

Optimization of skin testing

II. Evaluation of concentration and cutoff values, as compared with RAST and clinical history, in a multicenter study*

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In this multicenter study we evaluated the results of a previous study (part I) in a relatively large Dutch patient population, using the two previously tested allergens (house-dust mite and grass pollen) and two other standardized allergens (tree pollen and cat dander). The obtained skin test results were expressed as a histamine ratio and compared with RAST and clinical history (CH). The sensitivity and specificity were calculated at different cutoff values of the skin tests. The optimum cutoff values of 0.7 intracutaneous tests (ICT) and 0.4 skin prick tests (SPT) resulted in a predictive value for the detection of allergic sensitization of 83% (RAST) and 77% (CH), and 91% (RAST) and 86% (CH), for the ICT and SPT, respectively. As the ICT and SPT were performed in different centers, the results of these methods cannot be compared. No systemic side-effects of the skin tests were recorded. These results generally correspond well with the predictions regarding safety and predictive value of part I of this study, in which a limited number of patients was studied. In conclusion, through the use of a limited number of standardized allergens in a small group of patients, it may be possible to predict a safe and efficacious concentration for routine skin testing and to extrapolate from these results to other standardized allergens.

Clinical aspects

In this multicenter study we tested the optimum skin test concentrations of allergens calculated in part I of the study and further validated the cutoff values. Two allergens (house-dust mite and grass pollen) tested in part I, and two other standardized allergens (tree pollen and cat dander) were studied at concentrations of 30 BU/ml for intracutaneous tests (ICT) and 3000 BU/ml for skin prick tests (SPT). The wheal size, expressed as a histamine ratio (HEWS), gave the greatest diagnostic capacity for the detection of allergic sensitization, with cutoff values of 0.7 (ICT) and 0.4 (SPT). The data suggested that the allergen concentration of 3000 BU/ml for SPT was suboptimal. By this method, total predictive values of 83% and 91% were obtained, as compared with RAST, and 77% and 86%, as compared with clinical history, for ICT and SPT, respectively. There were no systemic side-effects in the 497 (ICT) and 361 (SPT) patients undergoing skin testing. The test indicators corresponding to optimal skin test performance in a small group of patients (part I) were seen to give a good diagnostic capacity and safety in a large multicenter patient population.

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The diagnosis of allergy largely depends on the clinical history (CH) and usually needs to be confirmed by other methods, such as skin tests and specific IgE determinations (RAST). For an optimal diagnostic capacity of a skin test, it is probably advantageous to use standardized allergens in combination with a well-evaluated method (7). However, the concentration used of the allergen and the cutoff value also determine the predictive value obtained for detection of sensitization.

Only a few authors report on the predictive value of the skin prick test (SPT) (8, 9, 11, 14, 17). Most authors use a cutoff limit of 3 mm (4, 6, 9, 11, 17); others use 1 mm (19) and 4 or 5 mm (2, 14). A predictive value of 40–90% was reported. Little is known about the criteria for a positive intracutaneous skin test (ICT). In a few studies, a wheal greater than 5 mm was used as the criterion for positivity (1, 3, 18), but none of these studies have estimated the cutoff value of the ICT more precisely. A few authors report poor results of the ICT (3, 4, 15, 18, 19), while one author reports a concordance between ICT and RAST of 80% (1), using birch pollen, timothy pollen, and dog epithelium allergens. None of these studies have related the CH to the outcome of the ICT.

In this multicenter study, we tested the optimum concentrations of allergen and the cutoff values of ICT and SPT calculated in the first part of the study, using two identical and two other standardized allergens in the same concentrations. The hypothesis we set out to test in this second part of the study was that we could predict the diagnostic precision for the detection of allergic sensitization and the safety of the skin tests by the results of a small patient population and a limited number of allergens studied in part I.

Material and methods

Patients and study design

Patients who had been newly referred to specialists of six (ICT) centers, and six other (SPT) allergy centers in The Netherlands, were selected for the ICT (497) or SPT (361). The age range of the tested patients was 15–78 years (mean 37.6 years) (ICT) and 15–76 years (mean 39.5 years) (SPT).

The study was conducted between April 1987 and August 1988. Antihistamines and oral corticosteroids were withheld for 48 h before skin testing, in accordance with the report on skin test standardization (12).

An allergen-specific CH was recorded before skin testing and graded as one of the following: 0) negative; 1) doubtful; 2) positive. When possible, specific IgE determinations were performed for

house-dust mite, grasses, tree pollen, and cat dander by Phadebas RAST (Pharmacia AB, Uppsala, Sweden). The results were divided into five RAST classes, and RAST class ≥ 1 was regarded as positive.

Allergen preparations

Standardized extracts (ALK, Hørsholm, Denmark, obtained through Laboratorium Diephuis BV, Groningen, The Netherlands) of five grasses (*Alopecurus pratensis*, *Dactylus glomerata*, *Lolium perenne*, *Phleum pratense*, and *Festuca pratensis*), three trees (*Alnus glutinosa*, *Betula verucosa*, and *Corylus avellana*), house-dust mite (*Dermatophagoides pteronyssinus*), and cat dander were used throughout the study in concentrations of 30 BU/ml (ICT) and 3000 BU/ml (SPT). All allergen extracts were dissolved in phosphate-buffered saline pH 7.4, and contained 0.5% phenol with 0.03% human serum albumin (ICT) or 50% glycerol (SPT). The solutions were kept at 4–8°C for a maximum of 3 (ICT) and 6 (SPT) months and used at ambient temperature.

Skin testing

The positive control was histamine phosphate (base) 0.01 mg/ml (ICT) and histamine dihydrochloride (base) 3 mg/ml (SPT); the diluent was used as a negative control. The ICT and SPT were performed as described in part I of this study. The results were expressed as histamine-equivalent wheal sizes (HEWS); the mean wheal diameter (MWD) divided by the mean histamine wheal size.

Statistical analysis

All statistical parameters were calculated, and Student's *t* test and the one-way variance analysis were performed by an SPSS/PC + computer program (13). Correlations were expressed as Spearman's *rho*.

The sensitivity and specificity of SPT and ICT, as compared with RAST or CH, were calculated at four different cutoff values by means of HEWS. The cutoff values used were 0.5, 0.7, 1.0, and 1.2 and 0.4, 0.5, 0.75, and 1.0 for ICT and SPT, respectively. From the results of these calculations, an optimal cutoff value representing the highest sensitivity and specificity was estimated.

The predictive value of the different methods and the number of false positive (FP) and false negative (FN) results were also calculated. In these calculations, ICT HEWS > 0.7 , SPT HEWS > 0.4 , RAST class ≥ 1 , and CH = 2 were regarded as positive results.

Table 1. Correlations (Spearman's ρ) between ICT/SPT and RAST or CH calculated for the wheal and wheal index

		ICT		SPT		RAST
		MWD	HEWS	MWD	HEWS	
Grasses	RAST	0.82	0.81	0.77	0.80	0.49
	CH	0.50	0.50	0.66	0.68	
Trees	RAST	0.59	0.51	0.77	0.82	0.51
	CH	0.27	0.28	0.46	0.46	
DP ¹	RAST	0.70	0.70	0.83	0.86	0.42
	CH	0.28	0.28	0.63	0.62	
Cat	RAST	0.64	0.65	0.87	0.88	0.43
	CH	0.44	0.46	0.61	0.61	

¹ *Dermatophagoides pteronyssinus*.

Results

In this study the mean histamine wheal diameter was 10.6 mm (range 9.9–11.8 mm, CV (coefficient of variation) 16.2%) for ICT, and 5.6 mm (range 4.8–6.6, CV 29.0%) for SPT. In 95.8% (ICT) and 97.3% (SPT) of the patients, the negative control gave no reaction. In the other patients, the mean wheal di-

ameter of the negative control was 4.5 mm (range 2.5–8.5, CV 32.4%) for ICT and 2.8 mm (range 1.0–7.0, CV 60.7%) for SPT.

Because of a wheal size ≥ 5 mm (ICT) or ≥ 2.5 mm (SPT) to the negative control, the results of 8 (ICT) and 14 (SPT) were excluded. No patients had a wheal size of the positive control < 5 mm.

In Table 1 the correlations (Spearman) between

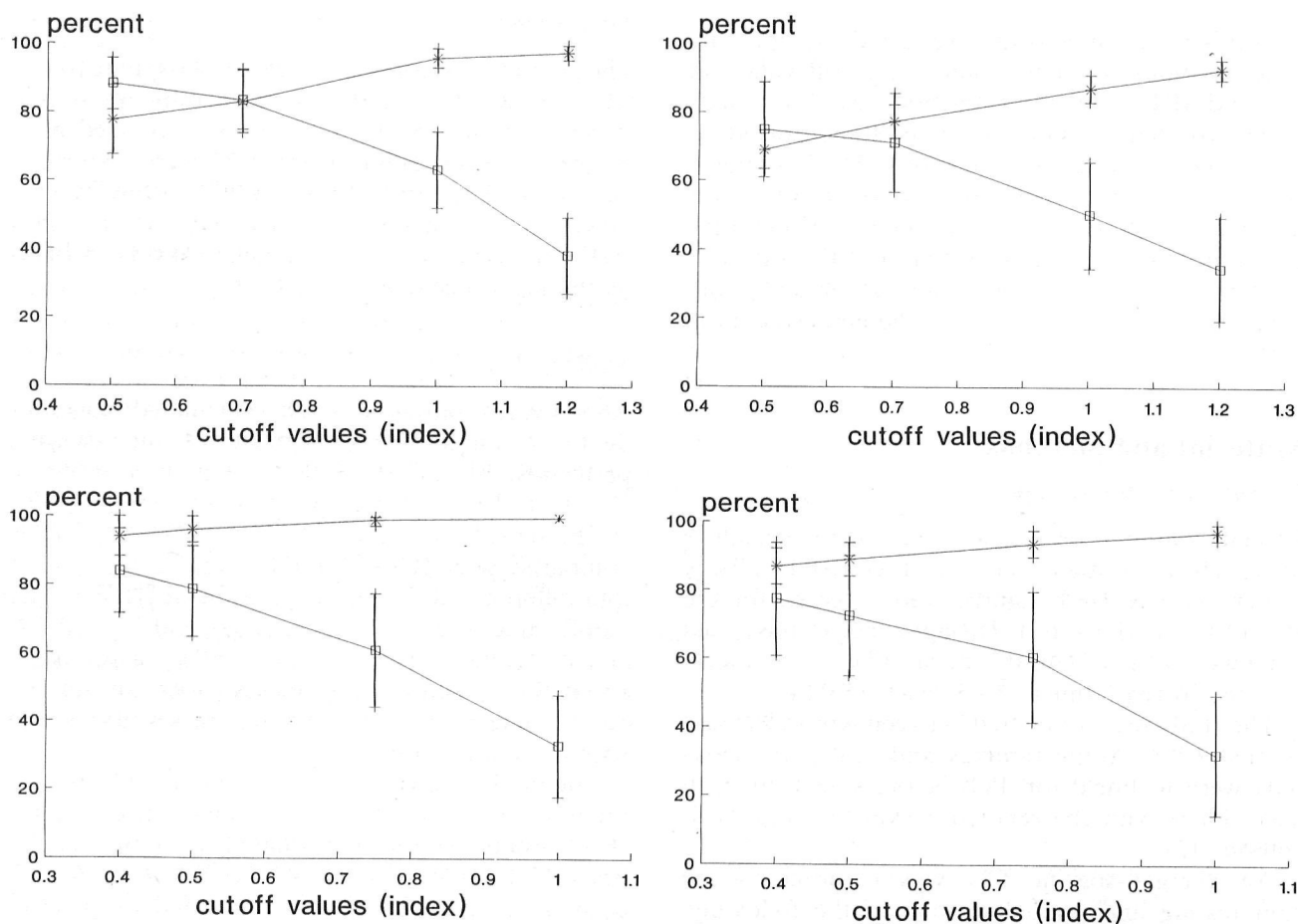


Fig. 1. Sensitivity (□), specificity (*) and confidence limits (95%) of ICT, as compared with RAST (A) and CH (B); and SPT, as compared with RAST (C) and CH (D), for different cutoff values.

Table 2. Correlation between ICT, SPT, and RAST

No. of patients	ICT		SPT		
	Neg	Pos	Neg	Pos	
RAST Neg	69	4	56	4	Grasses
Pos	15	62	13	28	
Neg	17	2	61	2	Trees
Pos	2	2	3	12	
Neg	46	26	61	5	DP ¹
Pos	12	85	3	52	
Neg	88	16	48	3	Cat
Pos	9	42	2	28	

¹ *Dermatophagoides pteronyssinus*.

Table 3. Correlation between ICT, SPT, RAST, and CH

No. of patients	ICT		SPT		RAST		
	Neg	Pos	Neg	Pos	Neg	Pos	
CH Neg	195	54	123	21	114	67	Grasses
Pos	12	39	5	30	7	47	
Neg	233	27	135	15	66	12	Trees
Pos	8	11	3	7	0	8	
Neg	143	94	127	22	90	76	DP ¹
Pos	20	48	13	48	18	61	
Neg	206	47	136	20	131	46	Cat
Pos	7	28	7	21	8	28	

¹ *Dermatophagoides pteronyssinus*.

ICT/SPT and RAST and CH for both MWD and HEWS are depicted. The correlation between RAST and CH was also calculated. SPT has a good correlation with RAST (0.7–0.9) and a moderate correlation with CH (0.5–0.7). ICT also has a good correlation with RAST (0.6–0.8), and a moderate correlation with CH (0.3–0.5). The correlation between RAST and CH ranged between 0.4 and 0.5.

The sensitivity and specificity of SPT and ICT at different cutoff values are depicted in Fig. 1. The mean standard deviation ranged from 3.8% to 9.7%, for the sensitivity, and from 1.2% to 5.1%, for the specificity. The optimal cutoff values were 0.7 and 0.4 for ICT and SPT, respectively, at equal sensitivity and specificity. The concordance between the different methods and the amount of false positive (FP) and false negative (FN) results are presented in Tables 2, 3, and 4. As can be seen from Tables 2 and 3, RAST and ICT give a relatively high value of FP results, as compared with CH. With all four allergens, SPT gives a relatively low value of FN and FP results.

Table 4 shows that the overall concordance between ICT and RAST was 83%, and between ICT and CH, 77%. For SPT, the concordance was 91% and 86% with RAST and CH, respectively. The

concordance between RAST and CH was 73%. For all allergens the concordance between ICT and RAST or CH was high, except for the concordance between ICT and CH for house-dust mite (63%). SPT had a high concordance for all four allergens. RAST also had a high concordance for all allergens, except for house-dust mite (62%).

All mean wheal sizes of the allergens were smaller than 30 mm (ICT) and 15 mm (SPT), and no systemic side-effects were reported.

Table 4. Concordance between ICT, SPT, RAST, and CH

Concordance (%)	ICT	SPT	RAST	
RAST	87	83	–	Grasses
CH	78	86	69	
RAST	83	94	–	Trees
CH	88	89	86	
RAST	78	93	–	DP ¹
CH	63	83	62	
RAST	84	94	–	Cat
CH	81	85	75	
Mean				
RAST	83	91	–	
CH	77	86	73	

¹ *Dermatophagoides pteronyssinus*.

Discussion

In this paper, we evaluated the results of the first part of this study in a relatively large patient population, using four standardized allergens.

When one performs a skin test, it may be important to use well-standardized allergens in combination with a well-described technique to obtain reproducible results (7). In recent years little attention has been given to the use of optimum test concentrations of allergens.

In part I of this study, it was concluded that the optimum concentration of the ICT was 30 BU/ml; this concentration was 3000 BU/ml for the SPT.

In the evaluation of a skin test, it is also important to establish an optimum cutoff value at which an optimum concordance is obtained with other allergy indicators, such as RAST and CH. In this study we calculated the sensitivity and specificity at different cutoff values for the HEWS, using RAST or CH. An optimum cutoff value, which gives the least FP and FN results, can be estimated from the results plotted in Fig. 1.

From these calculations it was concluded that a cutoff value of the HEWS of 0.7 (ICT) and 0.4 (SPT) gave the optimum sensitivity and specificity. It should be noted that the lines of the specificity and sensitivity of SPT do not intersect, indicating that the optimum value is not reached with this concentration and that a somewhat higher concentration should be used for routine skin testing. However, the differences are small.

The cutoff values found in this part of the study were slightly lower than those reported in part I, where 0.87 (ICT) and 0.53 (SPT) were found to be optimum cutoff values. This small difference is probably caused by a difference in the degree of sensitization between the two populations.

The concordances of 91% (RAST) and 86% (CH) found for SPT are comparable to the results of others (1, 3, 9, 11, 17). Petersson et al. (17) found a concordance between SPT and RAST of 87% for birch and 94% for timothy; Ahlstedt et al. (1) found a concordance between SPT and RAST of 80% for birch pollen, timothy pollen, and dog epithelium; Brown et al. (3) obtained a concordance of 89.2% between SPT and RAST for Bermuda grass pollen.

Only a few authors have related the results of SPT with CH. Petersson et al. (17) reported a concordance of 85.5% (birch) and 76.8% (timothy) for SPT, and Vanto et al. (21) reported a concordance of 66.7% (dog) between SPT and CH.

The concordance of ICT with RAST (83%) and with CH (77%) is much better than the concordance reported by other investigators. In the past a low concordance of ICT with CH and RAST (3, 4, 14, 18, 19) was reported. This low concordance (many

FP reactions) is commonly ascribed to a high degree of nonspecific reactions observed in ICT. It is thought that this lack of specificity may be related to the concentration of the antigen employed (16, 18, 20) and the presence of irritants. These problems may have been overcome by using highly purified, standardized allergens containing no irritants, in combination with a documented injection technique.

From this study, it can be concluded that it is possible to obtain an ICT with a good predictive value, if the concentration of the standardized allergen and the cutoff value of the skin test are chosen according to this method.

The concordance of ICT and RAST with CH in the case of house-dust mite is lower (63% and 62%, respectively) than for the other allergens. The reason for this relatively high number of FP results is probably the fact that it is more difficult to obtain a convincing history with house-dust mite than with the other allergens. However, SPT has a good concordance for all four allergens, probably because SPT is less sensitive than ICT and RAST.

When the sensitivity, specificity, and predictive value are compared with the results of part I of this study, it can be seen that there is good agreement between the results of both studies. In part I, ICT had a predictive value of 87% (in this study 83%), and SPT had a predictive value of 79% (in this study 91%).

The difference in the predictive value of SPT may be ascribed to the variability inherent in all allergy tests, including RAST and skin tests, and to a difference in the sensitization pattern between the populations of both studies. The results of the two new allergens (tree pollen and cat dander) agree well with the results of the two test allergens (house-dust mite and grasses) used in both studies.

RAST is regarded as less sensitive than SPT and ICT. In this study this view is not confirmed, probably because the RAST class 1 group was included in the positive result group. With RAST an FN result is obtained in 4.2% of the patients; for ICT, 4.0% and for SPT, 3.7% FN results are obtained. Our finding of 19% FP for ICT contrasts with the results of others. Brown et al. (3) reported 96%, and Reddy et al. (18) found 100% FP ICT results, as compared with RAST, in patients with a negative SPT.

SPT has a good correlation with RAST and a moderate correlation with CH. For grass pollen and house-dust mite, ICT also has a good correlation with RAST and a moderate correlation with CH, whereas the correlation for tree pollen and cat dander is poorer in this study. When the correlations of RAST and skin tests are compared with CH, it can be seen that RAST correlates less well with CH, as compared with the two skin tests.

The two skin tests correlated better with CH and RAST than was reported by others (3, 4, 5, 10, 14, 18). There is not much difference between the correlations for the MWD and HEWS.

In conclusion, with this method of studying a limited number of standardized allergens in a small group of patients, it is possible to choose a safe and efficacious concentration for routine skin testing and to extrapolate these results to other well-standardized allergens.

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