

Performance of specific immunoglobulin E tests for diagnosing occupational asthma: a systematic review and meta-analysis

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ABSTRACT

Objectives To determine the test performance parameters for the retrievable range of high-molecular-weight (HMW) and low-molecular-weight (LMW) occupational allergens and to evaluate the impact of allergenic components and the implementation of measures for test validation.

Methods A protocol with predefined objectives and inclusion criteria was the basis of an electronic literature search of MEDLINE and EMBASE (time period 1967–2016). The specific inhalation challenge and serial peak flow measurements were the reference standards for the specific IgE (sIgE) test parameters. All of the review procedures were reported according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

Results Seventy-one studies were selected, and 62 entered meta-analysis. Pooled pairs analysis indicated a sensitivity of 0.74 (95% CI 0.66 to 0.80) and specificity of 0.71 (95% CI 0.63 to 0.77) for HMW allergens and a sensitivity of 0.28 (95% CI 0.18 to 0.40) and specificity of 0.89 (95% CI 0.77 to 0.95) for LMW allergens. Component-specific analysis improved the test parameters for some allergens. Test validation was handled heterogeneously among studies.

Conclusion sIgE test performance is rather satisfactory for a wide range of HMW allergens with the potential for component-specific approaches, whereas sensitivity for LMW allergens is considerably lower, indicating methodological complications and/or divergent pathomechanisms. A common standard for test validation is needed.

INTRODUCTION

At least 10% of all adult new-onset asthma cases worldwide are caused by exposure to substances in the workplace.¹ Since many of these cases are preventable, reliable diagnosis is important for appropriate intervention, treatment and prevention: otherwise, persistent exposure of affected subjects to the allergen may accelerate the decline in lung function.² The disease burden of occupational asthma (OA) also has a socioeconomic impact with notable direct and indirect costs.³ However, when attempting to distinguish between irritant, specific IgE (sIgE)-mediated asthma and asthma caused by an agent with an unknown pathomechanism, diagnosis is not always easy.⁴

The allergen-specific inhalation challenge test (SIC) is widely acknowledged as the gold standard for the diagnosis of OA⁵; however, because it requires expertise, expense and time, it is limited to

Key messages

What is already known about this subject?

- The performance of specific inhalation challenge, the gold standard test for the diagnosis of occupational asthma, is restricted to specialised centres, resulting in great demand for alternative methods relying on the measurement of specific IgE levels.
- Information was lacking regarding the extent to which we can rely on the specific IgE measurement data.

What are the new findings?

- Our systematic review shows a rather satisfactory pooled sensitivity of 0.74 and specificity of 0.71 for specific IgE tests applied to high-molecular-weight allergens.
- The pooled sensitivity is generally lower (0.28) and the pooled specificity is generally higher (0.89) for low-molecular-weight allergens than for high-molecular-weight allergens.
- Both commercial and in-house assays are limited with respect to allergenic antigen extracts, especially for low-molecular-weight allergens.
- Methods for validating in-house tests are heterogeneous and lack common methodological quality control standards.

How might this impact on policy or clinical practice in the foreseeable future?

- This article expands our knowledge of the diagnostic reliability of targeted specific IgE tests for the diagnosis of occupational asthma.
- Such tests are especially useful, when specific inhalation challenge is not feasible.
- The establishment of needed common standards for in-house and commercial specific IgE tests to produce more reliable and comparable results should be expected.

specialised centres. This restricted availability and the numerous contraindications and complications, particularly in patients with advanced disease,⁶ as well as the incidence of false-negative⁴ and false-positive⁵ results, have placed great demands on research for alternative methods relying on in vitro tests that measure sIgE levels in serum.

sIgE tests are available in most laboratories and allergy assessment units and mostly rely on the demonstrable capture of IgE by preparations of

immobilised allergens of various purities. These tests can be performed easily and now represent an important diagnostic aid in the diagnosis of the allergic IgE-mediated type of OA.⁷ An appropriate sensitivity for detection of type I sensitisation to high-molecular-weight (HMW) allergens with corresponding asthma is generally accepted and makes the sIgE test an established part of a practical and stepwise diagnostic approach. However, the specificity of the test often limits its diagnostic value.⁴ In contrast to HMW antigens, there is evidence for reduced sensitivity of sIgE tests for low-molecular-weight (LMW) antigens, and this might be exacerbated by the unclear involvement of sIgE in the pathological mechanisms of OA caused by these agents. Furthermore, the generation of detectable LMW antigen-protein complexes as a sIgE-binding structure is difficult and is not always as successful as it could be.

However, to what extent can we rely on the sIgE test data? A previous systematic review determined a pooled sensitivity of 73.3% and a specificity of 79.0% for HMW and a sensitivity of 31.2% and a specificity of 88.9% for LMW allergens based on relatively few studies.⁸ Although recent systematic reviews for other indications of sIgE tests, such as food allergy,⁹ are available, no extensive and detailed analysis of sIgE tests for OA has been presented.

The aim of the current study was to conduct a systematic review with meta-analysis of the use of the sIgE test in OA diagnosis with respect to both HMW and LMW allergenic available antigens in relation to the current gold standard, SIC. The test performance parameters for the HMW and LMW sIgE tests were based on a broad body of data that also permitted analyses of single antigens and groups of allergenic antigens. The effects of methodological differences, especially the use of the fluorescence enzyme-labelled assay carrier polymer system (CAP) and radioallergosorbent test (RAST), and the extent of standardisation with in-house and commercial antigens were also investigated. In addition to essentially complete allergen preparations, the potential of component-resolved analysis was studied.

This article expands knowledge of the diagnostic reliability of targeted, sIgE serum tests for the diagnosis of OA. Such tests are especially useful when specific inhalation challenge is not feasible.

The outcome of this systematic review will be used to develop the future guideline 'Immunological methods for diagnosis and monitoring of IgE-mediated allergy caused by industrial sensitizing agents (IMExAllergy)' within a multidisciplinary, international task force.¹⁰

METHODS

This systematic review was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.¹¹ The methods also followed the guidelines for systematic reviews of diagnostic test accuracy provided by the Cochrane group.¹² The protocol and report are provided in the supplementary material. The search was conducted in EMBASE and MEDLINE. Further resources included reference lists of systematic reviews and meta-analyses of the search results based on relevant objectives and collections of the authors. Articles that reported the results of HMW and LMW allergen tests and of tests that used components of allergens were included according to the predefined inclusion criteria. A detailed explanation of the methods is provided in online supplementary appendix 1.

RESULTS

Characteristics of the included studies

Most of the included studies involved diagnostic cohorts; a few were case-control studies. Many cohorts had few participants. Insufficient quality of reporting in some individual studies partially limited the description of study characteristics. The studies from which data were extracted met the aforementioned inclusion criteria, including presence of the reference standard and sIgE test results (see table 2.5 and 2.6 in online supplementary appendix 2).

Results of the search and selection process

The results of database searching combined with 60 articles from citation lists and the authors' collections yielded 4939 studies after deduplication. We excluded all but 236 records because of thematic irrelevance by initial title/abstract screening and found 83 eligible studies based on full-text assessment (figure 1). Causes for exclusion based on the consecutively applied eligibility criteria were type of intervention in 33 cases, target condition in 20 cases, type of study in 2 cases, age in 6 cases, reference standard in 62 cases and outcome in 29 cases. One full text could not be obtained. Twelve initially eligible studies were not considered for analysis after a duplicate publication check was performed. Finally, 71 studies were included in the systematic review (53 studies involving HMW allergens and 18 involving LMW allergens), and 62 of these with 2138 test results were included in the meta-analysis. A detailed flow chart is provided in figure 2.1 in online supplementary appendix 2.

What is the test performance for HMW allergens? (see table 1 for the summary of results).

Bivariate analysis of HMW sIgE tests

Data from 20 studies were used in the bivariate analysis; 28 tests and 914 results yielded an estimated sensitivity of 0.74 (95% CI 0.66 to 0.80), and a false-positive rate of 0.29 (95% CI 0.23 to 0.37) and a specificity of 0.71 (95% CI 0.63 to 0.77). The area under the curve (AUC) was 0.778, indicating moderate discriminatory power (figure 2A).

Separate pooling of sensitivity of HMW sIgE tests

All 44 studies providing sensitivity (61 different tests and 697 results) were also pooled for separate analysis of sensitivity of the sIgE test for HMW allergens. The overall sensitivity was 0.77 (95% CI 0.71 to 0.82) (see figure 2.20 in online supplementary appendix 2). Pooled tests included the following methods: CAP (predominantly fluorescence enzyme-labelled assays, ImmunoCAP; 17 tests), RAST (30 tests), mixed (2 tests; CAP ca. 10% and RAST ca. 90% of the results), ELISA (4 tests), reverse enzyme immunoassay (REIA; 4 tests) and immunoblot (4 tests).

What are the test performances for individual and groups of HMW allergens? (see table 1 for the summary of results and figures 2.13–2.19 in online supplementary appendix 2 for additional forest plots).

Cereal flours

Studies with cereal flours, particularly wheat and rye, provided most of the HMW sIgE tests for analysis. The pooled sensitivity for this group was 0.78 (95% CI 0.68 to 0.86) (figure 3A). Tests were conducted with CAP (four tests), RAST (eight tests) and mixed (two tests; CAP ca. 10% and RAST ca. 90% of the results) methods.

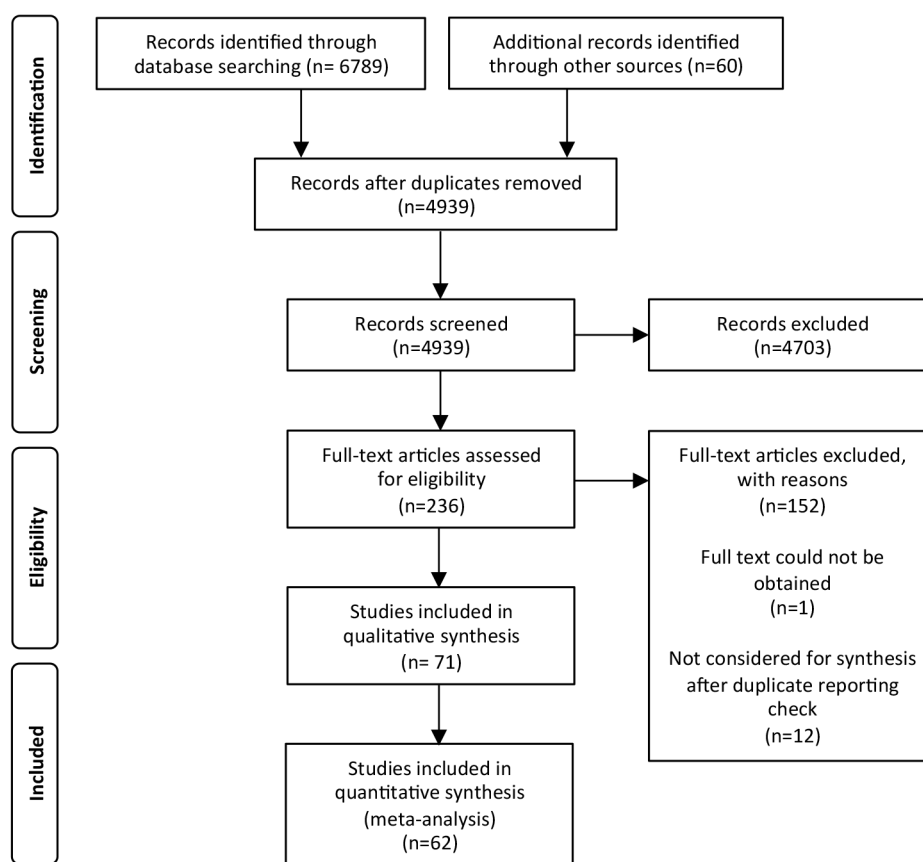


Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses study flow diagram.

The particular sensitivity for wheat was 0.79 (95% CI 0.65 to 0.88) based on 249 results; for rye, it was 0.84 (95% CI 0.65 to 0.94) based on considerably fewer data of 85 results (see figure 2.13 and 2.14 in supplementary appendix 2).

Bovine allergens

Bovine allergen sIgE tests had a pooled sensitivity of 0.86 (95% CI 0.64 to 0.95) based on 22 test results. The allergens were bovine dander in two tests and cow epithelia in one (see figure 2.15 in online supplementary appendix 2). A larger study that used the CAP fluorescence enzyme immunoassay (FEIA) and commercial bovine dander allergen provided a specificity of 1.0 (26 results).¹³ CAP methods were used with commercial allergens in two studies and with in-house allergens in the other.

Enzymes

Sensitivity for enzymes was 0.73 (95% CI 0.61 to 0.83) (figure 3B). The most frequently employed individual tests were alpha-amylase from *Aspergillus oryzae*, with a pooled sensitivity of 0.72 (95% CI 0.52 to 0.86), and cellulase from *Aspergillus niger* resp. of fungal origin, with a sensitivity of 0.72 (95% CI 0.42 to 0.90) (see figure 2.16 and 2.17 in online supplementary appendix 2).

Other enzymes that were used as part of the calculation were pectinase and glucanase from *Aspergillus* and *Trichoderma* species,¹⁴ glucoamylase from *A. niger*, xylanase from mould (not specified),¹⁵ lysozyme from chicken egg,¹⁶ porcine pancreatic extract (alpha-amylase and lipase)¹⁷ and papain, bacterial protease and hemicellulase of unspecified origin.¹⁵

The test methods varied: CAP FEIA,^{16 18} Phadezym RAST,^{15 19} RAST,^{14 20} ELISA,^{17 21} immunoblotting²¹ and REIA^{18 22} were used. With the exception of two CAP FEIA tests that used commercial antigen preparations of lysozyme¹⁶ and alpha-amylase,¹⁸ the investigations used in-house antigen preparations.

Latex

The separately pooled sensitivity for latex was 0.88 (95% CI 0.42 to 0.99) based on 3 tests and 104 results (see figure 2.18 in online supplementary appendix 2). CAP FEIA was used in the two most recent studies; RAST was used in a small study of four participants with just 3% of test results in this calculation. All antigen preparations were commercially manufactured.

Insects

Two studies investigated sIgE to extracts of insect larvae from either the mealworm beetle *Tenebrio molitor* or the common greenbottle fly *Lucilia caesar*. All eligible participants handled live fishing bait. The pooled sensitivity was 0.86 (95% CI 0.42 to 0.98) based on two tests and five results (see figure 2.19 in online supplementary appendix 2). Both studies used RAST test kits and in-house antigen preparations.

Moulds: *Alternaria alternata* (synonym *A. tenuis*)

Two studies from Spain reported sIgE tests for the mould *Alternaria alternata*, which is part of the compendium of causative agents of OA²³ and is potentially relevant for greenhouse workers.²⁴ The larger cohort (positive and negative reference

Table 1 Summary of findings**Specific IgE (sIgE) tests for high-molecular-weight (HMW) allergens****Pooled pairs analysis**

HMW	Sensitivity (95% CI)	Number of studies	Number of tests	Number of test results	Included allergens
	0.74 (0.66 to 0.80)	20	28	914	Latex, rye, wheat, oilseed rape, whole castor bean, grain dust, bovine dander, <i>Lucilia caesar</i> larvae, <i>Alternaria alternata</i> , papain, bacterial protease, pectinase, alpha-amylase, cellulase, hemicellulase
	Specificity (95% CI)				
	0.71 (0.63 to 0.77)				
	Area under the curve				
	0.778				

Separately pooled sensitivity

Allergen	Sensitivity (95% CI)	Number of studies	Number of tests	Number of test results	Heterogeneity I ² (95% CI)
HMW overall	0.77 (0.71 to 0.82)	44	61	697	40.4% (18.8% to 56.2%)
Cereals (wheat and rye)	0.78 (0.68 to 0.86)	11	14	348	49.3% (6.2% to 72.6%)
Wheat	0.79 (0.65 to 0.88)	9	9	249	52.7% (0.0% to 77.8%)
Rye	0.84 (0.65 to 0.94)	4	4	85	42.3% (0.0% to 80.6%)
Bovine allergens	0.86 (0.64 to 0.95)	3	3	22	0.0% (0.0% to 73.3%)
Enzymes	0.73 (0.61 to 0.83)	10	18	69	0.0% (0.0% to 10.9%)
Alpha-amylase	0.72 (0.52 to 0.86)	4	5	29	0.0% (0.0% to 77.3%)
Cellulase	0.72 (0.42 to 0.90)	3	3	13	0.0% (0.0% to 81.9%)
Latex	0.88 (0.42 to 0.99)	3	3	104	74.0% (13.0% to 92.2%)
Insect larvae	0.86 (0.42 to 0.98)	2	2	5	0.0%

sIgE tests for low-molecular-weight (LMW) allergens**Pooled pairs analysis**

LMW	Sensitivity (95% CI)	Number of studies	Number of tests	Number of test results	Included allergens
	0.28 (0.18 to 0.40)	9	13	512	PMDA, PhA, HDI, MDI, TDI, glutaraldehyde, Ni-Resin
	Specificity (95% CI)				
	0.89 (0.95 to 0.77)				
	Area under the curve				
	0.566				

Separately pooled sensitivity

Allergen	Sensitivity (95% CI)	Number of studies	Number of tests	Number of test results	Heterogeneity I ² (95% CI)
LMW overall	0.34 (0.23 to 0.46)	18	27	723	78.3% (69.0% to 84.9%)
Diisocyanates combined	0.27 (0.19 to 0.39)	11	18	307	58.4% (29.9% to 75.3%)
HDI	0.21 (0.09 to 0.41)	3	4	77	60.8% (0.0% to 86.9%)
TDI	0.25 (0.10 to 0.51)	6	6	91	68.5% (25.5% to 86.7%)
MDI	0.42 (0.22 to 0.66)	6	6	48	46.8% (0.0% to 78.9%)
Anhydrides	0.81 (0.46 to 0.95)	2	3	11	0.0% (0.0% to 82.2%)

HMW, high-molecular-weight; HDI, hexamethylene diisocyanate; LMW, low-molecular-weight; MDI, methyldiphenyl diisocyanate; Ni, nickel; PA, plicatic acid; PhA, phthalic anhydride; PMDA, pyromellitic dianhydride; sIgE, allergen-specific IgE; TDI, toluene diisocyanate.

standards, 65 results) provided a sensitivity of 0.92 and a specificity of 0.59.²⁵ The smaller cohort (positive and negative reference standards, 14 results) yielded a lower sensitivity of 0.75 (based on only 4 results) but a higher specificity of 0.9.²⁶ This contrast might be partially explained by imprecision and by selection bias in the larger cohort since proof of sensitisation based on either the skin prick test (SPT) or the presence of sIgE to *Alternaria* was used, with subsequent exaggeration of sensitivity and underestimation of specificity. The test kits, Phadezym RAST and FEIA, both used commercial allergens.

What is the test performance for LMW allergens? (see [table 1](#) for the summary of results).

Bivariate analysis of LMW sIgE tests

Data from nine studies entered the bivariate analysis. Thirteen tests and 512 results yielded an estimated sensitivity of 0.28 (95% CI 0.18 to 0.40), a false positive rate of 0.11 (95% CI 0.05 to 0.23) and a specificity of 0.89 (95% CI 0.77 to 0.95). The AUC was 0.566, indicating low discriminatory power ([figure 2B](#)).

Separate pooling of sensitivity of LMW sIgE tests

All 18 studies that at least provided sensitivity, consisting of 27 different tests and 723 results, were also separately pooled for analysis of the sensitivity of the sIgE test for LMW antigens. The overall sensitivity was 0.34 (95% CI 0.23 to 0.46). Tests were conducted using the CAP (3 tests), RAST (16 tests) or ELISA (8

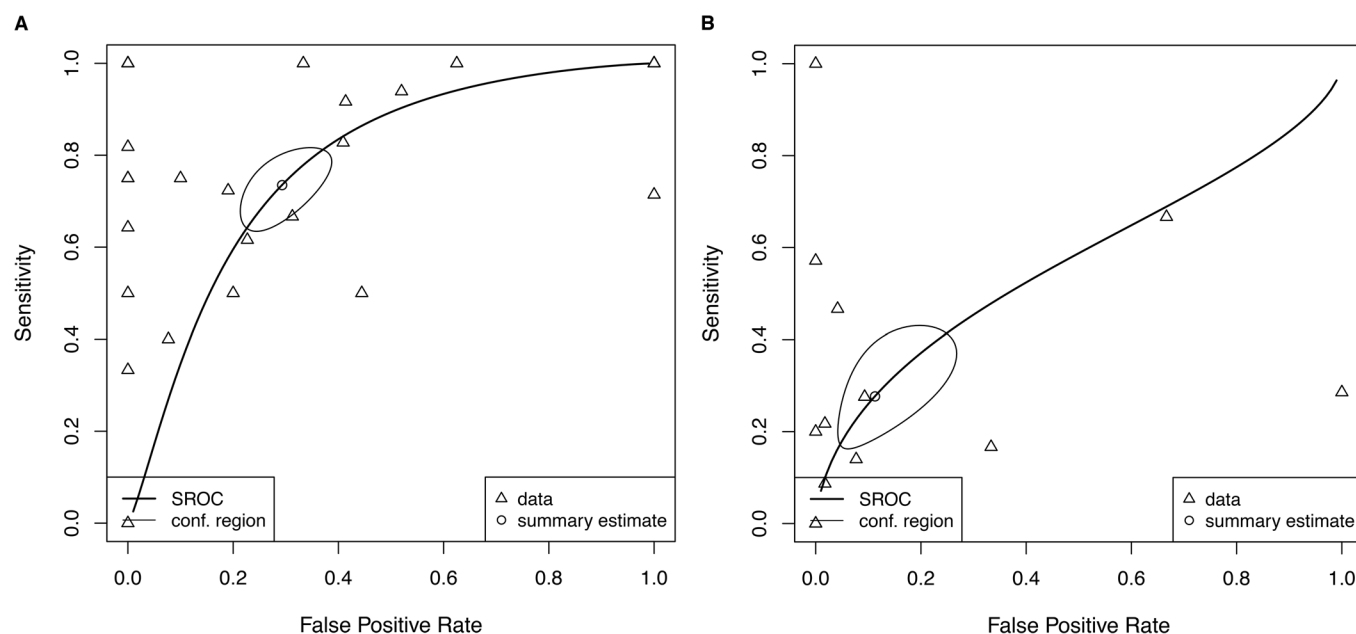


Figure 2 (A) Summary ROC curve of the pooled available pairs of sensitivity and specificity for HMW allergens sIgE tests from primary studies. (B) Summary ROC curve of the pooled available pairs of sensitivity and specificity for LMW allergens sIgE tests from primary studies. The summary ROC curves have corresponding ellipsoids indicating the conf. regions around the summary estimates of sensitivity and specificity; these are marked by a central circle. Scattered triangles indicate the results of the particular studies. Conf. region, confidence region; HMW, high-molecular-weight; LMW, low-molecular-weight; SROC, summary receiver operating characteristic.

tests) methods (see figure 2.21 in online supplementary appendix 2 for forest plot).

What are the test performances for individual or groups of LMW allergens? (see table 1 for the summary of results and figures 2.22–2.25 in online supplementary appendix 2 for additional forest plots).

Diisocyanates

Most LMW sIgE tests were performed for the group of diisocyanates. The separately pooled sensitivity for this group was 0.27 (95% CI 0.19 to 0.39) based on 18 tests and 307 results (figure 3C). Analysis of pooled pairs indicated a high specificity of 0.94 (95% CI 0.88 to 0.97) for a subset of the applicable datasets (see figure 2.26 in online supplementary appendix 2 for a summary ROC curve). Individual sensitivities to hexamethylene diisocyanate (HDI) and toluene diisocyanate (TDI) were similar, 0.21 (95% CI 0.09 to 0.41) and 0.25 (95% CI 0.10 to 0.51), respectively. Methylene diphenyl diisocyanate (MDI) sIgE tests revealed a higher pooled sensitivity of 0.42 (95% CI 0.22 to 0.66). Both commercial and in-house preparations were used for MDI, TDI and HDI. With the exception of two comparative studies, ELISA replaced RAST in the more recent studies for all groups of diisocyanates. In-vapour conjugates with human serum albumin (HSA) elicited higher sensitivity than in-solution conjugates for MDI-HSA using CAP,²⁷ while for HDI-HSA a RAST test achieved the best discriminatory power with an in-solution preparation.²⁸

Anhydrides

Sensitivity for anhydrides was high at 0.81 (95% CI 0.46 to 0.95), although only 11 test results were generated. Phthalic anhydride (PhA), pyromellitic dianhydride (PMDA)²⁹ and tetrachlorophthalic anhydride (TCPA)⁴ were the specific allergens. Phadezym RAST with an in-house antigen preparation was the

method used for PhA and PMDA, and Pharmacia RAST with a commercial antigen preparation was used for TCPA³⁰ (see figure 2.25 in online supplementary appendix 2).

Glutaraldehyde, chloramine T

A small study used a RAST test with an in-house antigen preparation for eight participants with symptoms suggestive of OA caused by glutaraldehyde. The results indicated rather low sensitivity (0.29) and poor specificity, with the only reference standard for a negative subject testing as a false positive.³¹ Chloramine T was investigated in a study with six symptomatic patients and provided a sensitivity of 0.67.³²

Metals

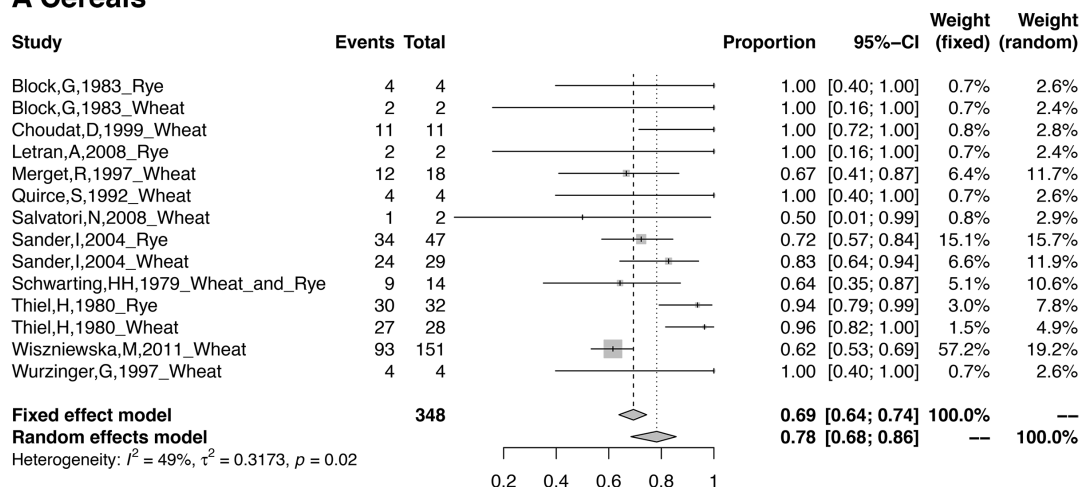
One RAST³³ study with few eligible participants investigated sIgE in hard metal asthma. Cobalt (Co, 12 participants) and nickel (Ni, 8 participants) coupled to HSA or resin resulted in sensitivities of up to 0.58 for Co and 0.57 for Ni. There was one true negative result for Ni.

Wood

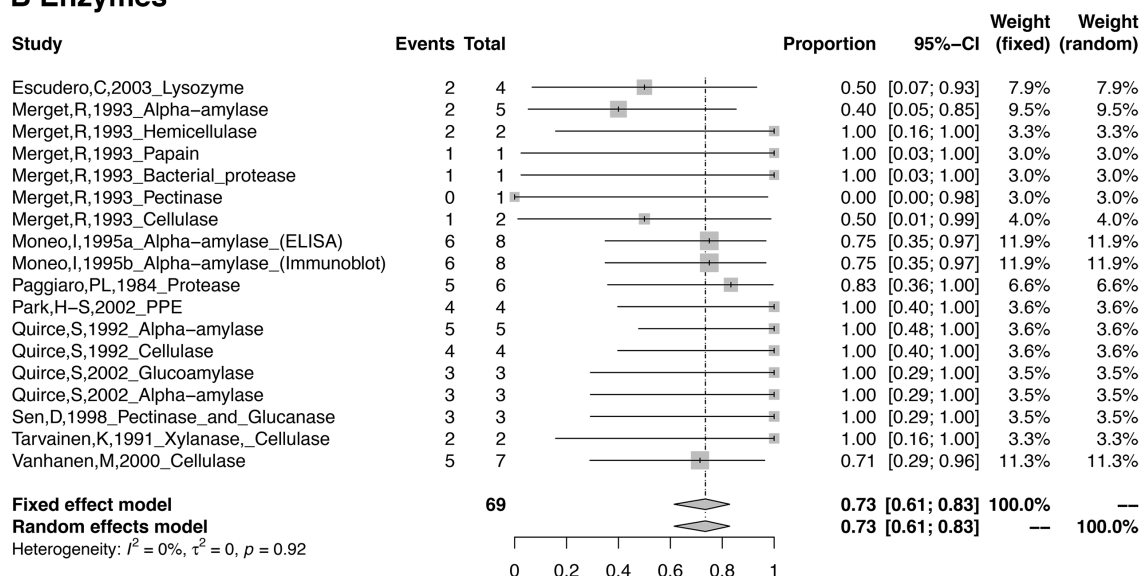
sIgE testing related to wood was considered an exception, because it involves both HMW and LMW antigen preparations. The investigated extracts were either HMW from African maple,³⁴ Tanganyika anigré³⁵ or iroko wood³⁶ or LMW plicatic acid (PA) from western red cedar coupled to HSA.³⁷

Two studies including few participants with confirmed OA for the HMW antigens iroko wood³⁶ and Tanganyika anigré³⁵ (nine and three participants, respectively) had negative sIgE test results, whereas all four participants in the study of African maple conducted with REIA were true positives.³⁴ Unlabelled African maple induced inhibition in a REIA inhibition assay and confirmed detection of sIgE. Inhibition also indicated cross-reactivity with ramin wood extract.

A Cereals



B Enzymes



C Diisocyanates

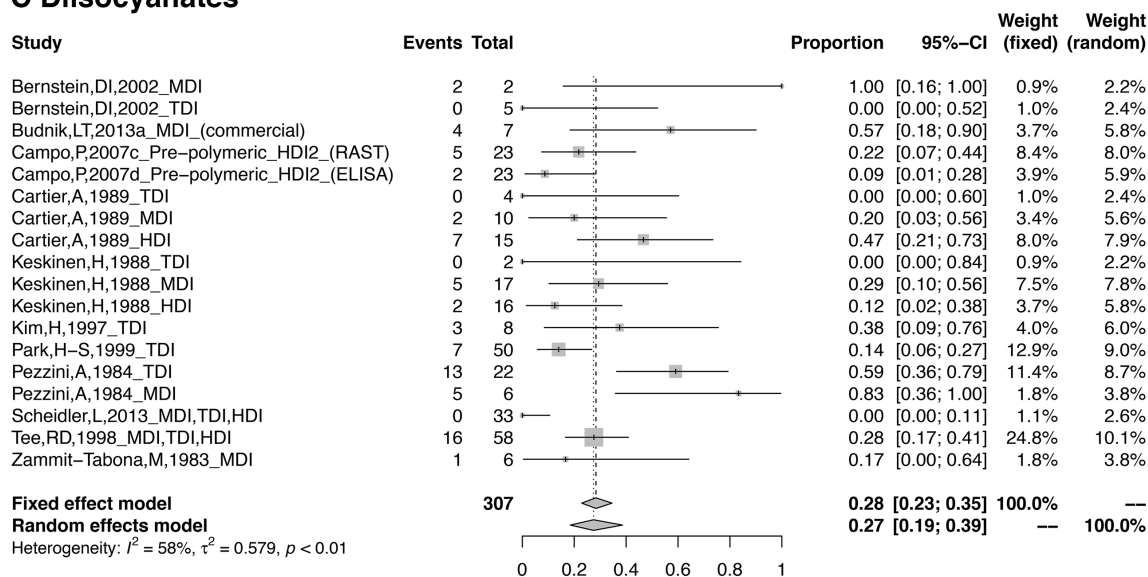


Figure 3 Forest plots of separately pooled sensitivities of specific IgE tests for (A) cereals (wheat and/or rye), (B) enzymes and (C) diisocyanates. HDI, hexamethylene diisocyanate; MDI, methyldiphenyl diisocyanate; PPE, porcine pancreatic extract; RAST, radioallergosorbent test; TDI, toluene diisocyanate.

sIgE against PA coupled to HSA was detected in 9.6% of participants with confirmed OA,³⁷ although sIgE to PA-HSA is considered unlikely to play a role in the pathomechanism of western red cedar asthma.³⁸

Is there a difference in test performance between sIgE test methods?

Direct comparison of RAST and CAP using the bivariate approach was possible for the HMW test results; it yielded almost inverse summary receiver operating characteristic (SROC) curves for the pooled pairs, with RAST sensitivity of 0.65 (95% CI 0.53 to 0.75), CAP sensitivity of 0.84 (95% CI 0.68 to 0.93), RAST specificity of 0.76 (95% CI 0.86 to 0.62) and CAP specificity of 0.63 (95% CI 0.75 to 0.49). Interpretability is limited due to the rather small dataset for CAP and the low numbers of participants for RAST. Summary estimates were well separated, but the confidence ellipsoids overlapped. CAP showed a slightly higher AUC (0.775 vs 0.709), which might indicate a marginally higher discriminatory power (see figure 2.27 in online supplementary appendix 2). Separate pooling of sensitivities of the larger HMW dataset, including all studies that at least provided results for reference standard positive subjects, yielded 0.73 (95% CI 0.61 to 0.82) for RAST and 0.84 (95% CI 0.73 to 0.91) for CAP (Δ 0.11). This might confirm the difference indicated by the bivariate calculation (Δ 0.19). The eligible LMW test data were insufficient to permit comparison using the bivariate approach. We generally considered potentially influential factors for sensitivity of LMW sIgE tests, for example, the individual allergens, to be unequally distributed within the test method groups; this invalidated the pooling of the results.

ELISA was used less frequently for both HMW and LMW allergens. Separately pooled sensitivities of 0.71 (95% CI 0.48 to 0.87) for HMW and 0.26 (95% CI 0.12 to 0.47) for LMW were at the lower ends of the CIs for the overall results (see figure 2.28–2.33 in online supplementary appendix 2). Different allergen-specific sensitivities in this subgroup and imprecision due to the lower number of included results could affect this disparity.

What is the test performance for recombinant or highly purified allergens?

Several studies have investigated the use of recombinant or highly purified natural components as well as the use of conventional extracts as allergens.

Three studies with few reference standard positive subjects reported the same results in subjects with OA with purified components and with the corresponding extracts. Tests with ovomucoid, ovalbumin and commercial egg white produced the same sensitivity as tests in which the extracts were used (0.8).³⁹ sIgE to conalbumin and lysozyme was less frequently found (the sensitivities were 0.6 and 0.4, respectively).³⁹ sIgE against the highly purified components ricin, ricinus agglutinin and dericinus of castor beans was detected in all three sera that were positive for the complete allergen, including one false-positive result.⁴⁰ An identical sensitivity of 1.0 was also reported for four subjects for the two enzymes alpha-amylase and lipase from porcine pancreatic extract.¹⁷

Other results demonstrated that components of soybean (highly purified Gly m 1/2 OD) and the fungus *A. alternata* (recombinant Alt a1) did not meet the test performance parameters of the extracts. While the sensitivity to soybean components

was very low (0–0.25) in four subjects, a sIgE test with recombinant Alt a 1 (rAlt a 1) that produced a sensitivity of 0.87 was reported for 15 subjects with proven allergic asthma.²⁵ One diseased subject with a negative result for rAlt a 1 had a positive CAP test to *Alternaria* extract.²⁵ Alt a 1 is exclusively located in the cell walls of spores of *A. alternata* and binding epitopes of this component for sIgE have been reported.⁴¹

The application of recombinant components in parallel with extracts using both reference standard positive and negative subject sera provided insight into the resolved sIgE-response pattern of OA caused by latex.

Unlike the crude latex extract, single recombinant components did not produce high sensitivity. However, all of the recombinant components exceeded the specificity of the crude extract, with the highest result of 0.96 for rHev b 1 and rHev b 11. The authors suggested better predictability for a positive SIC using the combination of results for sIgE against rHev b 5 plus b 6.01/6.02.⁴²

Purified bovine dander antigen BDA 20 elicited the same reactivity as bovine epithelial extract (100%) in 11 subjects and is denominated a major cow allergen.⁴³ Another study concluded that sIgE against purified Bos d 2 in nine subjects predicted the result of SIC better than RAST to a bovine standard allergen, but the authors did not provide a cut-off.⁴⁴ The RAST test standard allergen was presumably cow dander extract (however, this was not clearly stated in the manuscript). Bos d 2 and BDA 20 have the same 20 kDa molecular weight, and BDA 20 has now been identified as lipocalin.⁴⁵

Seven studies provided prevalences for sIgE against components of sera of selected subjects with exclusively positive sIgE results for the corresponding extracts. Studies of allergens contributing to baker's asthma reported single wheat components with prevalences of up to 61.54% (natural Tri a 14) in sIgE positive sera.⁴⁶ Prevalences for the wheat components Tri a 14 (range 44.44%–61.54%),^{46–48} thiol reductase (41.67%)⁴⁹ and WTAI-CM16 (53.33%)⁴⁸ were relatively high. A mixture of wheat components yielded the highest reactivity for baker's asthma with 63.64%.⁵⁰ For rye, most sera reacted to RAI-3 (33.33%).⁵¹

sIgE to durum wheat could not be detected in one of two subjects who had shown a positive SIC result against a mix of spring and durum wheat species.⁵²

Information on the individual tests is provided in table 2.6 in online supplementary appendix 2.

Standardisation

The number of in-house and commercial allergen preparation tests that were used in the quantitative analysis was equally balanced for HMW allergens. In contrast, the majority of 14 studies for LMW agents used in-house preparations, and there were only 7 commercial preparations. When authors used commercial tests, they predominantly relied on validation by the manufacturer, and of the 34 commercial tests, only 12 included controls for comparison of the results. The authors who used in-house allergen preparations validated the tests using heterogeneous measures. Controls were used to determine the positivity threshold for approximately half of the 67 in-house tests. The number of sera ranged from up to 5 in 4 cases to >20 in 10 cases.

In summary, 53 in-house tests reported an indiscriminate set of control groups with wide-ranging values. Of these groups, 19 included atopic and/or non-occupational allergic controls. Inhibition tests confirmed the specific binding of detectable sIgE

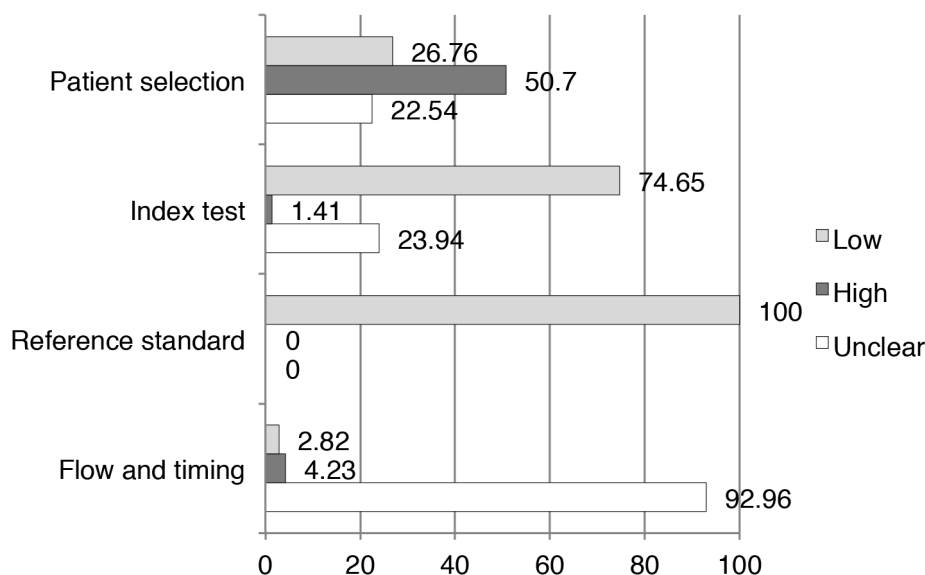


Figure 4 Results of the QUADAS-2 quality assessment for the risk of bias. The risk of bias for the four domains was rated as high, low or unclear in each study according to the QUADAS-2 guidance for assessment (online supplementary appendix 1, part 1.18). QUADAS, Quality Assessment of Diagnostic Accuracy Studies.

antibodies in the sera of eligible subjects for 38 tests. Validation with HSA and other substances was reported for half of the in-house tests, predominantly for LMW agents (see table 2.7 in online supplementary appendix 2).

Risk of bias in individual studies

We assessed the risk of bias systematically using the Quality Assessment of Diagnostic Accuracy Studies-2 tool³³ (see figure 4; for details online supplementary appendix 2, table 2.8).

Patient selection

Assessment of risk of bias through patient selection resulted in the most frequent rating of high risk of all domains for over 50% of the included studies. Only 26.76% of the studies were associated with low risk, and the remaining studies did not provide sufficient information.

Signalling questions indicated in detail that the main adverse issues related to selection were confirmed OA with proven sensitisation to the causative antigen at baseline and the sampling method. Selective inclusion of subjects with a confirmed diagnosis occurred in 24 studies. A positive sIgE test for the causative antigen as an obligatory inclusion criterion was only accepted for individual components, and a positive result as an optional criterion was only present in three of the quantitatively analysed studies. However, just 8 of 16 studies that failed to avoid a positive specific sIgE test and/or SPT result were entered into the meta-analysis and effectively raised the risk of selection bias. Sampling was unclear in a majority of 40 studies that might include convenient portions that could have increased the risk of selection bias, whereas sampling was appropriate (consecutive or random) in 29 studies.

Index test

Risk of bias introduced by the index test was determined to be low in 74.65% of studies, representing the presence of a predefined positivity threshold in the majority of studies. Risk was unclear in 23.94% of the studies and high in just 1.41%.

Reference standard

A completely low risk of bias through the use of a reference standard reflected the corresponding predefined inclusion criterion described in the definition of the eligible diagnostic methods. The majority of 64 studies used SIC with occupational agents as a reference standard. Four studies used serial peak expiratory flow (PEF) only, two studies used SIC/workplace challenge or serial PEF and one study performed both SIC/workplace challenge and serial PEF for each participant.

We considered the results of both the index test and the reference standard adequately objective and excluded blinding from the quality assessment.

Flow and timing

The domain flow and timing resulted in a predominantly unclear risk of bias for 92.96% of studies, mainly due to a lack of reporting of the interval between the reference and the index test. Apart from four studies that introduced a high risk with partial and/or differential verification, bias was low.

Risk of bias across studies

Multiple publication bias

A rigorous screening minimised the risk of multiple publication bias (for details, see online supplementary appendix 2, part 2.3).

For additional information on citation bias, publication bias, applicability, handling of test results from the same population and heterogeneity, see online supplementary appendix 2, part 2.10.

DISCUSSION

Limitations

Taking into account the limitations of the studies, the rigorous application of the eligibility criteria provided an extensive set of data and a differentiated analysis that covers sIgE tests for various causative allergens of OA. The full limitations section can be found in online supplementary appendix 3.

Implications for diagnosis

Relevant and reliable performance over a wide range of HMW allergen tests is evident and verifies the recommended role of these tests in diagnostic workups.⁷ Despite low sensitivity for LMW allergens, positive results for both HMW-sIgE and LMW-sIgE tests are strongly indicative of OA when used in combination with corresponding clinical and occupational case histories. In doubtful situations in which there are contradictory, missing or unclear findings, serial peak flow with spirometric measurements conducted in the workplace and on days off and/or a specific inhalation challenge test in the workplace are to be recommended.

Implications for research

Further identification and assessment of the relevant components of HMW allergens might lead to improvements in the diagnostic value and standardisation of sIgE tests for OA.

Promising methodological approaches involving LMW allergen sIgE tests that generated higher sensitivity could be reproduced with appropriate numbers of subjects, for example, by the use of isocyanate-HSA compounds prepared more physiologically in vapour form (instead of in solution). Additionally, research on alternative in vitro tests with the potential for higher discriminatory power, such as cellular approaches,^{54 55} requires investigation.

The evaluation of a composite diagnostic approach with sIgE testing and, for example, lung function testing in the workplace should be considered as an alternative to specific inhalation challenge tests.

For a discussion of further aspects, see the supplementary material in online supplementary appendix 3.

CONCLUSIONS

sIgE tests for HMW allergens have moderate discriminatory power when they have pooled estimates of sensitivity and specificity of approximately 0.7. The use of several groups of allergens produced better results than the pooled estimates, and some groups of allergens produced a lower performance that requires deeper consideration of the pertinence of the approach, for example, rat and mouse danders.

The results for LMW allergens indicate poor discriminatory power with generally low heterogeneous sensitivities and high homogeneous specificities. A positive result is highly indicative of OA, whereas a negative test cannot exclude the condition due to the high incidence of false negative results. Possible exceptions are sIgE reactions against anhydrides and chloramine T.

As a recent option, CAP is an appropriate method, and ELISA may offer a cheaper alternative without substantial loss of test performance.

Component-resolved analysis provides additional information that can be used in the diagnosis of OA caused by some HMW allergens; sIgE testing for individual components can be used to increase specificity and combined with component-specific results to improve predictions from specific inhalation challenge tests. When identified and assessed for their particular test performance and allergenic relevance, suitable components can be added to an extract to enhance test accuracy. This approach was successfully applied to latex-allergic subjects, but it was unsuccessful in the diagnosis of wheat/rye allergies.

A common standard for the validation of sIgE tests has thus far resisted establishment. However, now is the time for reliable and comparable results with in-house as well as commercially available testing to become routine.

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