manuscript for intellectual content.

References

- Agarwal R. Allergic bronchopulmonary aspergillosis. *Chest* 2009; 135:805–26.
- Agarwal R, Chakrabarti A, Shah A *et al.* Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. *Clin Exp Allergy* 2013; 43:850–73.
- Armstead J, Morris J, Denning DW. Multi-country estimate of different manifestations of aspergillosis in cystic fibrosis. *PLoS One* 2014; 9:e98502.
- Agarwal R, Aggarwal AN, Gupta D, Jindal SK. *Aspergillus* hypersensitivity and allergic bronchopulmonary aspergillosis in patients with bronchial asthma: systematic review and meta-analysis. *Int J Tuberc Lung Dis* 2009; 13:936–44.
- DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; 7:177–88.

- 6 Kulczycki LL, Mueller HH, Shwachman H. Respiratory allergy in patients with cystic fibrosis. *JAMA* 1961; 175:358–64.
- 7 Mearns M, Longbottom J, Batten J. Precipitating antibodies to *Aspergillus fumigatus* in cystic fibrosis. *Lancet* 1967; 1:538–9.
- 8 Allan JD, Moss AD, Wallwork JC, McFarlane H. Immediate hypersensitivity in patients with cystic fibrosis. *Clin Allergy* 1975; 5:255–61.
- 9 Bardana EJ Jr, Sobti KL, Cianciulli FD, Noonan MJ. *Aspergillus* antibody in patients with cystic fibrosis. *Am J Dis Child* 1975; 129:1164–7.
- 10 Counahan R, Mearns MB. Prevalence of atopy and exercise-induced bronchial lability in relatives of patients with cystic fibrosis. *Arch Dis Child* 1975; 50:477–81.
- 11 Warren CP, Tai E, Batten JC, Hutchcroft BJ, Pepys J. Cystic fibrosis–immunological reactions to *A. fumigatus* and common allergens. *Clin Allergy* 1975; 5:1–12.
- 12 Warner JO, Taylor BW, Norman AP, Soothill JF. Association of cystic fibrosis with allergy. *Arch Dis Child* 1976; 51:507–11.
- 13 Silverman M, Hobbs FD, Gordon IR, Carswell F. Cystic fibrosis, atopy, and airways lability. *Arch Dis Child* 1978; 53:873–7.
- 14 Carswell F, Oliver J, Silverman M. Allergy in cystic fibrosis. *Clin Exp Immunol* 1979; 35:141–6.
- 15 Nelson LA, Callera ML, Schwartz RH. Aspergillosis and atopy in cystic fibrosis. *Am Rev Respir Dis* 1979; 120:863–73.
- 16 Ormerod LP, Thomson RA, Anderson CM, Stableforth DE. Reversible airway obstruction in cystic fibrosis. *Thorax* 1980; 35:768–72.
- 17 van Asperen PP, Mellis CM, South RT. Respiratory allergy in cystic fibrosis. *Aust Paediatr J* 1980; 16:53–6.
- 18 Clarke CW, Hampshire P, Hannant C. Positive immediate skin tests in cystic fibrosis: a possible role for *Pseudomonas* infection. *Br J Dis Chest* 1981; 75:15–21.
- 19 Laufer P, Fink JN, Bruns WT *et al.* Allergic bronchopulmonary aspergillosis in cystic fibrosis. *J Allergy Clin Immunol* 1984; 73:44–8.
- 20 Schonheyder H, Jensen T, Hoiby N, Andersen P, Koch C. Frequency of *Aspergillus fumigatus* isolates and antibodies to *Aspergillus* antigens in cystic fibrosis. *Acta Pathol Microbiol Immunol Scand B* 1985; 93:105–12.
- 21 Wonne R, Hofmann D, Posselt HG, Stover B, Bender SW. Bronchial allergy in cystic fibrosis. *Clin Allergy* 1985; 15:455–63.
- 22 Feanny S, Forsyth S, Corey M, Levison H, Zimmerman B. Allergic bronchopulmonary aspergillosis in cystic fibrosis: a secretory immune response to a colonizing organism. *Ann Allergy* 1988; 60:64–8.
- 23 Forsyth KD, Hohmann AW, Martin AJ, Bradley J. IgG antibodies to *Aspergillus fumigatus* in cystic fibrosis: a laboratory correlate of disease activity. *Arch Dis Child* 1988; 63:953–7.
- 24 Schonheyder H, Jensen T, Hoiby N, Koch C. Clinical and serological survey of pulmonary Aspergillosis in patients with cystic fibrosis. *Int Arch Allergy Appl Immunol* 1988; 85:472–7.
- 25 Zeaske R, Bruns WT, Fink JN *et al.* Immune responses to *Aspergillus* in cystic fibrosis. *J Allergy Clin Immunol* 1988; 82:73–7.
- 26 Knutsen AP, Hutcheson PS, Mueller KR, Slavin RG. Serum immunoglobulins E and G anti-*Aspergillus fumigatus* antibody in patients with cystic fibrosis who have allergic bronchopulmonary aspergillosis. *J Lab Clin Med* 1990; 116:724–7.
- 27 Nicolai T, Arleth S, Spaeth A, Berteles-Harms RM, Harms HK. Correlation of IgE antibody titer to *Aspergillus fumigatus* with decreased lung function in cystic fibrosis. *Pediatr Pulmonol* 1990; 8:12–5.
- 28 Simmonds EJ, Littlewood JM, Evans EG. Cystic fibrosis and allergic bronchopulmonary aspergillosis. *Arch Dis Child* 1990; 65:507–11.
- 29 Hutcheson PS, Rejent AJ, Slavin RG. Variability in parameters of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *J Allergy Clin Immunol* 1991; 88:390–4.
- 30 el-Dahr JM, Fink R, Selden R, Arruda LK, Platts-Mills TA, Heymann PW. Development of immune responses to *Aspergillus* at an early age in children with cystic fibrosis. *Am J Respir Crit Care Med* 1994; 150:1513–8.
- 31 Marchant JL, Warner JO, Bush A. Rise in total IgE as an indicator of allergic bronchopulmonary aspergillosis in cystic fibrosis. *Thorax* 1994; 49:1002–5.
- 32 Mroueh S, Spock A. Allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *Chest* 1994; 105:32–6.
- 33 Nikolaizik WH, Moser M, Cramer R *et al.* Identification of allergic bronchopulmonary aspergillosis in cystic fibrosis patients by recombinant *Aspergillus fumigatus* I/a-specific serology. *Am J Respir Crit Care Med* 1995; 152:634–9.
- 34 Becker JW, Burke W, McDonald G, Greenberger PA, Henderson WR, Aitken ML. Prevalence of allergic bronchopulmonary aspergillosis and atopy in adult patients with cystic fibrosis. *Chest* 1996; 109:1536–40.
- 35 Hutcheson PS, Knutsen AP, Rejent AJ, Slavin RG. A 12-year longitudinal study of *Aspergillus* sensitivity in patients with cystic fibrosis. *Chest* 1996; 110:363–6.
- 36 Nikolaizik WH, Cramer R, Blaser K, Schoni MH. Skin test reactivity to recombinant *Aspergillus fumigatus* allergen I/a in patients with cystic fibrosis. *Int Arch Allergy Immunol* 1996; 111:403–8.
- 37 Wojnarowski C, Eichler I, Gartner C *et al.* Sensitization to *Aspergillus fumigatus* and lung function in children with cystic fibrosis. *Am J Respir Crit Care Med* 1997; 155:1902–7.
- 38 Geller DE, Kaplowitz H, Light MJ, Colin AA. Allergic bronchopulmonary aspergillosis in cystic fibrosis: reported prevalence, regional distribution, and patient characteristics. Scientific Advisory Group, Investigators, and Coordinators of the Epidemiologic Study of Cystic Fibrosis. *Chest* 1999; 116:639–46.
- 39 Nepomuceno IB, Esrig S, Moss RB. Allergic bronchopulmonary aspergillosis in cystic fibrosis: role of atopy and response to itraconazole. *Chest* 1999; 115:364–70.
- 40 Torres-Lozano C, Nunez-Nunez ME, Pedroza-Melendez A, Lezana FJ, Huerta-Lopez JG. Respiratory allergy in Mexican patients with cystic fibrosis. *Allergol Immunopathol (Madr)* 1999; 27:294–7.
- 41 Cimion B, Carrere J, Chazalotte JP, Ginies JL, Chabasse D, Bouchara JP. Bronchopulmonary mycoses in cystic fibrosis. Results of a five-year longitu-

- dinal study. *J Mycol Med* 2000; 10:128–35.
- 42 Henry M, Bennett DM, Kiely J, Kelleher N, Bredin CP. Fungal atopy in adult cystic fibrosis. *Respir Med* 2000; 94:1092–6.
 - 43 Mastella G, Rainisio M, Harms HK *et al.* Allergic bronchopulmonary aspergillosis in cystic fibrosis. A European epidemiological study. Epidemiologic Registry of Cystic Fibrosis. *Eur Respir J* 2000; 16:464–71.
 - 44 Skov M, Koch C, Reimert CM, Poulsen LK. Diagnosis of allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis. *Allergy* 2000; 55:50–8.
 - 45 Taccetti G, Procopio E, Marianelli L, Campana S. Allergic bronchopulmonary aspergillosis in Italian cystic fibrosis patients: prevalence and percentage of positive tests in the employed diagnostic criteria. *Eur J Epidemiol* 2000; 16:837–42.
 - 46 Maiz L, Cuevas M, Quirce S *et al.* Sero-logic IgE immune responses against *Aspergillus fumigatus* and *Candida albicans* in patients with cystic fibrosis. *Chest* 2002; 121:782–8.
 - 47 Casaulta C, Fluckiger S, Cramer R, Blaser K, Schoeni MH. Time course of antibody response to recombinant *Aspergillus fumigatus* antigens in cystic fibrosis with and without ABPA. *Pediatr Allergy Immunol* 2005; 16:217–25.
 - 48 Ritz N, Ammann RA, Casaulta AC, Schoeni-Affolter F, Schoeni MH. Risk factors for allergic bronchopulmonary aspergillosis and sensitisation to *Aspergillus fumigatus* in patients with cystic fibrosis. *Eur J Pediatr* 2005; 164:577–82.
 - 49 Skov M, McKay K, Koch C, Cooper PJ. Prevalence of allergic bronchopulmonary aspergillosis in cystic fibrosis in an area with a high frequency of atopy. *Respir Med* 2005; 99:887–93.
 - 50 Almeida MB, Bussamra MH, Rodrigues JC. ABPA diagnosis in cystic fibrosis patients: the clinical utility of IgE specific to recombinant *Aspergillus fumigatus* allergens. *J Pediatr (Rio J)* 2006; 82:215–20.
 - 51 Kraemer R, Delosea N, Ballinari P, Gallati S, Cramer R. Effect of allergic bronchopulmonary aspergillosis on lung function in children with cystic fibrosis. *Am J Respir Crit Care Med* 2006; 174:1211–20.
 - 52 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; 62:287–91.
 - 53 Carneiro AC, Lemos AC, Arruda SM, Santana MA. Prevalence of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis in the state of Bahia, Brazil. *J Bras Pneumol* 2008; 34:900–6.
 - 54 Chotirmall SH, Branagan P, Gunaratnam C, McElvaney NG. *Aspergillus*/allergic bronchopulmonary aspergillosis in an Irish cystic fibrosis population: a diagnostically challenging entity. *Respir Care* 2008; 53:1035–41.
 - 55 Latzin P, Hartl D, Regamey N, Frey U, Schoeni MH, Casaulta C. Comparison of serum markers for allergic bronchopulmonary aspergillosis in cystic fibrosis. *Eur Respir J* 2008; 31:36–42.
 - 56 Rapaka RR, Kolls JK. Pathogenesis of allergic bronchopulmonary aspergillosis in cystic fibrosis: current understanding and future directions. *Med Mycol* 2009; 47(Suppl. 1):S331–7.
 - 57 Simmonds NJ, Cullinan P, Hodson ME. Growing old with cystic fibrosis - the characteristics of long-term survivors of cystic fibrosis. *Respir Med* 2009; 103:629–35.
 - 58 Amin R, Dupuis A, Aaron SD, Ratjen F. The effect of chronic infection with *Aspergillus fumigatus* on lung function and hospitalization in patients with cystic fibrosis. *Chest* 2010; 137:171–6.
 - 59 Jubin V, Ranque S, Stremmer LBN, Sarles J, Dubus JC. Risk factors for *Aspergillus* colonization and allergic bronchopulmonary aspergillosis in children with cystic fibrosis. *Pediatr Pulmonol* 2010; 45:764–71.
 - 60 Paugam A, Baixench MT, Demazes-Dufeu N *et al.* Characteristics and consequences of airway colonization by filamentous fungi in 201 adult patients with cystic fibrosis in France. *Med Mycol* 2010; 48(Suppl. 1):S32–6.
 - 61 Mortensen KL, Jensen RH, Johansen HK *et al.* *Aspergillus* species and other molds in respiratory samples from patients with cystic fibrosis: a laboratory-based study with focus on *Aspergillus fumigatus* azole resistance. *J Clin Microbiol* 2011; 49:2243–51.
 - 62 Fillaux J, Bremont F, Murriss M *et al.* Assessment of *Aspergillus* sensitization or persistent carriage as a factor in lung function impairment in cystic fibrosis patients. *Scand J Infect Dis* 2012; 44:842–7.
 - 63 Sequeiros IM, Jarad N. Factors associated with a shorter time until the next pulmonary exacerbation in adult patients with cystic fibrosis. *Chron Respir Dis* 2012; 9:9–16.
 - 64 Baxter CG, Dunn G, Jones AM *et al.* Novel immunologic classification of *Aspergillosis* in adult cystic fibrosis. *J Allergy Clin Immunol* 2013; 132:560–6.e10.
 - 65 Baxter CG, Moore CB, Jones AM, Webb AK, Denning DW. IgE-mediated immune responses and airway detection of *Aspergillus* and *Candida* in adult cystic fibrosis. *Chest* 2013; 143:1351–7.
 - 66 Fillaux J, Bremont F, Murriss M *et al.* *Aspergillus* sensitization or carriage in cystic fibrosis patients. *Pediatr Infect Dis J* 2014; 33:680–6.
 - 67 Noni M, Katelari A, Dimopoulos G *et al.* Inhaled corticosteroids and *Aspergillus fumigatus* isolation in cystic fibrosis. *Med Mycol* 2014; 52:715–22.
 - 68 Parize P, Billaud S, Bienvenu AL *et al.* Impact of *Scedosporium apiospermum* complex seroprevalence in patients with cystic fibrosis. *J Cyst Fibros* 2014; 13:667–73.
 - 69 Sharma VK, Raj D, Xess I, Lodha R, Kabra SK. Prevalence and risk factors for allergic bronchopulmonary aspergillosis in Indian children with cystic fibrosis. *Indian Pediatr* 2014; 51:295–7.
 - 70 Jones AM, Horsley A, Denning DW. What is the importance of classifying *Aspergillus* disease in cystic fibrosis patients? *Expert Rev Respir Med* 2014; 8:389–92.
 - 71 Maguire CP, Hayes JP, Hayes M, Masterson J, FitzGerald MX. Three cases of pulmonary aspergilloma in adult patients with cystic fibrosis. *Thorax* 1995; 50:805–6.
 - 72 Brown K, Rosenthal M, Bush A. Fatal invasive *Aspergillosis* in an adolescent with cystic fibrosis. *Pediatr Pulmonol* 1999; 27:130–3.
 - 73 Shoseyov D, Brownlee KG, Conway SP, Kerem E. *Aspergillus* bronchitis in cystic fibrosis. *Chest* 2006; 130:222–6.
 - 74 Gernez Y, Dunn CE, Everson C *et al.* Blood basophils from cystic fibrosis patients with allergic bronchopulmonary aspergillosis are primed and hyper-responsive to stimulation by

- Aspergillus* allergens. *J Cyst Fibros* 2012; 11:502–10.
- 75 Moss RB. Allergic bronchopulmonary aspergillosis and *Aspergillus* infection in cystic fibrosis. *Curr Opin Pulm Med* 2010; 16:598–603.
 - 76 Agarwal R, Aggarwal AN, Gupta D. High-attenuation mucus in allergic bronchopulmonary aspergillosis: another cause of diffuse high-attenuation pulmonary abnormality. *AJR Am J Roentgenol* 2006; 186:904.
 - 77 Agarwal R, Gupta D, Aggarwal AN, Saxena AK, Chakrabarti A, Jindal SK. Clinical significance of hyperattenuating mucoid impaction in allergic bronchopulmonary aspergillosis: an analysis of 155 patients. *Chest* 2007; 132:1183–90.
 - 78 Agarwal R, Khan A, Gupta D, Aggarwal AN, Saxena AK, Chakrabarti A. An alternate method of classifying allergic bronchopulmonary aspergillosis based on high-attenuation mucus. *PLoS One* 2010; 5:e15346.
 - 79 Agarwal R, Maskey D, Aggarwal AN *et al.* Diagnostic performance of various tests and criteria employed in allergic bronchopulmonary aspergillosis: a latent class analysis. *PLoS One* 2013; 8:e61105.
 - 80 Morozov A, Applegate KE, Brown S, Howenstine M. High-attenuation mucus plugs on MDCT in a child with cystic fibrosis: potential cause and differential diagnosis. *Pediatr Radiol* 2007; 37:592–5.
 - 81 Stevens DA, Moss RB, Kurup VP *et al.* Allergic bronchopulmonary aspergillosis in cystic fibrosis—state of the art: cystic fibrosis foundation consensus conference. *Clin Infect Dis* 2003; 37 (Suppl. 3):S225–64.
 - 82 Fricker-Hidalgo H, Coltey B, Llerena C *et al.* Recombinant allergens combined with biological markers in the diagnosis of allergic bronchopulmonary aspergillosis in cystic fibrosis patients. *Clin Vaccine Immunol* 2010; 17:1330–6.
 - 83 O'Driscoll BR, Powell G, Chew F *et al.* Comparison of skin prick tests with specific serum immunoglobulin E in the diagnosis of fungal sensitization in patients with severe asthma. *Clin Exp Allergy* 2009; 39:1677–83.
 - 84 Liang KL, Su MC, Jiang RS. Comparison of the skin test and ImmunoCAP system in the evaluation of mold allergy. *J Chin Med Assoc* 2006; 69:3–6.
 - 85 van der Zee JS, de Groot H, van Swieten P, Jansen HM, Aalberse RC. Discrepancies between the skin test and IgE antibody assays: study of histamine release, complement activation in vitro, and occurrence of allergen-specific IgG. *J Allergy Clin Immunol* 1988; 82:270–81.
 - 86 Calabria CW, Dietrich J, Hagan L. Comparison of serum-specific IgE (ImmunoCAP) and skin-prick test results for 53 inhalant allergens in patients with chronic rhinitis. *Allergy Asthma Proc* 2009; 30:386–96.
 - 87 de Vos G. Skin testing versus serum-specific IgE testing: which is better for diagnosing aeroallergen sensitization and predicting clinical allergy? *Curr Allergy Asthma Rep* 2014; 14:430.
 - 88 Kanthan SK, Bush A, Kemp M, Buchdahl R. Factors effecting impact of *Aspergillus fumigatus* sensitization in cystic fibrosis. *Pediatr Pulmonol* 2007; 42:785–93.
 - 89 Peetermans M, Goeminne P, De Boeck C, Dupont LJ. IgE sensitization to *Aspergillus fumigatus* is not a bystander phenomenon in cystic fibrosis lung disease. *Chest* 2014; 146:e99–100.