

Specific Diagnosis of Exogenous Bronchial Asthma in Children

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Twenty-six children with exogenous asthma were studied by case history (CH), skin tests (ST), allergen-specific serum IgE (RAST), basophil histamine release (HR), and bronchial provocation tests (BPT). Nine standardized allergens were used from the following groups: house dust mite, animal dander, pollen, and mould fungi. For each allergen, the same batch was used throughout for all *in vivo* and *in vitro* studies, and the tests were carried out in a controlled, double-blinded manner, independent of each other. Different allergen concentrations were used and the results graded on a semi-quantitative scale. Approximately 120 comparable investigations, using *in vivo* and *in vitro* tests were carried out, followed by calculations of the sensitivity and specificity for each test alone, as well as for various combinations of tests, at various allergen-concentrations, and compared with the result of BPT, i.e. the true diagnosis. In this way the results could be used to directly compare the diagnostic value of each test, and combinations of tests. In general, a careful CH combined with ST gave the most reliable results. RAST was the most unreliable single analysis. When pollen or mite allergy was suspected, a combination of ST with either RAST or HR gave a further diagnostic reliability. HR as a single analysis did not seem to offer advantages over existing methods. None of the tests, or combinations of tests offered 100 % sensitivity and specificity. By altering the concentration of allergen, the sensitivity, or specificity could be increased, but only at the expense of a decrease in the other. BPT is still necessary in many instances when a specific diagnosis is required.

Key words: asthma bronchiale; bronchial provocation test; case history; diagnosis; histamine release from basophils; radioimmunoassay; sensitivity; skin prick test; specificity.

Accepted for publication 22 August 1985

Identification of an offending allergen in exogenous bronchial asthma in children cannot always be made by a careful case history (CH) combined with a skin prick test (ST). It is often necessary to perform a bronchial provocation test (BPT). This is, however, time-consuming and requires hospitalization for safety. It is therefore important to clearly establish to what extent BPT may be replaced by RAST, or the more recently developed technique based on release of histamine from leucocytes exposed to allergen (HR). The latter technique has been found to correlate well with BPT in adults (8) but experience in children is still limited. The

present study was undertaken to compare the sensitivity and specificity of the different *in vivo* and *in vitro* assays, including HR, while at the same time analysing the value of combining these tests.

MATERIAL AND METHODS

Patients

The study comprised 26 children (13 boys and 13 girls aged 7-14 years (mean 9.6)) with a history of extrinsic asthma. All had asthma as their main disease. None of the children had

ever received immunotherapy. After the CH had been obtained the children were hospitalized and BPT, ST, RAST and HR carried out. At time of study, none received systemic steroids. Bronchodilating medicine was suspended for at least 6 h, and aminophylline and antihistamines for at least 72 h prior to study.

Experimental design

A total of 122 comparable investigations between CH, ST, RAST, HR and BPT were carried out. All were performed in a blinded manner, independent of each other. CH were obtained by the same experienced clinical allergologist (O.Ø.). ST were done by the same experienced nurse, BPT by a few experienced investigators, and HR and RAST by the same investigator but without knowledge of the relationship between samples for the two tests. Results remained confidential until all analyses had been completed and scored as shown in Table 1.

Each child was provoked with the suspected allergens and at least one non-suspected allergen. Tests were made with nine different allergens from the following four groups; house dust mite, animal dander, pollen and mould fungi.

Allergen extracts

Standardized allergen extracts were supplied by the Allergological Laboratory A/S Copenhagen, and the same batches were used throughout the study. For each extract, ST, HR, RAST and BPT were done with preparations from the same original freeze-dried batch.

Skin prick test (ST)

ST were performed on the volar surface of the forearm with each extract in concentrations 0.01, 0.1, 1, and 5 HEP (Histamine Equivalent Prick) per ml (1). The wheals were recorded when the reactions were at maximum and compared with a positive reference containing histamine chloride, 1 mg/ml (Table 1). The contours of the wheals were marked with a felt-tipped pen and transferred to squared mm paper with a strip of tape. The size of the wheals was measured using the formula

$$\frac{(D + d)}{2} \text{ mm,}$$

where D is the biggest diameter and d is the perpendicular diameter.

Table 1
Score system

Score	Case history	Skin test	RAST	Histamine release	Bronchial provocation ⁴
0	Negative	< 75 % of histamine reference with 5 HEP ¹ extracts	allergy ³ class 0	< 20 % with 5 HEP extracts	negative
1	Doubtful	> 75 % of histamine reference with 5 HEP extracts	allergy class 1	> 20 % with 5 HEP extracts	> 20 % fall in FEV ₁ ² with 5 HEP extracts
2	Probable	> 75 % of histamine reference with 1 HEP extracts	allergy class 2	> 20 % with 1 HEP extracts	> 20 % fall in FEV ₁ with 1 HEP extracts
3	Certain	> 75 % of histamine reference with 0.1-0.01 HEP extracts	allergy class 3-4	> 20 % with 0.1-0.01 HEP extracts	> 20 % fall in FEV ₁ with 0.1-0.01 HEP extracts

¹ HEP = histamine equivalent prick (1).

² FEV₁ = forced expiratory volume in 1 sec.

³ Vanto et al. (6).

⁴ BPT using fungi were only carried out to a maximal concentration of 1 HEP.

Allergen-specific IgE (RAST)

Allergen-specific IgE was determined using a modified radioallergosorbent technique as described by Weeke (7) on sera stored at -20°C . The allergens were coupled to Alhydrogel® (Superfos, Vedbæk, Denmark) and the analyses performed as previously described and the results expressed in the allergy classes 0 to 4 (6). The difference between this RAST and Phadebas RAST is in essence that the allergen here is coupled to a gel whilst the allergen in Phadebas RAST is coupled to a paper disc. Vanto et al. (6) have made a comparison between the two tests and this showed a good correlation, although AL-RAST was more sensitive and less specific than Phadebas RAST.

Histamine release from basophil leucocytes (HR)

The method used was a fluorimetric determination of histamine released from EDTA stabilized blood (5). The blood cells were washed extensively as described in detail by Diamant & Patkar (2), and finally resuspended in buffered balanced salt solution to a final volume of twice the original blood sample. 1 ml samples were transferred to 3 ml plastic tubes, and 10 μl samples of aqueous allergen solutions at concentrations 0.001, 0.01, 0.1, 1, and 5 HEP added. The tubes were incubated at 37°C for 40 min, transferred to an ice bath for 10 min to stop the reaction, and centrifuged. 0.6 ml of the supernatants were transferred to Technicon cups and precipitated with an equal volume of 7% perchloric acid. At this stage the cups were stored at -20°C until further analysed. The histamine in the precipitate was estimated in a Technicon Autoanalyzer system (2, 4). Release is given as per cent of total histamine content of the blood sample as determined by perchloric acid precipitation of washed blood samples (mean of three determinations). The results were scored as shown in Table 1. For each patient, the release of histamine upon exposure to rabbit anti-human IgE at final concentrations of 25 μg , 5 μg , and 2.5 μg , instead of allergen, was determined.

Bronchial provocation test (BPT)

BPT were carried out by free inhalation of 1 ml samples of nebulized aqueous allergen extracts in increasing concentrations of 0.01, 0.1, 1, and 5 HEP, except mould fungi where 1 HEP was maximum. Before and after inhalation of each concentration, FEV₁ was recorded on an electronic spirometer (Spirotron®, Dräger) and the patient was asked for symptoms, and chest auscultation performed before raising the concentration one decade. A positive reaction was defined as a decrease in FEV₁ by 20% or more. The reaction was scored as shown in Table 1, but in the subsequent calculations of sensitivity (SE) and specificity (SP), all positive reactions, regardless of the score value, were regarded "positive", i.e. "the true diagnosis". Inhalation was started each time with phenol saline as negative control, and all patients showed a negative result with at least one allergen extract at maximum concentration. After BPT, peak expiratory flow rate (PEFR) was recorded every half hour for the next 6 h to detect any late reactions none of which were seen.

BPT was carried out with only one allergen per day. We regard the results from these tests as independent in spite of the sometimes short interval between tests. There is no evidence that a positive reaction one day increased the likelihood of a positive reaction the following day. Following a positive test result, 14 patients showed a BPT result that agreed with the result predicted from the CH. Only two showed an "unexpected" positive result, i.e. the CH score was 0, whilst eight showed an "unexpected" negative result. Positive and negative results were evenly distributed throughout the entire investigation, and we are satisfied that the interval between tests is sufficient.

Calculation of sensitivity (SE) and specificity (SP)

SE indicates how well each analysis, or analyses combination, can predict a positive BPT, i.e. make a correct diagnosis of the "ill" group. Conversely, SP indicates how well each analysis, or analyses combination, can predict a

negative BPT, i.e. make a correct diagnosis of the "well" group (8).

If a positive analysis result is confirmed by a positive BPT, then the analysis result is a "true positive" (TP) ("ill"). On the other hand, if BPT is negative, the analysis would have been "false positive" (FP) ("well"), and the analysis would have given a wrong diagnosis, because a "well" person would be classified as "ill".

If a negative analysis was confirmed by a negative BPT, the analysis result was "true negative" (TN) ("well"). If BPT was positive the analysis would be "false negative" (FN) ("ill"), and an "ill" person would be classified as "well".

Analysis	BPT	
	+	-
	+	-
	TP	FP
	FN	TN

Combining SE and SP gives a measure of how well a single analysis or a combination of different analyses can distinguish between ill and healthy subjects.

The terms calculated are

$$SE \% = \frac{TP \times 100}{TP + FN} \quad SP \% = \frac{TN \times 100}{TN + FP}$$

With a graded score system using a scale of 0-3, the boundary between "ill" and "well" can be arbitrarily set at 3 points on the scale. This produces a set of results which can be shown graphically as "discrimination curves" (Figs. 1 to 7). At the point shown as "A", patients with score 0 were classified as negative, while those with scores 1, 2, and 3 were classi-

fied as positive. At point "B", the limit was set between scores 1 and 2, and at "C", between 2 and 3. Each "discrimination curve" therefore consists of the set of three points; A, B, and C. It is clearly seen that A, which represents a limit at a low score, i.e. a weak reaction, gives the highest sensitivity. Conversely, point C, with the highest score-limit, represents the highest specificity.

RESULTS

Table 2 shows the distribution of score values for all allergens within each of the four analyses (CH, ST, RAST, HR) in relation to the BPT score values.

Fig. 1 shows the discrimination curves for each of the four assays using house dust mite. There are no points close to the ideal (BPT), although ST, HR, and CH were very specific (few FP) and RAST very sensitive (no FN at point A). ST was the best single analysis.

Fig. 2 shows the results when animal dander is used. These results are generally better than those obtained with dust mite. The results of ST are clearly the best, with high sensitivity and specificity (points A and B).

Fig. 3 shows the discrimination curves for pollen. None of the four analyses was in fact very reliable, showing low sensitivity but acceptable specificity at high score limits.

Fig. 4 shows the results for mould fungi. ST was highly specific and also highly sensitive (points A and B) whereas the other analyses, particularly RAST, showed low sensitivity.

The results for each of these four different

Table 2

Combination of results of case history (CH), skin tests (ST), allergen-specific serum IgE (RAST), and histamine release from basophil leucocytes (HR), with the results of bronchial provocation tests (BPT) for nine allergens in 26 children with exogenous asthma

BP score ¹	CH score ¹				ST score ¹				RAST score ¹				HR score ¹			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
0	25 ²	31	1	2	43	8	3	2	18	27	10	3	48	0	9	2
1	5	9	2	2	5	3	7	4	5	5	6	2	8	0	4	5
2	3	9	10	3	2	8	12	4	6	4	6	9	8	2	4	13
3	0	5	8	4	1	0	8	7	1	3	2	10	6	0	2	9

¹ Score value of a result is calculated as defined in Table 1.

² Figures represent the number of results with the given combination of score-value in BPT and the given test (here CH).

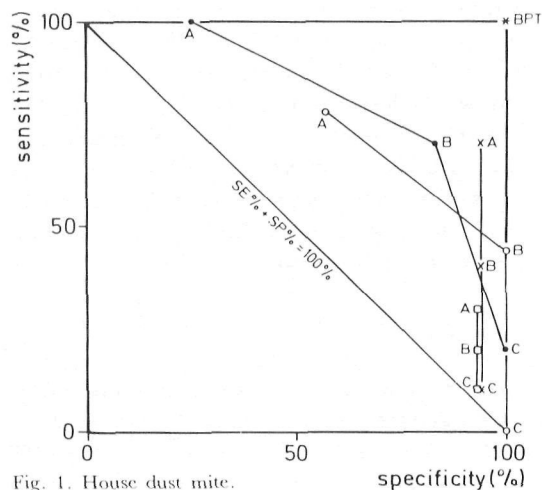


Fig. 1. House dust mite.

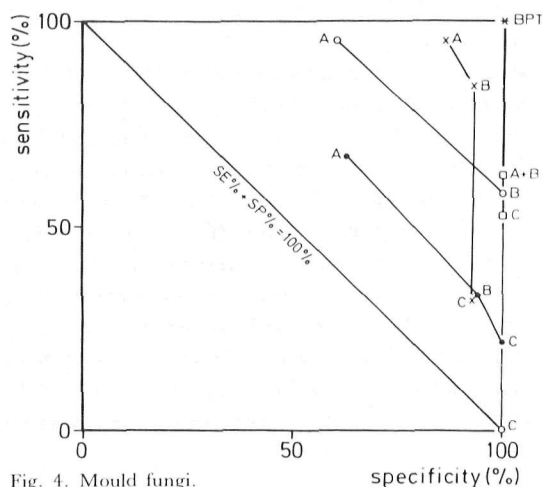


Fig. 4. Mould fungi.

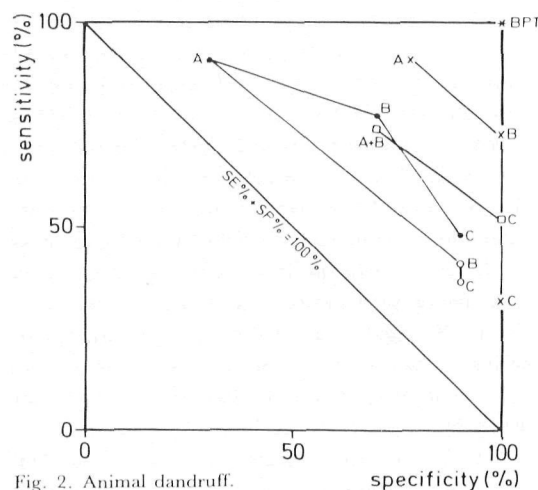


Fig. 2. Animal dandruff.

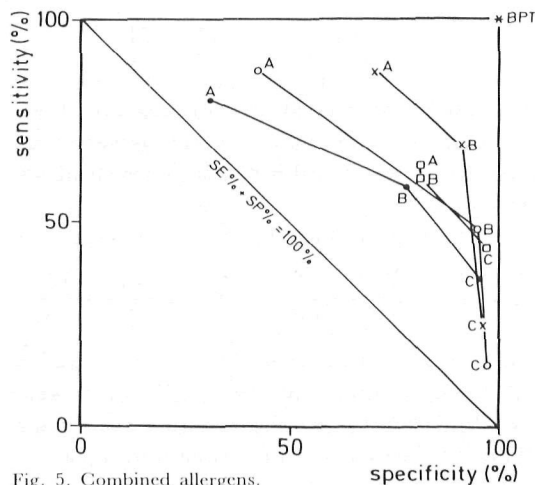


Fig. 5. Combined allergens.

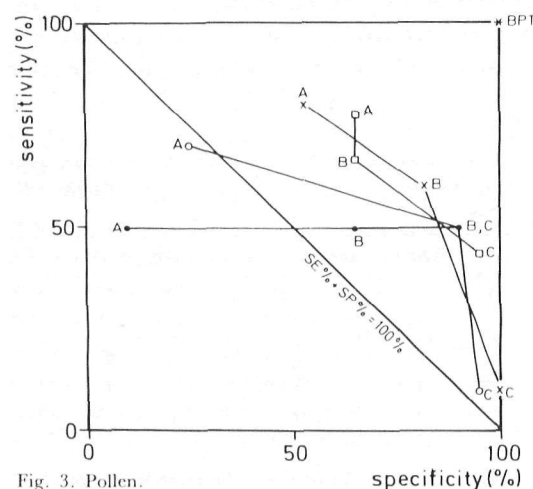


Fig. 3. Pollen.

Figs. 1-5. SE% (ordinate) and SP% (abscissa) for the individual analyses: CH, ○; ST, ×; RAST, ●; HR, □; and BPT, * for: House dust mite; animal dander; pollen; mould fungi; combined allergens.

A, B, and C represent the different limits between a positive and a negative result:

A: $0/1 + 2 + 3$, the limit is set between 0 and 1.

B: $0 + 1/2 + 3$, the limit is set between 1 and 2.

C: $0 + 1 + 2/3$, the limit is set between 2 and 3.

A positive result in BPT was regarded as the true diagnosis, regardless of the score value of the BPT result.

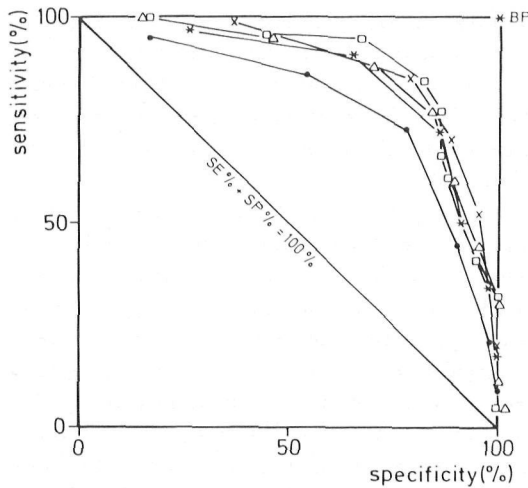


Fig. 6. SE % (ordinate) and SP % (abscissa) for the following analysis combinations: CH + ST, x; CH + RAST, ●; ST + RAST, *; CH + ST + RAST, △; and CH + ST + RAST + HR, □.

A, B, and C represent the different limits between a positive and a negative result as described for Figs. 1-5.

allergen groups have been combined and plotted as before (Fig. 5). The discrimination curves for the three analyses, HR, CH, and RAST, are similar. In general, ST is a very reliable analysis and clearly better than any of the others.

Since in clinical practice a number of different analyses are used in the examination of allergic patients, we have combined the results from the different analyses in various ways (Fig. 6). As might be expected, a combination of all analyses gives the best result. The most reliable combinations all include ST, as could be expected from the results of the individual assays. The least reliable combination is that of CH and RAST.

It might be argued that BPT was too sensitive when the limit was set between no reactivity and reactivity at the highest HEP concentrations resulting in false positive BPT, which could jeopardize the results of the other tests. The SE and SP were therefore re-calculated for the combined ST at the three different score limits of reactivity in BPT: the original limit between score 0 and 1, between 1 and 2,

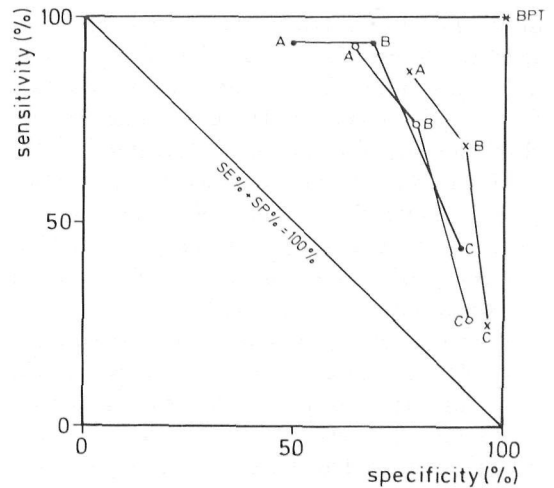


Fig. 7. SE % (ordinate) and SP % (abscissa) for ST for combined allergens. A, B, and C represent the different limits between a positive and a negative result for ST.

The three curves represent different score limits of reactivity in BPT:

- x: 0/1 + 2 + 3, the limit is set between 0 and 1.
- : 0 + 1/2 + 3, the limit is set between 1 and 2.
- : 0 + 1 + 2/3, the limit is set between 2 and 3.

and finally between 2 and 3. Fig. 7 shows that as the limit decreases, the ST becomes more sensitive, but at the expense of loss of specificity. The discrimination curve is displaced upwards and to the left. Comparable results were found with CH, HR, and RAST (data not shown).

DISCUSSION

In the present study, all analyses were carried out prospectively in a defined group of children in a controlled, double-blinded manner. For each allergen, the same batch was used for all *in vivo* and *in vitro* tests. The importance of the study is, therefore, that the results permit a direct comparison of the diagnostic value of the different tests – as well as combinations of tests – available to the allergologist. A rather complex picture was disclosed, with differences from test to test and from allergen group to allergen group. However, some more general conclusions can be drawn. Thus, when only one test is applied, the ST appears to be the most reliable. RAST, on the other hand, was the

most unreliable single analysis, and only reasonably dependable when the allergen was house dust mite. None of the tests was satisfactory in pollen allergy. However, most of the children were multiallergic and besides asthma, some had rhinitis, including pollen rhinitis, but possibly not pollen asthma. Nasal provocation tests were not done in this study and a high frequency of "false positive" BPT has been shown in hay fever patients (3).

Previous studies in adults have shown the novel HR to be of considerable diagnostic aid (8). This was not confirmed in the present study in children. However, the studies are not directly comparable. First, the adult study was done with a method which did not include automatized analyses (8). Second, the limit of positive reaction at 20% release may be too high. Diamant (2) has used 8% as the limit, and this would have increased the sensitivity in this study. Further, some of the false negative results with HR were in patients who reacted weakly to anti-IgE. This control measure may be of possible value in the interpretation of the reliability of the HR. It appears, however, that the value of HR needs to be further clarified in controlled studies like the present.

It is possible that BPT was too sensitive (too many false positive) with 5 HEP extracts, and that reactions at this concentration do not represent "the true diagnosis. This problem cannot be solved by the present study. It is clear from the data that there is a relationship between the strength, i.e. the score value of BPT and the score value of other tests. In other words, the sensitivity and specificity of a test with a given antigen concentration depend on the limit set for a positive BPT, i.e. "the true diagnosis". This is most simply illustrated by the data given in Fig. 7, showing that as the criteria for a positive diagnosis at BPT are tightened, i.e. the patient must react at a lower concentration, then the sensitivity of the ST is increased, but at the expense of loss of specificity. The criteria and the limits thus have a reciprocal influence. It is interesting to note that as more and more data are accumulated in a discrimination curve (Fig. 6), the middle part, which represents an intermediate limit,

becomes successively closer to 100% specificity and sensitivity. Deviations from this point may increase either sensitivity or specificity, but only at the expense of the other. Exactly the same is true of the limit for a positive BPT, i.e. the reference.

It is important for the allergologist to understand this complex relationship. If BPT is not used, the various *in vivo* and *in vitro* tests can be used singly, or in combinations, to give approximate predictions of how the patient will react to inhalation of the allergen. The reliability of this approximation depends upon proper selection of allergen concentration, proper evaluation of the relationship between reactivity in the test or tests, and level of reactivity in BPT, i.e. "the true diagnosis".

When looking at the figures it is obvious that 1 HEP and 5 HEP concentrations give very different results. With 1 HEP there is a high risk of false negatives, while with 5 HEP there is a high risk of false positive results. In our department we now use a 3 HEP concentration as standard in ST with the allergens used in this study. RAST and HR are used only rarely. In cases where there is a discrepancy between CH and ST, or the results from the ST need corroboration, the BPT is used.

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