

Original article

Assessing skin prick tests reliability in ECRHS-I

Introduction: Atopy, the clinical definition of an immunoglobulin E (IgE) high-responder, can be documented either by the detection of IgE antibodies in serum or by a positive skin prick test. Epidemiological studies use different reaction sizes to define a skin test reaction as positive or negative. The aim of the study was to identify the best cut-off level for skin prick tests.

Method: Using the data collected during the European Community Respiratory Health Survey (ECRHS I) the association of serum allergen-specific IgE and skin prick tests [*Dermatophagoides pteronyssinus* (Der p), cat, timothy grass and *Cladosporium*] were assessed.

Results: The proportion of the 11 355 subjects (49.9% men), with positive skin tests ranged from 10.4% (cut-off > 5 mm) to 20.9% (cut-off > 0 mm) for Der p, 3.5–10.2% for cat, 9.3–16.6% for timothy grass and 0.2 and 2.3% for *Cladosporium*. For identifying subjects with detectable specific IgE (> 0.35 kU/l) the most appropriate cut-off appeared to be over 0 mm for Der p, cat and timothy grass (Youden Index over 0.81). However, the relationship between serum IgE and skin prick test for *Cladosporium* was weak (Youden index under 0.56).

Conclusion: In epidemiological studies, a single method may be chosen to assess allergenic sensitivity. A cut-off level of over 0 mm for skin prick tests was best at identifying those with allergen-specific IgE.

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Atopy, the clinical definition of an immunoglobulin E (IgE) high-responder, can be documented either by the detection of IgE antibodies in serum or by a positive skin prick test (1). Serum-specific IgE is considered positive if the level is higher than the lowest detection limit of the assay. However, some patients with a true IgE-mediated allergic reaction tests may have undetectable serum-specific IgE (2). Selection of a reaction size at which to declare a skin test reaction as positive requires a compromise between acceptable levels of sensitivity and the specificity of the test. Reactions regarded as indicative of clinical allergy are usually over 3–5 mm in diameter (2–5) although there are some earlier propositions that small wheals of 1–2 mm with flare and itching represent a positive immunologic response and there is also the presence of specific IgE antibodies without clinical relevance (6). Other definitions of positivity exist, including the ratio of histamine wheal size to the allergen wheal size (2, 7), a summary index combining the reactions to several antigens (8), and the area of the wheal or erythematous reaction (9).

Skin prick tests and serum-specific IgE do not have the same biological and clinical relevance and are not interchangeable (10, 11). Both methods examine the

existence of sensitization to given allergens, although their presence does not always coincide with clinically significant allergic disease. Skin prick tests (mean wheal diameter over 3 mm) have been shown to be more sensitive but less specific than serum IgE tests to diagnose allergy in subjects presenting with asthma or rhinitis (2, 12). This lower sensitivity of serum IgE has been observed with both radioallergo sorbent test and with the CAP System (Pharmacia Diagnostics, Uppsala, Sweden).

The advantage of skin prick tests is that multiple tests can be performed relatively inexpensively but it is difficult to assess the most clinically relevant definition of positivity. On the other hand, while it is easier to achieve standardization of serum-IgE measurements, the expense of the test means that only a few allergens can be tested.

In the ECRHS, a study limited to the three UK centres has suggested the use of a cut-off point of > 0 mm, based on the greatest reproducibility of this cut-off when skin tests when carried out several times (13). However, in the present paper, we have used the data collected during European Community Respiratory Health Survey (ECRHS I), to define the best cut-off level for skin tests using serum-specific IgE as the gold standard.

Materials and methods

Population and inclusion criteria

The population of this study is based on the ECRHS I, a multi-centre international cross-sectional survey of the general population. Populations and subjects participating in the study have been described elsewhere (14, 15). Only centres which performed at least one serum allergen-specific IgE and one skin prick tests were included in the study.

Serum-specific IgE and skin prick tests

Serum-specific IgE levels were measured using the CAP System® (Pharmacia Diagnostics) in a central laboratory (16) (except for the Australian centre). The allergen-coated Phazet® (Pharmacia Diagnostics) was used for skin testing (17). A histamine positive control and a saline negative control were also skin tested to reduce false positives and negatives. The performance of skin tests is described in the supplementary file. Four allergens [*Dermatophagoides pteronyssinus* (Der p), timothy grass pollen and cat; *Cladosporium herbarum*] were tested. Any additional test performed using only one of the methods has not been included in the present study.

Assessing specificity and sensitivity of skin test in the ECRHS I survey

The association of serum allergen-specific IgE and skin prick tests for the four allergens tested using both methods was assessed. Serum allergen-specific IgE was considered as positive if over 0.35 kU/l and was considered as the gold standard. The sensitivity, specificity and negative and positive predictive values of the skin prick tests were then assessed for different cut-off levels (0, 3 and 5 mm, ratio of the size of the test induced by the allergen to the size that elicited by histamine over 1).

Statistical analysis

To describe the population, qualitative variables were assessed using frequencies and percentages. As the distribution of quantitative variables (age, serum-specific IgE measurement, mean wheal diameter of skin prick tests) was not normal (using Shapiro-Wilk normality test and Skewness and Kurtosis tests), median and 25 and 75 percentiles were used for quantitative variables. Sensitivity and specificity of skin tests were expressed in percentages with 95% confidence intervals, as well as negative and positive predictive value. To assess predictive values, we needed to estimate in the general population the prevalence of subjects with positive serum allergen-specific IgE for the four main allergens. As the ECRHS study was a multi-centred survey performed in the general population, the prevalence was considered to be equal to that of the general population. Finally, a measure of the Youden index (sensitivity + specificity – 1) was assessed to estimate the optimal cut-off points of skin prick tests. The statistical analysis was performed using STATA 9.1® (Statacorp, College Station, TX, USA). The *P*-value for normality tests was considered significant if under 0.05.

Results

Participants

Overall, 18 794 participants in 37 centres and 17 countries, had undergone at least one serum allergen-specific IgE measurement or one skin prick test for the four most common allergens (Fig. 1). Among them, 692 subjects had to be excluded because of a variation in the protocol in the centre. In one centre, skin prick tests were not performed with the Phazet® (Pharmacia Diagnostics), and one had a technical failure (the major part of subjects had a positive negative saline control). Thus, the analysis

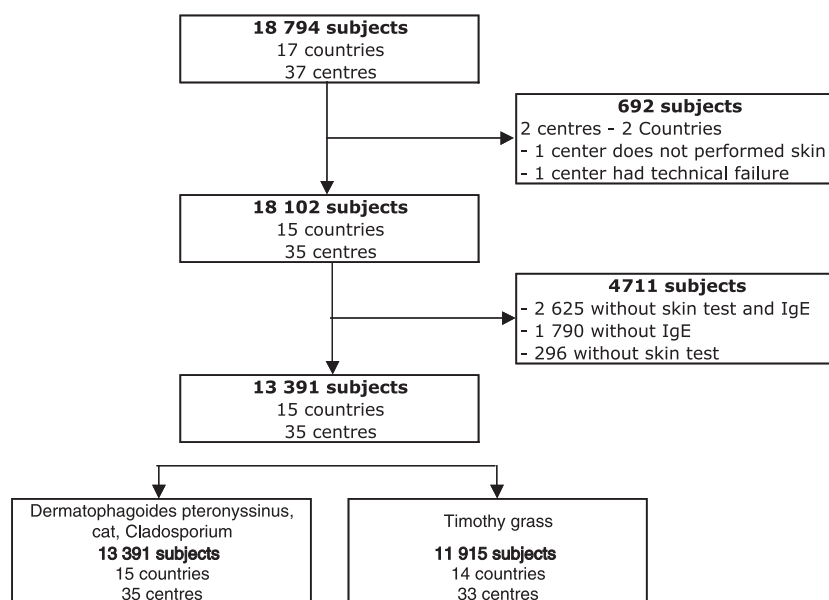


Figure 1. Flow chart of participants for the four most common allergens.

took into account only 35 centres in 15 countries. Moreover, 4711 other subjects were excluded: 2625 because either skin prick tests or serum allergen-specific measurements were not performed, 296 because of lack of all the skin prick tests, 1790 because of lack of serum-specific IgE measurements. These subjects came from all centres. Finally, the two centres from Germany could not perform timothy grass skin prick test because of registry restrictions. Analyses involving this allergen were therefore restricted to 33 centres from 14 countries (11 915 participants).

The median age of the 13 391 subjects [6750 (50.4%) men and 6641 (49.6%) women] studied was 33.8 (27.8–40.0) years. Only 1.4% of subjects had current asthma (attack of asthma within a year and/or used of medication for asthma); 23.0% had allergic rhinitis and 3.3% had both current asthma and allergic rhinitis.

Cut-off level of skin test positivity

Using different cut-offs, the proportions of positive skin prick tests in the study group ranged from 9.7% (cut-off > 5 mm) to 20.4% (cut-off > 0 mm) for Der p, 3.4–10.1% for cat, 9.6–17.1% for timothy grass pollen and 0.2–2.2% for *Cladosporium* (Table 1). When the ratio of the size of the test induced by the allergen to the size of that elicited by histamine was used, the findings are similar to those with a cut-off of 5 mm, except for *Cladosporium* the prevalence of which was > 0 mm cut-off (2.7%).

Using a positive serum allergen-specific IgE level > 0.35 kU/l as the gold standard, sensitivity and specificity for the skin prick test cut-off levels at 0 or 3 mm were good for Der p, cat and grass pollen (sensitivity was

over 53% and specificity over 93%, Youden index over 0.76; Table 2). Using another definition of skin prick test positivity (mean wheal diameter 5 mm or the ratio of the size of the test induced by the allergen to the size of that elicited by histamine) small increases in specificity were associated with substantial decreases in sensitivity. Sensitivity was poor for *Cladosporium* irrespective of skin prick tests definition (Youden index always under 0.55).

Discussion

Based on a study involving more than 11 000 subjects we have shown that, for an epidemiologic study, a mean wheal diameter of > 0 mm is the most appropriate definition of positive skin prick tests to assess allergic sensitization.

The aim of the study was an attempt to assess the optimal cut-off for skin prick tests for four allergens, assuming that the gold standard was serum allergen-specific IgE. Comparisons between *in vivo* and *in vitro* methods depend upon the quality and standardization of allergens used in both tests and to a lesser extent the method of skin testing used (2). In the ECRHS study, trained interviewers performed skin prick tests, and allergens of the same manufacturer standardized identically (Phazet and immunoCAP; Pharmacia Diagnostics) were used for both skin tests and measurement of specific IgE in serum. The allergens were prepared identically for both and it is unlikely that there are large differences between extracts, even though *Cladosporium* is a poorly known allergen and there may be some differences between batches used for the two tests. On the other hand, the binding of the allergens to the metal of Phazet and the solid phase of the IgE test may lead to some differences between tests. However, to our knowledge this has never been tested.

Thus, it is unlikely that differences are related to variability in allergens. Receiver operating characteristics curves are used in biomedical research to evaluate the effectiveness of biomarkers for distinguishing individuals with disease from those without. The Youden index, a function of sensitivity and specificity, is a commonly used measure of overall diagnostic effectiveness (18). These two methods identify the same cut-point as 'optimal' in certain situations but different cut-points in others. In the present study, we selected Youden index as the 'optimal' cut-point for given weighting with respect to the overall misclassification rate (19). Moreover, differentiating 0.3 from 0.4 mm was not useful, measurement error being too important for such precision. Thus, a mean wheal diameter of > 0 mm was found to be the most appropriate definition of positive skin prick tests to assess allergic sensitization for three main allergens (Der p, cat and timothy grass pollen), based on the Youden index. With a > 0 mm cut-off, specificity was over 93.1% and sensitivity over 63.4%. This is in accordance with the previous study

Table 1. Proportion of positive skin tests depending on definition of positivity

Skin tests	Negative	Positive
<i>Dermatophagoides pteronyssinus</i> , mm (%)		
0	10658 (79.6)	2732 (20.4)
3	11161 (83.4)	2229 (16.7)
5	12097 (90.3)	1293 (9.7)
Histamine	11946 (89.2)	1443 (10.8)
Cat, mm (%)		
0	12037 (89.9)	1354 (10.1)
3	12407 (92.7)	984 (7.4)
5	12935 (96.6)	456 (3.4)
Histamine	12678 (94.7)	712 (5.3)
Timothy grass, mm (%)		
0	9880 (82.9)	2036 (17.1)
3	10170 (85.4)	1746 (14.7)
5	10770 (90.4)	1146 (9.6)
Histamine	10589 (88.9)	1326 (11.1)
<i>Cladosporium</i> , mm (%)		
0	13097 (97.8)	294 (2.2)
3	13246 (98.9)	145 (1.1)
5	13364 (99.8)	27 (0.2)
Histamine	13023 (97.3)	367 (2.7)

Per cent: row per cent.

Table 2. Distribution of skin tests depending on cut-off size and the presence of serum allergen-specific IgE

	0 mm		3 mm		5 mm		Histamine	
IgE	–	+	–	+	–	+	–	+
<i>Dermatophagoides pteronyssinus</i>								
Negative	9934	735	10250	419	10538	131	10284	384
Positive	724	1997	911	1810	1559	1162	1662	1059
Sensitivity (%)	73.4 (72.7–74.1)		66.5 (65.7–67.3)		42.7 (41.9–43.5)		38.9 (38.1–39.7)	
Specificity (%)	93.1 (92.7–93.5)		96.1 (95.8–96.4)		98.8 (98.6–99)		96.4 (96.1–96.7)	
Positive PV (%)	73.1 (71.4–74.8)		81.2 (79.6–82.8)		89.9 (88.2–91.5)		73.4 (71.1–75.7)	
Negative PV (%)	93.2 (92.7–93.7)		91.8 (91.3–92.3)		87.1 (86.5–87.7)		86.1 (85.5–86.7)	
% Correct class	89.1		90.1		87.4		84.7	
Kappa static	0.66 ± 0.01		0.67 ± 0.01		0.52 ± 0.01		0.43 ± 0.01	
Youden index	0.83		0.81		0.71		0.68	
Cat								
Negative	11583	569	11836	316	12077	75	11753	398
Positive	454	785	571	668	858	381	925	314
Sensitivity (%)	63.4 (62.6–64.2)		53.9 (53.1–54.7)		30.8 (30–31.6)		25.3 (24.6–26)	
Specificity (%)	95.3 (94.9–95.7)		97.4 (97.1–97.7)		99.4 (99.3–99.5)		96.7 (96.4–97)	
Positive PV (%)	58 (55.3–60.6)		67.9 (65–70.8)		83.6 (80.2–87)		44.1 (40.5–47.7)	
Negative PV (%)	96.2 (95.9–96.6)		95.4 (95–95.8)		93.4 (92.9–93.8)		92.7 (92.3–93.2)	
% Correct class	92.4		93.4		93		90.1	
Kappa static	0.56 ± 0.01		0.57 ± 0.01		0.42 ± 0.01		0.27 ± 0.01	
Youden index	0.79		0.76		0.65		0.61	
Timothy grass								
Negative	9205	422	9366	261	9533	94	9245	381
Positive	675	1,614	804	1,485	1,237	1,052	1,344	945
Sensitivity (%)	70.5 (69.7–71.3)		64.9 (64–65.8)		46 (45.1–46.9)		41.3 (40.4–42.2)	
Specificity (%)	95.6 (95.2–96)		97.3 (97–97.6)		99 (98.8–99.2)		96 (95.6–96.4)	
Positive PV (%)	79.3 (77.5–81)		85.1 (83.4–86.7)		91.8 (90.2–93.4)		71.3 (68.8–73.7)	
Negative PV (%)	93.2 (92.7–93.7)		92.1 (91.6–92.6)		88.5 (87.9–89.1)		87.3 (86.7–87.9)	
% Correct class	90.8		91.1		88.8		85.5	
Kappa static	0.69 ± 0.01		0.68 ± 0.01		0.56 ± 0.01		0.44 ± 0.01	
Youden index	0.83		0.81		0.73		0.69	
<i>Cladosporium</i>								
Negative	12690	240	12831	99	12915	15	12578	351
Positive	407	54	415	46	449	12	445	16
Sensitivity (%)	11.7 (11.2–12.2)		10 (9.5–10.5)		2.6 (2.3–2.9)		3.5 (3.2–3.8)	
Specificity (%)	98.1 (97.9–98.3)		99.2 (99–99.4)		99.9 (99.8–100)		97.3 (97–97.6)	
Positive PV (%)	18.4 (13.9–22.8)		31.7 (24.1–39.3)		44.4 (25.7–63.2)		4.4 (2.3–6.4)	
Negative PV (%)	96.9 (96.6–97.2)		96.9 (96.6–97.2)		96.6 (96.3–96.9)		96.6 (96.3–96.9)	
% Correct class	95.2		96.2		96.5		94.1	
Kappa static	0.12 ± 0.01		0.14 ± 0.01		0.05 ± 0.01		0.01 ± 0.01	
Youden index	0.55		0.55		0.51		0.5	

Sensitivity, specificity, positive and negative predictive values (PV) are given in per cent with 95% confidence intervals.

performed in three English centres which showed that a cut-off of >0 mm was the most reproducible between fieldworkers (13).

The relationship between serum IgE and skin prick test for *Cladosporium* was weak (Youden index under 0.55) for unknown reasons. It is clear that mould allergens are largely uncharacterized, but as the same allergen was tested *in vivo* and *in vitro*, similar results would have been expected. Using other definitions of skin prick tests positivity (mean wheal diameter of 3 and 5 mm or the ratio of the size of the test induced by the allergen to the size of that elicited by histamine), small increases in specificity were associated with

substantial decreases and sensitivity and a fall in the Youden index.

Even if >0 mm seems to be the best cut-off, it is important to note that predictive values depend on the prevalence of sensitization in the population being considered. The positive predictive value will (in this range of results) be better with a high cut off and the negative predictive value with a low cut off. The proportion of subjects correctly classified will vary: a low cut off will tend to do relatively better in populations with a higher prevalence of sensitization (e.g. in clinics, ironically) and the high cut off will tend to do relatively better at low prevalences. In populations with a prevalence of

sensitization of 10% the > 3 mm wheal correctly classifies more people but at 20% the > 0 mm prevalence correctly classifies a greater proportion of the population (which is reflected in Table 2).

The present study is consistent with the suggestion of Pepys (6) that small wheals of 1–2 mm with flare and itching represent a positive immunological response and indicate the presence of specific IgE antibodies. Moreover, in this epidemiological study, the agreement between IgE and skin prick-puncture tests is similar to clinical studies (agreement ranges between 85% and 95% depending on allergens) (2, 20–22). The appropriate cut-off level for skin tests may however differ depending on the purpose of the test. In clinical practice skin prick tests are usually considered as indicative of clinical allergy when the mean wheal diameter is over 3–5 mm in diameter (2–5), but this is based on an association with clinically relevant symptoms believed to be because of the allergen. In epidemiological studies, the interest is more often on whether the patient has been sensitized.

In epidemiological studies, a cut-off level of over 0 mm for skin prick tests appeared to be the most discriminative. However, there are subjects with allergic sensitization as demonstrated by serum measures of allergen-specific IgE who have negative skin tests.

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Skin prick tests technique. The following procedure was adopted for skin testing. A skin test grid for application of Phazets was applied to the volar aspect of the forearm and taped. The lancet was held at 90° to the skin and pressed against the skin with the forefinger for at least 1 s. The lancet was withdrawn and results were read 15 min later by drawing around the perimeter of the wheal with a ballpoint pen, placing transparent tape against the skin and transferring the prints to a data collection sheet. The transferred image of the wheal was measured at its widest point and at 90° to the

diameter at its midpoint. Both diameters were recorded to the nearest whole millimetre and the mean of the two determined (mean wheal diameter).

Two strategies were adopted to reduce measurement error:

- 1 Prior to starting the study each fieldworker was required to perform at least two histamine tests on 20 volunteers and measure them following the protocol given above to achieve a coefficient of variability of <30%.

- 2 Each centre was visited during the study and five sample wheal images were measured by each fieldworker. Where the wrong method of measurement was being used (e.g. the two diameters not at right angles) fieldworkers were asked to review the records of all tests and re-measure the wheal images that had been kept on the transparent tape.

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