

Diagnostic value of a glass fibre-based histamine analysis for allergy testing in children

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The aim of this study was to compare the diagnostic value of common allergy tests with basophil histamine release in 124 children with symptoms of asthma. The patients were evaluated by case history, skin prick test, RAST-analysis, and basophil histamine release using a glass fibre-based histamine assay to 10 common inhalant allergens. The bronchial provocation test was used as a reference of "true" IgE-mediated asthma. To compare the various diagnostic parameters each absolute test value was classified into a scoring system. The concordance between the tests varied between 85–97 %. In general, the best concordance was found between basophil histamine release and RAST. Sensitivity, specificity and predictive values were calculated on the basis of 104 bronchial provocation tests. It was found that histamine release was the best single analysis, followed by RAST and prick testing. The sensitivity of RAST and histamine release was very high (1.00) for pollen and house dust mites. Histamine release showed a predictive value between 0.91 and 1.00 for pollen and house dust mites, thus indicating the possibility of omitting the bronchial provocation test. In the dander group histamine release gave the best sensitivity (0.91), however at the expense of specificity (0.64), whereas RAST and skin prick test gave a specificity of 1.00. In the mould group histamine release also showed the best diagnostic value. The combination of skin testing with histamine release or RAST was of no additional diagnostic help. It is concluded that the glass fibre-based histamine analysis, which makes routine histamine release testing possible, is a reliable diagnostic test in children.

Key words: allergy; bronchial asthma; bronchial provocation test; diagnosis; glass fibre; histamine; histamine release; RAST; skin prick test.

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CLINICAL ASPECTS

Basophil histamine release represents a clinically useful *in vitro* test for the understanding and diagnosis of atopic disease. Histamine release from basophils in whole blood can be determined now simply, quickly, and precisely with an automatized glass fibre-based histamine assay. Compared with other histamine release assays the method is technically simple and has special advantages due to small blood sample volume (25 µl whole blood/sample), high sample capacity and a performance time of 3 h.

At present, a careful case history (CH) combined with skin prick test (SPT) are the common means of diagnosing Type I allergy in children. In small children frequent respiratory tract infections often makes it difficult to determine if the child has a primary allergic condi-

tion, and the CH may not be very helpful, especially when the children have a predisposition to allergy. They constitute a diagnostically difficult group of patients and histamine release from basophil leukocytes (HR) or RAST may be a valuable diagnostic tool. In some cases a

bronchial challenge test (BPT) may be necessary to determine clinical allergy. However, in small children the BPT may not be technically possible and one is left with CH, SPT, and blood tests.

Several investigators have shown that HR is a sensitive procedure for the diagnosis of inhalant allergy in children (5, 7, 8, 13). The development of a glass fibre-based histamine analysis (10, 11) has recently made it possible to perform histamine release from basophils using very small amounts of whole blood. Since studies of the diagnostic value of histamine release in children are limited and to a certain degree contradictory (7, 8, 12, 13, 14) we wanted to determine the test's potential to identify children with atopic allergy to common inhalant allergens. Histamine results are compared with the other tests by comparing the agreement of positive and negative reactions of each test. In addition, specificity and sensitivity are compared using a score system and different "cut-off" limits in order to demonstrate how this affects the diagnostic value of the test.

MATERIAL AND METHODS

Patients and study design

From February to December 1988 124 children, 3-14 years old, who were referred to our outpatient Allergy Clinic at the Department of Pediatrics, suspected of having bronchial asthma were consecutively enrolled in the study. Other criteria of eligibility included "responding basophils" i.e. basophils responding to anti-IgE challenge with a histamine release of ≥ 0.2 ng/sample, no systemic steroid-treatment, and no prior immunotherapy. All children were instructed to discontinue bronchodilating drugs for at least 6 h and aminophylline or short half-life antihistamines for at least 24 h and long half-life antihistamines for 3 weeks prior to admission and testing. The children were evaluated by case history based on a questionnaire mailed to the parents before their first visit. At admission the questionnaire was reevaluated and all children were seen by

H.N. or K.S., and from the case history a diagnosis of exogenous asthma to pollen, animal dander, moulds and house dust mites was graded as shown below. In calculations, grades 2 and 3 were regarded as positive (3). Then SPT was carried out. Blood samples for RAST and HR analysis were collected prior to SPT and analysed within 24 h. Finally, a BPT was performed. These studies were reviewed and approved by the local ethics committee on human investigations and all parents gave their informed consent to their children's participation in the study.

Allergens and anti-IgE

The following allergens were used: grass pollen (*Phleum pratense*), birch, mugwort; dander from dog, cat and horse; moulds from wormwood (*Alternaria alternata*) and *Cladosporium herbarium*; mite extracts from *Dermatophagoides pteronyssinus*, and *Dermatophagoides farinae*. In SPT, HR, and BPT the same batch No. of freeze-dried extracts (Pharmalgen standardized by Pharmacia AB, Sweden and delivered by ALK, Denmark) in stock solutions of 100,000 BU/ml were used. To ensure maximum potency only fresh extracts reconstituted and diluted the same day of testing were used. For histamine release experiments the allergens were diluted in 50 % glycerol at 100,000 BU/ml and stored at 4°C for a maximum of 3 months. On the day of HR-testing allergens were diluted in Pipes-AMC (7) at final concentrations in the reaction mixture of 1, 100, and 1000 BU/ml. Anti-IgE (462,000 U/ml) from Behringwerke, Germany was used at final concentrations of 4, 400, and 4000 U/ml and the same batch of anti-IgE was used throughout the study.

Skin prick test method

Single prick tests were carried out on the volar surface of the patients' forearms with each extract at 10,000 BU ml⁻¹ using a prick test lancet with a 1 mm tip and shoulders. The same experienced nurse carried out the SPT. The

weals were marked when the reaction was at maximum (after 15 min) and compared with a positive reference containing histamine chloride, 10 mg/ml. An albumin diluent was used as a negative reference. The contours of the weals were encircled by means of a felt-tipped pen and transferred to squared mm paper with a strip of tape. The size of the weal was measured using the formula,

$$\frac{(D + d)}{2}$$

where D is the biggest diameter and d is the perpendicular diameter. Only weals ≥ 3 mm in diameter was regarded as positive and graded as shown below (16).

Allergen-specific serum IgE (RAST)

RAST was determined in duplicate and performed as Phadebas RAST® by the Central Laboratory at the Hospital of Silkeborg, Denmark, using the same raw material as Pharmalgen, but not the same batch No. as this prerequisite was not obtainable by the company. The results were expressed in Phadebas RAST Units (PRU) and scored as shown below.

Histamine release method

Heparinized whole blood, 0.1 ml/sample (diluted 1:3 in Pipes-AMC), was incubated in duplicate in glass fibre-prepared microtitreplates for 60 min at 37°C with 20 µl of allergen or anti-IgE. The plates were then rinsed and protein-residues enzymatically removed. Histamine analysis was performed as previously described (7) using a Perkin-Elmer spectrophotofluorometer and the results were graded as shown below.

Bronchial provocation test

With a time interval of at least 1 week two provocations per patient were performed. The

allergens chosen for BPT were selected with a preference for at least one allergen selected on the basis of CH and for an allergen giving contradictory test results. The peak expiratory flow (PEF) was measured with a Mini Wright peak flow meter and chest auscultation performed. The technique was carried out by using nebulized aqueous allergen extracts generated by a Wright nebulisator in increasing concentrations from 100 to 100,000 BU/ml. Before and after each inhalation at each concentration, PEF was recorded and the patient was asked for symptoms, and chest auscultation was performed before raising the concentration one decade. A drop in PEF of 20% or more was defined as a positive reaction. Inhalation was started each time with phenol saline as a negative control. After BPT, the patient was observed and PEF recorded every hour for the next 24 h. Two late reactions occurred and they were regarded as positives.

Score system

CH: 0 = negative
1 = doubtful
2 = probable
3 = convincing

RAST: 0 = < 0.35 PRU
1 = ≥ 0.35 –0.7 PRU
2 = > 0.7–3.5 PRU
3 = > 3.5 PRU

SPT: 0 = < 25% of histamine ref. with 10 mg/ml histamine
1 = $\geq 25\%$ of histamine reference
2 = $\geq 50\%$ of histamine reference
3 = $\geq 100\%$ of histamine reference

HR-test: 0 = < 0.2 ng histamine/sample
1 = ≥ 0.2 ng histamine/sample at 1000 BU/ml
2 = ≥ 0.2 ng histamine/sample at 100 BU/ml
3 = ≥ 0.2 ng histamine/sample at 1 BU/ml

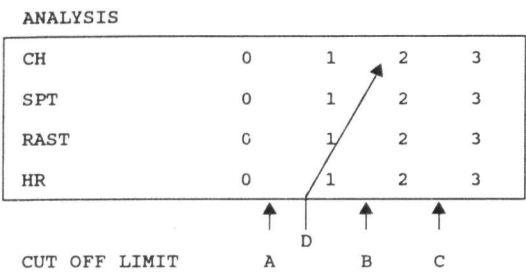


Fig. 1. Different cut-off limits (A-C) for the various test scores. D is used in calculations of predictive value and concordance as this reflects the common “cut-off” limits used in clinical practise.

Statistics and calculations

Two by two contingency tables were established to compare the concordance between each test applying McNemar’s chi-square test for paired alternatives with a continuity correction to improve the approximation. Please note, the lower this value, the better the fit. To evaluate the “clinical significance” of the diagnostic parameter results to predict clinical asthma, 95 % confidence intervals for a binomial proportion were estimated.

The diagnostic value of the various tests was determined by calculating the specificity and sensitivity of each test method and combination of tests according to Galen & Gambino (4). Test concordance (test efficiency), i.e. percent of all results that are true results whether negative or positive, was also computed. In this study the results of BPT are considered the “true” diagnostic test for determining the presence of clinical allergy. The sensitivity of the diagnostic test was therefore calculated by dividing the number of positive test results identified by the total number of positive BPT, and the specificity was calculated by dividing the number of negative BPT correctly identified by the test by the total number of negative challenges. The specificity and sensitivity were computed with different “cut-off” limits for each test score (Fig. 1). The predictive value positive is the probability that a person who has a positive test result has the disease. The predictive value negative is the probability that a person who has a negative test result does not

Table 1

Summary of eligible patients

	Atopics	Non-atopics	Total
Entered	78	46	124
Patients with non-responding basophils	6	4	10
Evaluable	72	42	114
Evaluable for BPT	57	—	57
Males	41	21	62
Females	31	15	46
Age (median)	10	7	
Age (range)	3-14	3-14	

have the disease. The predictive values were calculated according to Bayes’ theorem (4). Thus, predictive accuracy depends on the specificity and sensitivity of the test and the prevalence of clinical allergy in the group of patients studied. Therefore, the prevalence (number of confirmed positive cases divided by the total number of cases under study) was incorporated in the calculations.

RESULTS

SPT, RAST and histamine release to the 10 allergens in question were performed in all children (*n* = 124). In SPT a median of 4.3 mm (range 2-12) and a mean of 4.7 mm (SD±1.7 mm) in diameter of the weal was obtained with 10 mg/ml histamine reference.

Non-atopic controls

In 46 patients the CH of exogenous asthma was negative or doubtful. In 39 of these patients asthma symptoms appeared only in conjunction with respiratory infections or unspecific stimuli (smog, cold air, fumes) and seven patients experienced asthma only at exercise (verified by exercise test). In addition, they all showed negative test results in SPT, RAST, and HR-test. There was no indication of allergen BPT in these patients, since they suffered from non-allergic asthma and they were used as a control

group when comparing the concordance of the various tests. Four controls had non-responding basophils (i.e. basophils releasing < 0.20 ng histamine/sample to anti-IgE) and were excluded from the calculations (Table 1).

Atopic patients

Among the atopic patients a total of 104 BPT were performed and 57 children experienced 68 positive bronchial challenges. Three children were not tested because they were too young to cooperate (age < 4 years). Ten children refused the second provocation and eight children denied bronchial provocation. In four children BPT could not be carried out due to a dependency on continued medication with a fall of PEF after at least three attempts to discontinue the asthma medicine. Since BPT data for some of the individual allergens are too small for the following statistical calculations, the data were pooled in four different allergen groups: pollen (grass, birch, mugwort); dander (cat, dog, horse); moulds (*Cladosporium*, *Alternaria*); and mites (*D. pteronyssinus*, *D. farinae*).

Frequency of positive and negative test results

A total of 3524 tests were performed and the results of the various tests for the 10 allergens

are depicted in Table 2. The most frequent allergens giving a positive reaction were in the following order: grass, mites, and danders. Skin testing gave fewer positive test results than RAST and HR, and the number of positive RAST and HR tests were also reduced more than SPT by using a higher cut-off limit. In HR the dander allergens (especially dog dander) gave more positive test results in comparison with RAST and SPT; however, using a cut-off limit of score 1 for HR reduced the numbers by almost half, thus indicating that a higher cut-off limit for HR decreases the number of false positive test results.

Test concordance

Table 3 shows concordances between the various test results for each allergen group for all evaluable subjects. The concordance percentages as well as the McNemar's test values are also given. The best concordance between the tests varied for the various allergens according to cut-off chosen. Hence, concordance between the tests was high for pollen, moulds and mites, between 90 and 97 % depending on the cut-off value chosen. Concerning dander, discrepant lower values were found between HR and SPT or RAST and this was not significantly ($P > 0.05$) minimized when a cut-off score value of > 1 for HR was chosen.

Table 2

Results of the various tests performed in 114 children								
	No. positive tests				No. negative tests			
	BPT	RAST	SPT	HR	BPT	RAST	SPT	HR
Grass	14	41 (32)	25 (24)	31 (26)	7	73 (82)	89 (90)	83 (88)
Birch	2	12 (9)	9 (9)	11 (10)	2	102 (105)	105 (105)	103 (104)
Mugwort	3	11 (5)	4 (2)	5 (4)	–	103 (109)	110 (112)	109 (110)
Cat	2	21 (19)	16 (15)	21 (19)	3	93 (95)	98 (99)	93 (95)
Dog	7	24 (12)	16 (16)	40 (21)	6	90 (102)	98 (98)	74 (93)
Horse	2	10 (7)	11 (10)	23 (9)	2	104 (107)	103 (104)	91 (105)
<i>Alt.</i>	1	11 (8)	6 (5)	10 (9)	–	103 (106)	108 (109)	104 (105)
<i>Clad.</i>	5	13 (10)	7 (7)	8 (7)	5	101 (104)	107 (107)	106 (107)
<i>D. Pt</i>	30	35 (33)	32 (32)	39 (39)	10	79 (81)	82 (82)	75 (75)
<i>D. Fa</i>	2	31 (28)	31 (26)	29 (29)	1	83 (86)	83 (88)	85 (85)
Total	68	209 (163)	157 (146)	217 (173)	36	931 (977)	983 (994)	923 (967)

Numbers in parentheses indicate number of tests classified as positive or negative if a test score of ≥ 1 was considered positive.

Table 3
Concordances between the various tests

	HR vs RAST	HR vs SPT	RAST vs SPT
Pollen			
Concordance %	93 (97)	94 (94)	90 (94)
χ^2	10.2, $P < 0.01$	5.3, $P < 0.01$	21.4, $P < 0.01$
(χ^2)	(0.4, $P > 0.05$)	(8.5, $P < 0.01$)	(7.6, $P < 0.01$)
Dander			
Concordance %	86 (85)	85 (85)	91 (95)
χ^2	18.4, $P < 0.001$	33.3, $P < 0.001$	5.6, $P < 0.05$
(χ^2)	(44.2, $P < 0.001$)	(38.0, $P < 0.001$)	(0.2, $P > 0.05$)
Moulds			
Concordance %	96 (97)	94 (95)	93 (95)
χ^2	2.5, $P > 0.05$	2.8, $P > 0.05$	8.5, $P < 0.05$
(χ^2)	(0.1, $P > 0.05$)	(4.1, $P < 0.05$)	(4.1, $P < 0.05$)
Mites			
Concordance %	96 (95)	92 (93)	92 (94)
χ^2	1.1, $P > 0.05$	1.9, $P > 0.05$	0.8, $P > 0.05$
(χ^2)	(5.8, $P < 0.05$)	(1.1, $P > 0.05$)	(1.2, $P > 0.05$)

Numbers indicate results when cut-off limit A was used. Numbers in parentheses indicate results when cut-off limit D was used.

Table 4

Sensitivity, specificity, and predictive values for the various allergen groups

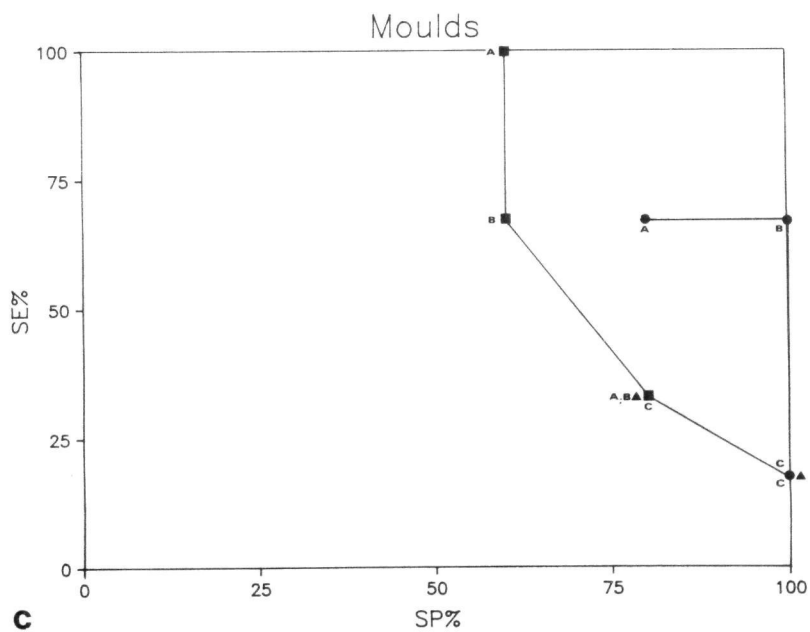
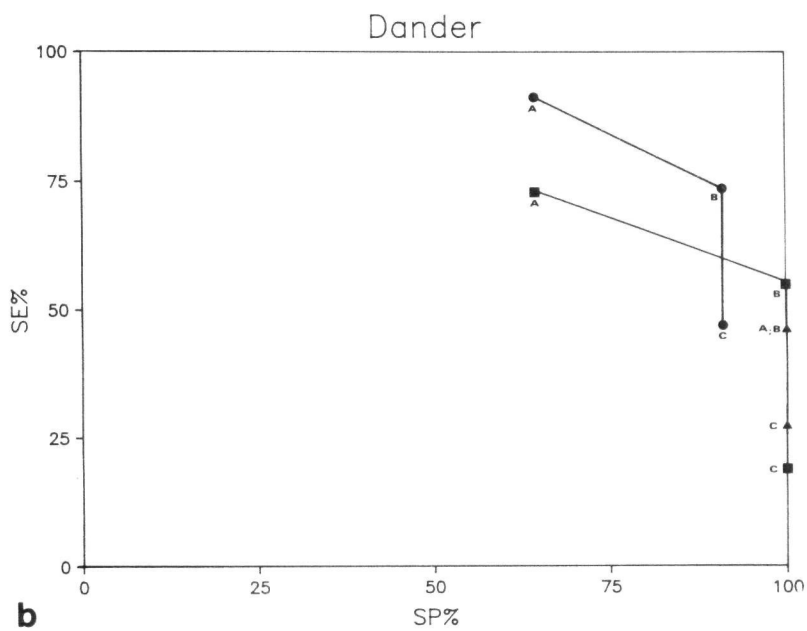
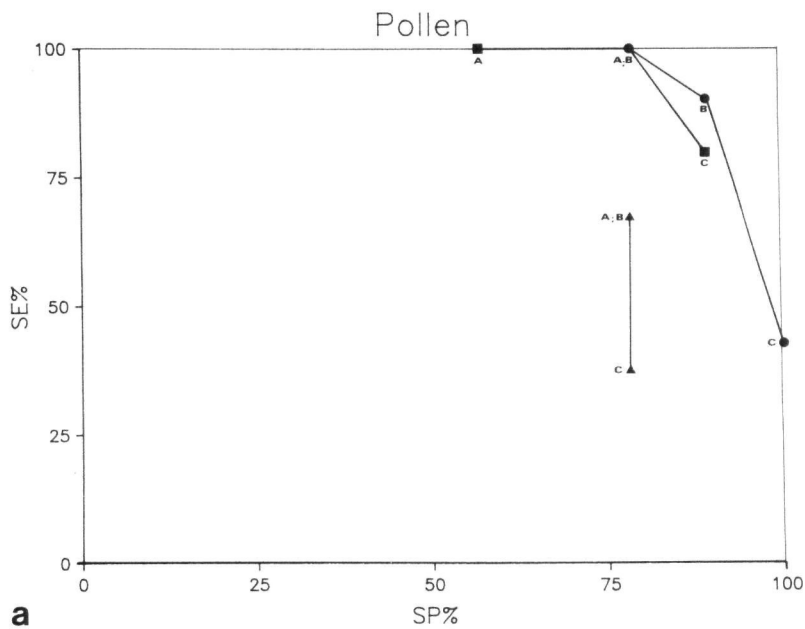
		CH	RAST	SPT	HR
Pollen 28*					
	se	0.79	1.00	0.68	1.00
(19)	sp	0.78	0.78	0.78	0.78
P 0.68	pp	0.88	0.91	0.87	0.91
	pn	0.64	1.00	0.53	1.00
Dander 22*					
	se	0.64	0.55	0.46	0.91
(11)	sp	0.64	1.00	1.00	0.64
P 0.50	pp	0.64	1.00	1.00	0.71
	pn	0.64	0.59	0.64	0.87
Moulds 11*					
	se	0.60	0.67	0.33	0.67
(6)	sp	0.80	0.60	0.80	0.80
P 0.55	pp	0.79	0.67	0.67	0.77
	pn	0.62	0.60	0.49	0.67
Mites 43*					
	se	0.66	0.84	0.78	1.00
(32)	sp	0.73	1.00	0.91	0.91
P 0.74	pp	0.87	1.00	0.96	0.97
	pn	0.43	0.69	0.59	1.00

*Indicates number of patients studied. Numbers in parentheses indicate number of positive BPT (P = prevalence; se = sensitivity; sp = specificity; pp = predictive value positive; pn = predictive value negative). The D cut-off limit was used for calculations.

When comparing the overall (all allergens) test concordance (test efficiency) with BPT, using a cut-off score value for CH > 1 , SPT ≥ 1 , RAST ≥ 1 , and HR-test ≥ 1 , the HR-analysis showed the best concordance with BPT ($P < 0.01$). Concordance percentages were in the following order BPT vs HR 89 %, BPT vs RAST 84 %, BPT vs CH 75 %, and BPT vs SPT 73 %. Concordances were not significantly changed if the D “cut-off” limit (see Fig. 1) was used ($P > 0.05$).

Diagnostic sensitivity and specificity

To further evaluate the various tests the sensitivity and specificity were computed according to different “cut-off” limits. In Fig. 2a, b, c, d the sensitivity (SE) and specificity (SP) of the diagnostic tests for each allergen group are depicted for the different cut-off values. Fig. 2a illustrates for the pollen group that both RAST and HR gave 100 % sensitivity with a cut-off score value of ≥ 1 or > 1 . All tests include few false positive test results (high specificity). Fig. 2b depicts the generally lower discriminative



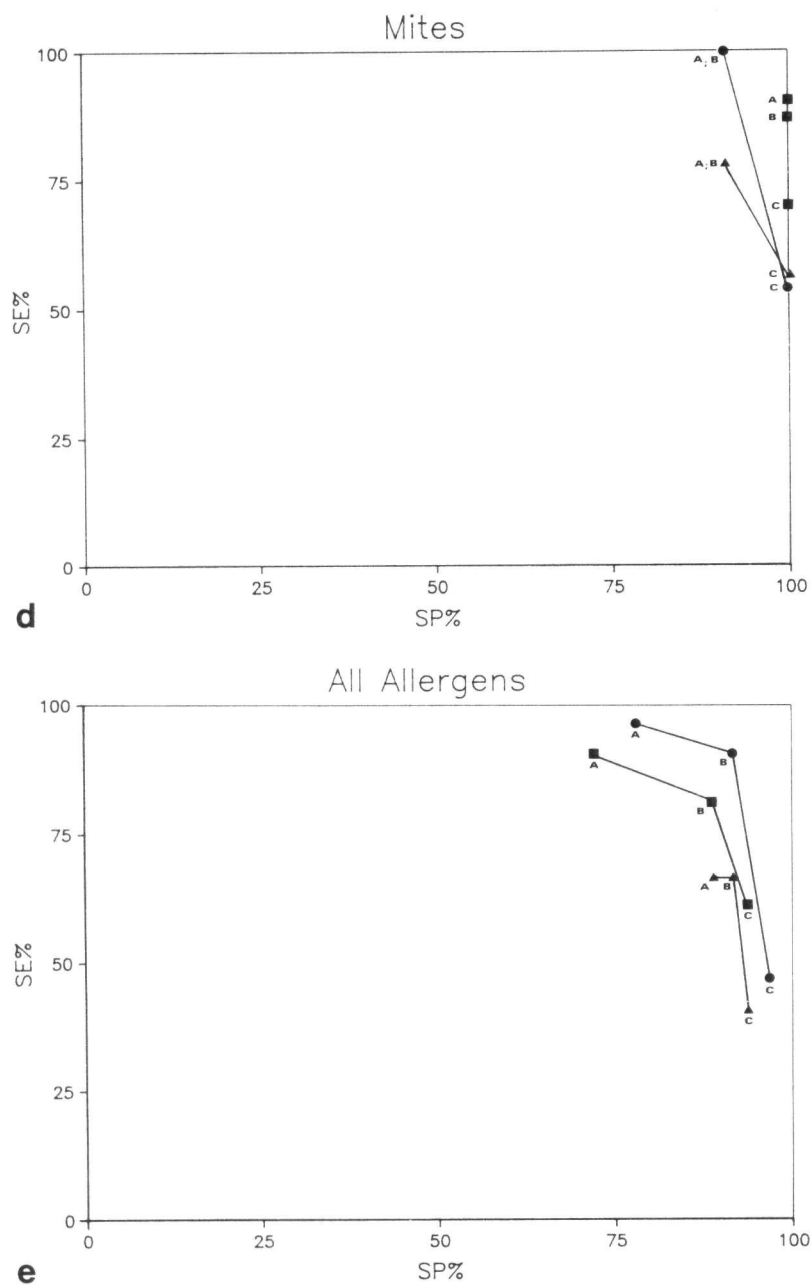


Fig. 2. Specificity (SP%) and sensitivity (SE%) in percent at different cut-off points (A-C) for HR (●--●), RAST (■--■), and SPT (▲--▲).

ability of the tests for dander. By employing HR the sensitivity is high (91 %) and with a similar specificity as RAST. SPT and RAST gave the best specificity (100 %), although at the expense of sensitivity. Fig. 2c (moulds) shows that RAST gives the highest sensitivity at score ≥ 1 . However, at a cut-off score value of > 1 HR implies better diagnostic reliability (higher sensitivity and specificity) than RAST. Fig. 2d (mites) shows that all tests have high diagnostic reliability, with HR giving the highest sensitivity (100 %) and RAST the best specificity (100 %). Fig. 2e illustrates the discrimi-

nant curves obtained for all tests when pooling the results obtained from all allergens. HR was the single analysis giving the best diagnostic sensitivity and specificity followed by RAST and SPT, respectively.

Diagnostic value

Table 4 depicts the calculated values of sensitivity (se), specificity (sp), predictive value positive (pp) and predicitive value negative (pn) for CH, RAST, SPT, and HR-test. The calcula-

tions are based on the common cut-off score values for the diagnostic parameters (CH > 1, HR ≥ 1, RAST > 1 and SPT > 1). The prevalence of the allergens giving a positive bronchial challenge in the population studied is also given. Consequently, when results of CH, SPT, RAST, or HR alone were compared the values for HR and RAST differed from those for CH and SPT. RAST and HR were similar in diagnostic value. However, HR was the single analysis giving the best predictive values, except in the dander group of allergens where SPT and RAST had a better positive predictive value than HR. CH was comparable to SPT in predictive value, although, in diagnosing house dust mite allergy SPT was better than CH. Finally, it was examined whether the combination of SPT and RAST or SPT and HR-test would increase the diagnostic values. It was found on comparing the sensitivity of the single analysis with a combination of tests that sensitivity decreased and only a marginal increase in

specificity was obtained. The predicitive values did not increase (results not shown).

Diagnostic gain

To evaluate and compare the “clinical significance” of the diagnostic tests and CH the diagnostic gain of using BPT was calculated (Table 5). Based on the confidence intervals the HR-test was marginally better than the RAST analysis in diagnosing mite allergy; however, for pollen allergy RAST and HR were similar when a cut-off score of > 1 for RAST was chosen. When a cut-off score of > 1 for HR was used for dander and moulds the HR test was also of higher diagnostic gain than RAST. Both CH and SPT were of significantly less value than HR and RAST.

DISCUSSION

The present study was performed to evaluate the use of histamine release in the diagnosis of

Table 5
Diagnostic gain in percent of BPT compared with the various diagnostic parameters

		Bronchial provocation test							
		Pollen		Dander		Moulds		Mites	
		pos	neg	pos	neg	pos	neg	pos	neg
CH	pos	15 (15)	2 (2)	7 (7)	4 (1)	5 (3)	1 (1)	25 (21)	3 (3)
	neg	4 (4)	7 (7)	4 (4)	7 (10)	1 (3)	4 (4)	7 (11)	8 (8)
Gain %		21 (21)		36 (23)		18 (36)		23 (35)	
95 %-limits		7-42 (7-42)		17-57 (8-45)		3-52 (10-70)		12-37 (22-52)	
SPT	pos	13 (13)	2 (2)	5 (5)	0 (0)	2 (2)	1 (1)	25 (25)	1 (0)
	neg	6 (6)	7 (7)	6 (6)	11 (11)	4 (4)	4 (4)	7 (7)	10 (11)
Gain %		29 (29)		27 (27)		45 (45)		19 (16)	
95 %-limits		14-48 (14-48)		12-47 (12-47)		16-76 (16-76)		8-33 (7-31)	
RAST	pos	19 (19)	4 (2)	8 (6)	4 (0)	6 (4)	2 (2)	29 (27)	0 (0)
	neg	0 (0)	5 (7)	3 (5)	7 (11)	0 (2)	3 (3)	4 (6)	10 (10)
Gain %		14 (7)		32 (23)		18 (36)		9 (14)	
95 %-limits		4-33 (2-25)		14-55 (8-45)		3-52 (10-70)		3-23 (5-28)	
HR	pos	19 (17)	2 (1)	10 (8)	4 (1)	4 (4)	1 (0)	32 (32)	1 (1)
	neg	0 (2)	7 (8)	1 (3)	7 (10)	2 (2)	4 (5)	0 (0)	10 (10)
Gain %		7 (11)		23 (18)		27 (18)		2 (2)	
95 %-limits		2-25 (3-28)		8-45 (5-40)		6-60 (3-52)		0-11 (0-11)	

Numbers indicate results when cut-off limit A was used and numbers in parentheses indicate results when cut-off limit D was used.

inhalant allergy in children by a new histamine assay allowing routine testing on whole blood. The study confirms previous studies that histamine release and RAST may be superior to SPT (13, 14, 15), and it may also reflect that RAST and HR have increased their diagnostic value as both tests have been recently improved. In general, the study indicated that the HR-test was superior to the other diagnostic parameters except the BPT, which in this study was the reference for the "true diagnostic test". Since HR was found to have excellent negative predictive accuracy for pollen and house dust mite allergy the test is useful for excluding this type of inhalant allergy. As there were few patients with mould allergy in this material, the results should be interpreted with this reservation in mind. As regards the HR-test classifies too many false positives at score ≥ 1 ; however a cut-off value of > 1 will increase its diagnostic value. The SPT results in this study may have been influenced by the prick technique, the use of single prick tests, and using only one concentration (1, 6). The influence of drugs is considered to be negligible since it was a prerequisite that the children were free from drugs at testing and only three children were using antihistamines. However, the metabolism of drugs in children may vary and the time limits chosen in this study may have been too short. We believe the high validity of SPT found in other studies is more likely due to the use of quantitative SPT performed in duplicate (1, 6). In the present study, however, the method was carried out as is widely done in out-patient clinics and probably reflects everyday practise.

In conclusion, the development of a microtiter application of basophil histamine release has made it possible to routinely carry out the histamine release test more conveniently and faster than conventional histamine release methods (2, 9). It seems that apart from research purposes one of the major applications for basophil histamine release is as an aid in the diagnostic investigation of suspected inhalant allergy. This study indicates that basophil histamine release has a similar or better diagnostic value than conventional allergy tests.

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