

Basophil histamine release in the diagnosis of house dust mite and dander allergy of asthmatic children

Comparison between prick test, RAST, basophil histamine release and bronchial provocation

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The aim of the study is to compare the glass fibre-based basophil histamine release test with skin test (Phazet®), RAST (Phadebas®) and bronchial provocation test in children with allergic asthma. The study comprised 68 selected children with a case history of extrinsic allergic asthma to danders (cat and dog) and house-dust mite. Skin prick test, RAST, and histamine release were performed in all children and the bronchial provocation test was used as a reference of "true allergic asthma". A total of 81 allergen bronchial challenges were performed and 44 children experienced 49 positive provocations. In 2.9% (2/68) of the children histamine release could not be performed due to technical difficulties (low histamine release with anti-IgE). Concordances in the range 76-87% were observed with no significant difference between the tests. The highest concordance (87%) was found between histamine release and bronchial provocation test followed by skin prick test vs bronchial provocation (84%) and RAST vs bronchial provocation (80%). The sensitivity and specificity were calculated for each test. All tests showed sensitivities in the range 90-94% and no significant difference between them was observed. The specificity of histamine release, skin prick test, and RAST was 0.78, 0.69, and 0.63, respectively. The specificity of histamine release was better than RAST demonstrated by 95% confidence intervals. In conclusion, it was found that the histamine release test is a convenient diagnostic method and the study indicates a diagnostic value comparable to the common diagnostic methods in clinical allergy.

Key words: asthma; allergy; bronchial provocation; basophil; children; histamine; histamine release; RAST; skin test.

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CLINICAL ASPECTS

Recently, several new *in vitro* allergy tests have been introduced to the clinician, e.g. the fluoro-allergo-sorbent test (FAST), the Pharmacia CAP system, and the chemiluminescent immunoenzymometric system (MAST CLA). Basically, they all measure specific IgE in serum. Another diagnostic principle is the basophil histamine release test which measures a biological signal in the form of released histamine from sensitized basophils in a blood sample from the patient. However, the conventional histamine release test has been confined to basic research and scientific studies due to its complicated technique, but the introduction of a simplified automatized glass fibre-based histamine assay now allows large-scale testing for diagnostic purposes.

The basophil histamine release test has been found a valuable method for the diagnosis of inhalant allergy in children (2, 3, 5, 10). However, the conventional histamine release

methods have been of limited use in the daily routine of allergy diagnosis due to technical limitations.

Recently, a new glass fibre-based histamine assay (3, 7, 8) has been developed and made commercially available. This histamine assay makes use of allergen-coated microtitre plates which allow rapid routine testing with very small sample volumes of blood. Hence, we wanted to compare histamine release (HR) with skin prick test (SPT), RAST, and bronchial provocation test (BPT) in children with allergic asthma to three common allergens.

MATERIAL AND METHODS

Patients and study design

Between February 1988 and June 1989, children 6–18 years old, with a case history and clinical symptoms of extrinsic asthma to cat and/or dog danders or house dust mite were enrolled in the study. The case history was recorded and SPT carried out. Blood samples for RAST and HR were collected prior to SPT. Finally, the BPT was performed with an allergen selected on the basis of case history. The study was approved by the local ethics committee on human investigations and all parents or guardians gave their informed consent.

Allergens and anti-IgE

The following allergens were used: dander from the dog and cat and house-dust mite-extract from *Dermatophagoides pteronyssinus*. In all tests (HR, SPT, RAST and BPT) the same raw extracts (Pharmalgen), although, different batch No., were used. For HR, freeze-dried Pharmalgen extracts were delivered by Pharmacia (Sweden) and then air-dried into the microtiter wells. Anti-IgE was obtained from Behringwerke, Germany. For BPT, cat and dog dander extracts (Pharmalgen) were delivered by ALK, Denmark and were used at dilutions between 50,000 to 200 BU/ml. As an exception the house dust mite extract used for BPT was obtained from Dome-Hollister-Stier and used at

increasing concentrations from 1000 to 50 BUN/ml (1000 BUN/ml = 50,000 BU/ml). The upper concentration used for BPT was selected according to the manufacturer's recommendations. The allergens were diluted in isotonic saline on day of use and then discarded.

Bronchial provocation test

Forced expiratory volume in 1 s (FEV_1) was measured with a Vitalograph and chest auscultation performed. The patients inhaled 1 ml of nebulized aqueous allergen material in increasing concentrations as mentioned above. Inhalation was started each time with isotonic saline as a negative control. Before and after each inhalation of each concentration, FEV_1 was recorded and the patient questioned about symptoms, and chest auscultation was performed before raising the concentration one decade. A drop in FEV_1 of 20% or more was defined as a positive reaction. The provocations were continued until either a positive reaction was observed or up to the highest concentration. After BPT, PEF was recorded every hour for the next 8 h to detect any late reactions.

Skin prick test method

Single prick tests were performed on the volar surface of the patient's forearms with the Phazet test (Pharmacia). Phazet is a 1 mm lancet coated with standardized Pharmalgen allergens and has previously been shown to have an allergen extract strength of 100,000 BU/ml (13). The weals were read after 15 min and compared to a histamine reference, and uncoated Phazet lancets were used as negative controls. The contours of the weals were circled with a felt-tipped pen and transferred to squared mm paper with a strip of tape.

A SPT reaction > 50% of the histamine reference and a weal ≥ 3 mm in diameter was considered a positive test result (13).

Allergen-specific serum IgE

RAST was performed as Phadebas RAST and the results were expressed in RAST-classes 0–4.

A RAST class ≥ 2 was considered a positive test result.

HR-test

All patients had 5 ml heparinized blood drawn for the HR test. The next day, histamine release from basophil leukocytes was performed as described elsewhere (5). Briefly, 25 μ l of whole blood is applied to allergen-coated microtiter wells and each allergen is tested at six concentrations from 500 BU/ml to 0.5 BU/ml. After 1 h incubation at 37°C the plates are rinsed and protein residues are enzymatically removed (4). Histamine bound to the microfibrils is measured spectrophotofluorometrically with a Gilson fluorometer. Patients with non-responding basophils, i.e. basophils releasing < 15 ng/ml of histamine at anti-IgE challenge, were excluded from the calculations, since their test results are inconclusive. In this study the histamine release results were scored according to the answers given by the company. Thus, a HR score > 0 , i.e. a significant allergen-induced histamine release ≥ 30 ng/ml in any concentration, was considered positive.

Statistics and calculations

Two by two contingency tables were established to compare the concordance between each test. The results of BPT indicate the “true diagnosis”, i.e. the presence of clinical allergy. Hence, the sensitivity of the laboratory tests was computed by dividing the number of positive test results

identified by the total number of positive BPT to a given allergen, and the specificity was calculated by dividing the number of negative BPT correctly identified by the laboratory test by the total number of negative challenges of a given allergen. The relevance of computed differences between sensitivity and specificity was estimated on the basis of 95 % confidence limits.

RESULTS

The study comprised 68 patients with extrinsic asthma, 43 were males and 25 females. The median age was 13 (range: 6–18). Two patients (2.9 %) had non-responding basophils and were excluded from the study. A total of 81 BPT were performed and 44 children experienced 49 positive bronchial challenges.

Table 1 shows the distribution of test results between the various diagnostic parameters. Thus, the most frequent allergen giving a positive test result was in decreasing order: house dust mite, cat and dog danders. In general, RAST and SPT gave more positive test results than HR and BPT.

Table 2 reports the concordance between the tests. In decreasing order a concordance of 87 % (69/79) was observed between BPT and HR; SPT vs BPT of 84 % (68/81); RAST vs HR of 84 % (66/79); BPT vs RAST of 80 % (65/81); SPT vs HR of 76 %; and RAST vs SPT of 72 % (58/81, to make the table legible these results are not shown). No significant difference as determined by 95 % confidence intervals was observed between the tests.

Table 3 shows the computed specificity, sensitivity, and 95 % confidence intervals of each

Table 1
Results of the various diagnostic tests for each allergen

	House dust mite		Cat		Dog		Total	
	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
BPT	7	28	12	13	14	7	33	48
SPT	7	28	9	16	19	12	25	56
RAST	7	28	8	17	9	12	24	57
HR	5	26	10	11	8	9	23	46

Numbers indicate number of positive or negative test results among 81 determinations/test except for HR in which test results from two non-responding patients were excluded.

Table 2

Concordances between the various tests							
		SPT		RAST		HR	
		Pos	Neg	Pos	Neg	Pos	Neg
BPT	Pos	46	3	45	4	44	3
	Neg	10	22	12	20	7	25
Concordance %		84		80		87	
HR	Pos	43	8	46	4		
	Neg	11	17	9	20		
Concordance %		76		84			

test when “pooling” the data for the three allergens. Hence, SPT gave the highest sensitivity (0.94) followed by RAST (0.92) and HR (0.90). However, the 95 % confidence intervals obtained with the various tests overlapped each other, thus, the differences were not significant. In contrast, HR showed a significantly better specificity of 0.78 compared with RAST (0.63). No difference between HR and SPT (0.69) was demonstrated.

DISCUSSION

Until recently, the histamine release test has not been carried out routinely and its diagnostic value is not fully known. To keep the study easily comparable to everyday practise a complex scoring system was not employed and the limits chosen for positive BPT, SPT and RAST are those generally accepted (5, 9, 11, 12, 13). In this study extracts from different batches were used. However, the extracts were obtained from the same source (Pharmalgen) except the house dust mite extract from Dome-Hollister-Stier used for BPT, although, the raw materials used in Pharmalgen and Dome-Hollister-Stier were bought from the same company (Allergon, Sweden). It was expected that this would influence the concordances to a greater extent than demonstrated. However, the concordances computed are in agreement with other reports (3, 5, 9). This is probably because the different tests, despite the use of various extracts, to a high degree measure the same phenomenon when performing the tests in a selected group of patients.

Table 3

Sensitivity and specificity of the various tests			
	RAST	SPT	HR
All allergens 49 ² (81) ¹ Sensitivity	0.92 (0.84–0.95)	0.94 (0.88–0.98)	0.90 (0.84–0.98)
Specificity	0.63 (0.52–0.69)	0.69 (0.62–0.78)	0.78 (0.70–0.86)

Values in parentheses are the 95 % confidence limits.
¹Indicates number of patients studied. ²Indicates number of positive BPT.

It was found that HR is a highly specific method demonstrating a better specificity than RAST, thus, indicating that HR is better than RAST in confirming clinically manifest allergy. A comparison with SPT (Phazet) showed no such difference. The sensitivity of the various tests was of similar value (> 90 % sensitivity) and no significant difference was observed between them. These values are high and well within the range reported in previous studies in children and adults (1, 2, 5, 10, 11, 12). However, different threshold values which determine a positive or negative test result may cause variation in the results reported (5, 12). The threshold value for HR (30 ng histamine/ml) was selected by the company before the study was initiated and, unfortunately, it was not possible to evaluate the data using a lower threshold value. It seems relevant, however, since the present limit of methodological variation is 15 ng/ml (3 ± SD). As recently demonstrated, a lower threshold value may increase the sensitivity of HR without affecting the specificity (5).

In summary, the HR test seems to be a valuable routine histamine release test for children. The study indicates a diagnostic value comparable to RAST and SPT; however, it should be confirmed in a prospective follow-up study.

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