

Specific Diagnosis of Exogenous Bronchial Asthma in Adults

Comparison between Case History, Intracutaneous Test, RAST, Histamine Release from Basophil Leucocytes, and Bronchial Provocation

OLE ØSTERBALLE, HANS-JØRGEN MALLING and BENT WEEKE

Medical Department TA and the Laboratory for Clinical Allergy,
Rigshospitalet, Copenhagen, Denmark

Twenty-two adults with extrinsic bronchial asthma were examined with 26 different allergens from five groups: house dust + mite (30 BP), animal dandruff (30 BP), pollen (12 BP), mould fungi (11 BP), and feather + cotton (10 BP). The same allergen batch was used for each allergen extract in all analyses.

Based on the results of the single analyses: case history (CH), intracutaneous test (ST), RAST, histamine release from basophil leucocytes (HR), and the results of the analysis combinations and the "true diagnosis" bronchial provocation (BP), the sensitivity and specificity (SE and SP) were calculated and shown graphically. An evaluation of the diagnostic reliability of the single analyses and analysis combinations was then possible.

Generally, HR was the best individual analysis with very few false positive values and false negative values, followed by RAST, ST, and CH. None of them was able to predict allergy for feather + cotton. Otherwise, BP was positive when HR and RAST reached scores 2 and 3 with the exception of RAST for animal dandruff. As regards ST, we had to reach score 3 in order to predict positive BP. CH would only provide the diagnosis with a maximum score in the pollen group. BP was positive in any analysis combination resulting in score 6 or more. ST appeared to be the most sensitive of the individual analyses. All the patients with ST score 0 showed negative BP for all allergen groups, whereas a corresponding sensitivity for HR score 0 was only achieved with animal dandruff, and for RAST score 0 for pollen only.

In spite of the new analyses, BP is in many cases necessary if a definite diagnosis is required.

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

Accepted for publication 3 October 1978

The most important tools of allergology have traditionally been a good case history (CH) combined with a skin test (ST).

Since CH + ST have not always proved adequate, when required we supplement our investigations with nasal (NP) or bronchial (BP) provocation and regard the result of the provocation as the "true diagnosis". Unfortunately, the provocations take some

time. A patient can be provoked with only one allergen a day, and the investigation must therefore be reserved for specially selected allergens.

In an attempt to find other ways to obtain the "true diagnosis" research in recent years has resulted in the development of new allergen-specific investigations, first the RAST-analysis, and recently it has been

shown (6, 12) that histamine release from basophil leucocytes (HR) seems well correlated with BP.

Previously, several investigations have shown a relation between CH, ST, RAST, and BP (1, 2, 3, 4, 5, 8, 9, 10, 11, 13, 14).

In this study the analyses CH, ST, RAST, and HR were evaluated both singly and combined, based on the sensitivity and specificity, and graded according to their diagnostic reliability.

MATERIAL AND METHODS

Patients

Twenty-two adults with an exogenous bronchial asthma were examined, seven men and 15 women, aged 16–49 years. All the patients had bronchial asthma as their main symptom. In addition, 18 of them suffered from allergic rhinitis, four from atopic dermatitis, and two from urticaria. None of them showed symptoms of gastrointestinal allergy and none had complicating diseases of heart or lungs, or diseases affecting the serum proteins.

All the patients were admitted to hospital in connection with a bronchial provocation. At the time of examination none of them was receiving any systemic steroid treatment. Bronchodilating spray and prophylactic medicine were suspended for at least 6 h prior to the examination, and neither aminophylline nor anti-histaminics had been given for 24 h.

The patients had not previously received immunotherapy with the allergen extracts used in the examination. Four had previously received immunotherapy with allergen extracts belonging to other groups than those used here.

Investigations

Ninety-three comparable investigations were made between CH, ST, RAST, HR, and BP. Tests were made with 26 different allergens from five groups: house dust+mite (30 BP),

animal dandruff (30BP), pollen (12BP), mould fungi (11 BP), and feather+cotton (10 BP). The result of the BP was regarded as the "true diagnosis".

Case history (CH)

The case history was based on repeated talks with the patients during their admission and were carried out by different doctors. Information on the relation between exposition and asthma symptoms was evaluated by the authors independently, and the scores were graded as shown below. As regards the less well-defined allergens such as house-dust, mould fungi, and feather, the case history gave only 1 point which corresponded to a doubtful relation.

Skin test (ST)

The skin test was carried out as an intracutaneous test using aqueous allergen extracts. ST was performed on the volar surface of the forearm with extracts increasing in concentration by factor 10 from 1:1.000.000 = 10^{-6} w/v to a concentration which provoked an urticaria reaction in the skin, corresponding to a histamine reference 0.1 mg/ml, or at most 10^{-2} . The urticaria reactions were read after 10–20 min when the reaction was at maximum, the contours of the urticaria were marked with a phenol pen and using a strip of tape transferred to squared millimetre paper.

The concentration of the extract which produced an urticaria reaction equal to a histamine reference = 1 HEC (histamine equivalent intracutaneous) was used as a result.

Allergen specific IgE

Allergen specific IgE was found by RAST, according to Cheska et al. (7). The dialysed allergen extracts, 10^{-2} , were mixed with cyanogen bromide-activated pieces of filterpaper (5 mm diam.) in proportion 0.1 ml extract to five pieces of paper. At a later analysis, 0.05 ml serum was added to

one of the pieces of paper, and after a reaction time of at least 3 h at room temperature any eventual allergen-specific IgE had bound to the allergen coupled papers. Bound IgE was now found by adding ^{125}I anti-IgE (Pharmacia, Phadebas RAST) and by counting the paperbound activity in a gamma counter. Counts of a blind test were compared with counts of a reference system consisting of timothy grass pollen extract attached to paper, and serum from a timothy allergic patient. By relating blind tests to this reference system of 100 SU (sorbent units) and to employed serum diluted 1+0, 1+4, 1+24, 1+49 we achieved a clinical grading in the following allergy classes:

- Allergy class 0 < 2 SU = no allergy
- Allergy class 1 < 4 SU = doubtful allergy
- Allergy class 2 < 20 SU = weak allergy
- Allergy class 3 < 100 SU = moderate allergy
- Allergy class 4 $\geq$ 100 SU = strong allergy

All analyses were double controlled and the results were set as the mean value. The error of analysis for the single allergen extracts ranged from 10 to 20% of relative deviation.

Histamine release from basophil leucocytes (HR)

HR has been previously described in detail (12). We used approximately 4 ml whole blood per allergen. The newly tapped venous blood was anti-coagulated with EDTA. After diluting with 0.9% sodium chloride the EDTA-blood was centrifuged in Ficoll-Hypaque and the monocellular layer isolated and washed. The cellular suspension was then divided into a number of glass tubes and the allergen dilutions added. After 40 min incubation at 37°C the allergen had bound to the basophil leucocytes and histamine was released. The supernatant was removed and the remaining histamine in the cells estimated. The percentage histamine release in relation to the total content of histamine in the cells was then recorded. A characteristic curve was obtained by expressing the released histamine in % of total histamine (ordinate) with four dilutions of the allergen extract

(abscissa), e.g. allergen extracts at dilutions 10^{-9} , 10^{-7} , 10^{-5} , and 10^{-3} .

A positive release of 30% or more of the total cell content was defined as positive.

The allergen dilution which resulted in a release of 30% was used as the result. All stages of the analyses were double-controlled.

Bronchial provocation (BP)

BP was effectuated with 1 ml aqueous extract in phenol saline atomized with a Pari-atomizer and an inhalation instrument of the type "Inhalier Boy". By activating the atomizer only when the patient inspired (push-button for self-service) the whole extract was atomized during inspiration, and 1 ml was inhaled in approximately 5 min. Allergen extracts of increasing concentrations from 10^{-6} to 10^{-2} were used. Positive reaction was defined by a fall in FEV₁ (forced expiratory volume in one second) of 20% and more. BP was carried out with only one allergen a day. Inhalation with phenol saline was included as a negative control, and most of the patients had negative BP with at least one allergen extract at the highest concentration 10^{-2} .

In high concentrations (10^{-2}) the allergen extract used did not produce any unspecific positive BP in asthma patients with a different allergy (no unspecific irritant effect).

Allergen extracts

Allergen extracts were supplied by Allergologisk Laboratorium A/S, Copenhagen. All extracts had been dialysed by the manufacturer; molecules of less than 8,000 Daltons were removed together with unspecific irritants. Extracts with the same batch-number were used throughout the investigation. In RAST and HR extracts were diluted in 0.9% sodium chloride and stored at -20°C for less than a year. In BP and ST extracts were diluted with phenol saline (0.4% phenol/0.9% sodium chloride) at concentrations 10^{-2} – 10^{-6} (w/v). The strongest

extracts 10^{-2} and 10^{-3} were stored for up to 6 months at 4°C . The weaker extracts were made up regularly and used within approximately 3 months, stored at 4°C .

SCORE SYSTEM

Case history (CH)

- 0 = *Do not know* whether symptoms were related to the allergen in question.
- 1 = *Doubtful*, as exposure to the allergen only rarely provoked symptoms.
- 2 = *Probable*, as the allergen *often* provoked symptoms.
- 3 = *Certain*, as the allergen *always* gave symptoms in connection with exposure.

Skin test (ST)

- 0 = negative
- 1 = 1 HEC with extract dilution 10^{-2}
- 2 = 1 HEC with extract dilution 10^{-3} and 10^{-4}
- 3 = 1 HEC with extract dilution 10^{-5} and weaker.

Radioallergosorbent test (RAST)

- 0 = negative (allergy class 0)
- 1 = doubtfully positive (allergy class 1)
- 2 = moderately positive (allergy class 2)
- 3 = strongly positive (allergy classes 3 and 4).

Histamine release (HR)

- 0 = negative HR with less than 30% release
- 1 = positive with 30% release with extract dilution 10^{-3}
- 2 = positive with 30% release with extract dilution 10^{-5} and 10^{-7}
- 3 = positive with 30% release with extract dilution 10^{-9} and weaker.

Bronchial provocation (BP)

- 0 = negative
- 1 = positive with extract dilution 10^{-2}

2 = positive with extract dilution 10^{-3} and 10^{-4}

3 = positive with extract dilution 10^{-5} and weaker.

Sensitivity and specificity

In this study these values were used to evaluate whether the single and combined analyses were reliable enough to use for obtaining a correct diagnosis – “ill” or “well”.

Sensitivity (SE) indicates how well the single analyses, or analysis combinations, can predict positive BP, i.e. make a correct diagnosis in the “ill” group (positive BP).

Specificity (SP) indicates conversely, how well the single analyses, or analysis combinations, can predict negative BP, i.e. make a correct diagnosis in the “well” group (negative BP).

If a positive analysis result was confirmed

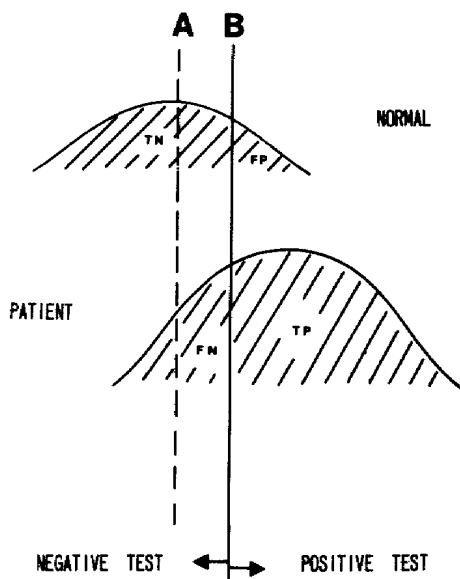


Fig. 1. A population of “well” TN+FP and “ill” TP+FN is divided up into two subgroups by means of an analysis with low (A) and high (B) score limits. For details, see text.

by a positive BP, the analysis result was "true positive" (TP) ("ill"). On the other hand, if BP was negative, the analysis result would be "false positive" (FP) ("well"), and the analysis had given a wrong diagnosis, because a healthy person without BP had been regarded as ill.

If a negative analysis was confirmed by a negative BP, the analysis result was "true negative" (TN) ("well"). If BP was positive, the analysis would be "false negative" (FN) ("ill"), and an ill person without BP would be recorded as healthy.

This is illustrated in Fig. 1.

Fig. 1 illustrates a population of healthy people (TN+FP) and a population of ill people (FN+TP). The vertical lines exemplify the chosen score limits. If a strong degree of certainty is required for a positive analysis value the limit for the single analyses is placed between score 2 and score 3 (B), thus eliminating most of the FP at the expense of many FN. If, on the other hand, one wants to use the analysis to detect as many ill people as possible (TP), the score limit is placed between score 0 and score 1 (A). The single analyses thus get a minimum of FN, but often a great many FP.

The combination of SE and SP indicates how well an analysis or analysis combination can separate the ill from the healthy people. As this allows for several score limits, it can be illustrated graphically (Figs. 2 and 3). The chief aim is to completely separate the curves (Fig. 1) for "ill" and "well", so that by selecting a score limit 100% SE and 100% SP

is achieved. At worst, the curves will overlap, showing that the analysis (analysis combination) is unable to separate "ill" from "well", and you will get an 0-discrimination curve (Figs. 2 and 3), where each point on the curve means that $SE\% + SP\% = 100\%$.

The following formulas can be used:

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

RESULTS

In Tables 1-4 the single analyses and their combinations are related to BP by use of grading. The numbers in the Tables represent the number of BP at different score combinations.

By estimating SE and SP for the single analysis the score limits were set between score 0/1, 1/2, and 2/3, respectively, which for the single analysis resulted in three combined SE/SP, i.e. points A, B, and C at scores 0/1+2+3, 0+1/2+3, and 0+1+2/3, respectively.

By combining two analyses 6 score limits appeared, and correspondingly three and four analysis combinations produced 9 and 12 score limits, respectively.

SE and SP were estimated for each score limit based on the analysis results shown in Tables 1-4, and in Figs. 2a and 3 with SE% as ordinate ("ill") and SP% as abscissa ("well") for the combined allergens. In Fig. 2b-f, SE% and SP% are shown for the individual allergen groups, but not in Table form.

Table 1

Number of BP with the combined allergens at different score combinations of BP and the single analyses

BP scores	CH scores				ST scores				RAST scores				HR scores			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
0	20	16	9	1	13	18	15	—	29	14	2	1	41	5	—	—
1	5	2	5	3	—	1	13	1	3	3	6	3	3	3	3	6
2	5	7	13	5	—	2	20	8	3	7	8	12	2	3	2	23
3	—	—	1	1	—	—	1	1	—	—	1	1	—	—	—	2

In Figs. 2 and 3 TN and TP (in % of all "well" and "ill", respectively) are marked on the left and below the curves, respectively, and FP and FN on the right and above the curves, respectively (the remaining percentage). The spaces on the left and below the curves up to the 0-discrimination curve illustrate how well the analysis in question can predict the result of BP (TN+TP), whereas the spaces on the right and above the curves correspond to the false results (FP+FN), where the diagnosis can only be achieved by combining the analyses or carrying out BP. The aim is, per definition, "a curve" for BP, which is indicated as a point on the top right corner with 100% TN and 100% TP.

If an analysis curve were placed further to the right and above the curve for a different analysis, the first analysis would be better able to predict the "true diagnosis" (=the result of BP).

Table 2
Number of BP with the combined allergens at different score combinations of BP and two analysis combinations.

BP scores	Analysis scores						
	0	1	2	3	4	5	6
CH + ST							
0	6	13	16	6	3	2	—
1	—	—	5	3	4	2	1
2	—	—	4	9	8	8	1
3	—	—	—	—	1	—	1
CH + RAST							
0	11	22	6	5	2	—	—
1	2	—	4	4	1	3	1
2	2	—	5	9	4	6	4
3	—	—	—	—	1	—	1
ST + RAST							
0	8	14	18	5	1	—	—
1	—	—	4	2	6	2	1
2	—	—	5	5	4	12	4
3	—	—	—	—	1	—	1

Table 3

Number of BP with the combined allergens at different score combinations of BP and three analysis combinations

BP scores	CH + ST + RAST scores									
	0	1	2	3	4	5	6	7	8	9
0	3	10	15	9	6	3	—	—	—	—
1	—	—	2	—	5	3	1	3	—	1
2	—	—	2	—	6	7	2	7	5	1
3	—	—	—	—	—	—	1	—	—	1

Table 4

Number of BP with the combined allergens at different score combinations of BP and four analysis combinations.

BP scores	CH + ST + RAST + HR scores												
	0	1	2	3	4	5	6	7	8	9	10	11	12
0	3	10	14	9	4	6	—	—	—	—	—	—	—
1	—	—	1	1	2	1	1	3	2	1	2	—	1
2	—	—	—	—	1	2	5	3	6	1	6	5	1
3	—	—	—	—	—	—	—	—	—	1	—	—	1

Fig. 2a shows that, in general, the curves for the individual analyses and the combined allergens are separated, and, therefore, the spaces between the single curves show an increasingly diagnostic reliability in the following order: CH, ST, RAST, and HR.

Fig. 2b shows that the discrimination curves for house dust+mite are completely separated.

As regards scores 2 and 3, RAST can predict positive BP, and much better than for the combined allergens. However, it is surpassed by HR which includes more "ill" (fewer FN). By employing HR the diagnostic reliability is nearly on a level with BP, as only a small space with FP+FN remains. The curve for the combination CH+ST+RAST+HR is not marked, but it appears to be 100% TN and 100% TP at score 6, and is thus on a level with BP.

Mite had 15 BP and the analyses used here have mainly the same SP % and SE % as the house dust+mite group.

Fig. 2c animal dandruff – illustrates that HR, unlike in the results for the combined allergens, does not have FN at score 0, and furthermore, only a few FP. In addition, CH and ST are almost just as satisfactory as the RAST-analysis at score 2 to 3.

Fig. 2d pollen group – illustrates that the RAST-analysis is far superior to the other analyses, having "changed places" with HR. CH has no FP (no mis-diagnoses) at score 3.

Fig. 2e mould fungi – illustrates that ST is the only test which does not mis-diagnose "ill" (FN) at score 0. On the other hand, HR, RAST, and CH have, in this case, 25% FN which thus cannot be diagnosed as "ill". BP is positive at HR and RAST at score 2, and ST at score 3.

Generally, ST implies a better diagnostic reliability than RAST, whereas CH is of no value as the curve is almost equal to the 0-discrimination curve.

Fig. 2f feather and cotton – illustrates that none of the analyses are useful for making a diagnosis.

Fig. 3 represents clinically relevant combinations. In general, the curves are here separated showing the best diagnostic reliability for the combination CH+ST+RAST+HR, followed by the combinations CH+ST+RAST, CH+RAST, and CH+ST, and the worst, the "reference" CH.

None of the combinations reach the level of BP, but that the diagnostic reliability was improved by combining the analyses is exemplified by the fact that none of the combinations have FP at higher score limits.

Thus CH+RAST were diagnostically reliable at score 5, corresponding to 83% score. The other combinations were diagnostically reliable at score 6, which for CH+ST corresponded to 100% score, for CH+ST+RAST 67% score, and for CH+ST+RAST+HR 50% score.

Correspondingly, all the combinations gave a definite "negative diagnosis" at score 0, except the combination CH+RAST which had 8% FN, i.e. "ill" not diagnosed. On the whole, the combination ST+RAST coincided with the combination CH+ST+RAST. For the sake of clarity, the ST+RAST curve was not drawn, but marked with points only, as this combination without CH has no clinical relevance.

The single analyses (Table 1) have been evaluated in order to find whether an increasing analysis score 1 to 3 results in correspondingly higher BP score. The number of BP expressed in percentage which with an increasing analysis score "move" from BP score 1 to BP score 2 and 3 are shown in Fig. 4. This Figure shows that an increase in BP scores from analysis score 2 to 3 takes place in HR, RAST, and ST.

Also, in the combined analyses (Tables 2–4), there is an increase in BP scores at the higher analysis score limits.

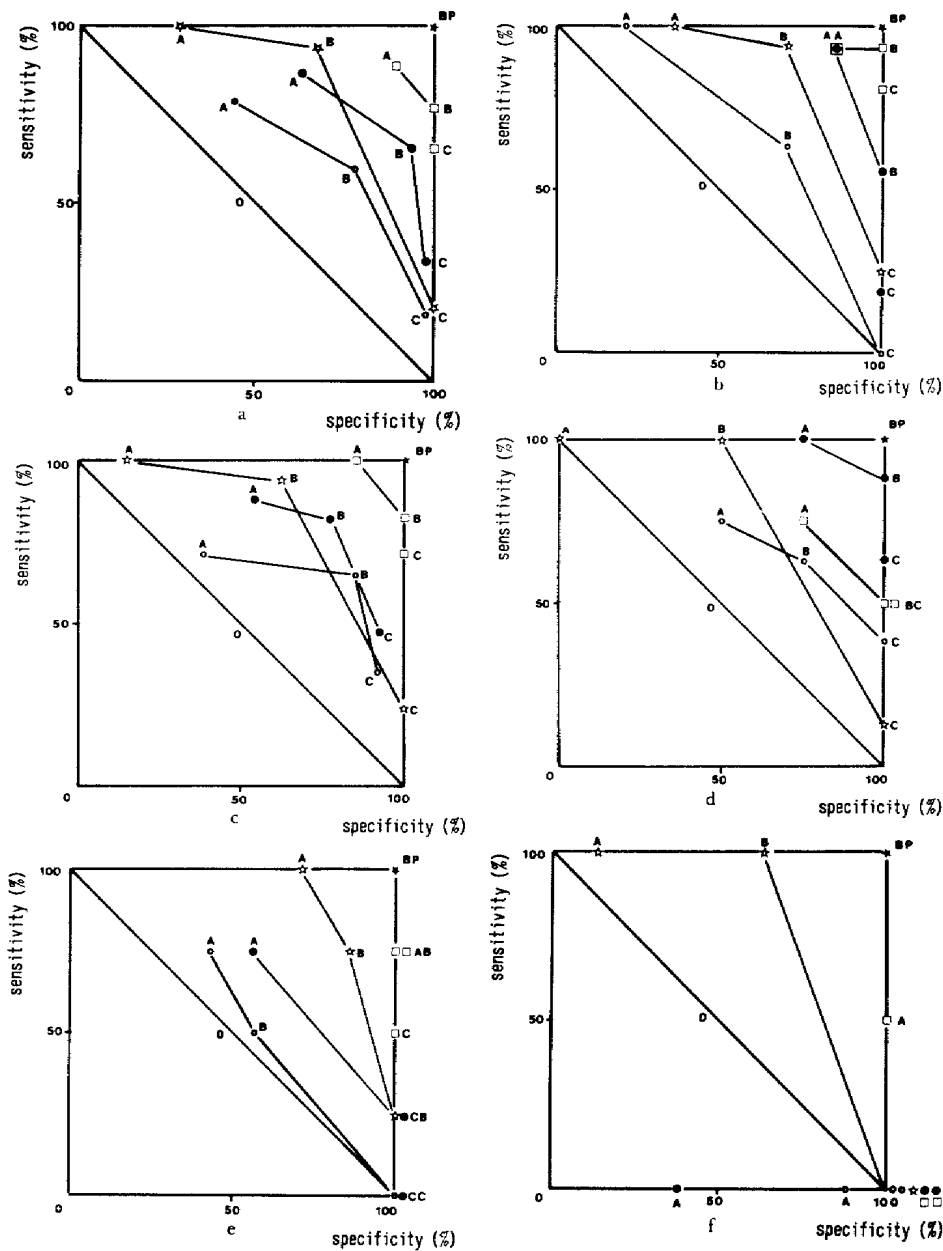


Fig. 2a-f. SE% (ordinate) and SP% (abscissa) for the individual analyses: CH, O; ST, $\star$; RAST, $\bullet$; HR, $=$; and BP, $\star$ for:
a: Combined allergens (93 BP). d: Pollen (12 BP).
b: House dust (15 BP)+house dust mite (15 BP). e: Mould fungi (11 BP).
c: Animal dandruff (30 BP). f: Feather + cotton (10 BP).

Score limits employed: 0/1 + 2 + 3 (A), 0 + 1/2 + 3 (B) and 0 + 1 + 2/3 (C).

For details, see text.

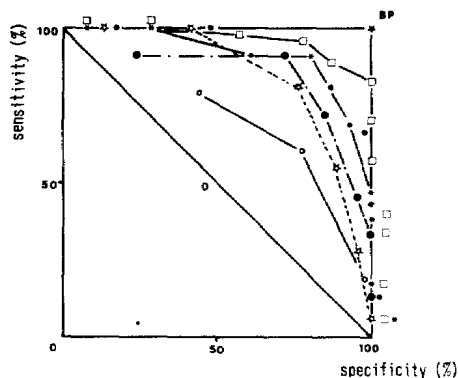


Fig. 3. SE % (ordinate) and SP % (abscissa) for the following analysis combinations: CH + ST, Δ ; CH + RAST, $\bullet$; ST + RAST, $\star$; CH + ST + RAST, $\square$; and 'reference' CH, $\circ$. Values of the combination ST + RAST are only stated with points.

For details, see text.

DISCUSSION

Through sensitivity and specificity (SE and SP) we have tried to evaluate the diagnostic value of some of the analyses available to the allergologist in the specific diagnosis of exogenous allergy. The reason being that SE and SP are both independent of the number of well and ill persons participating in the investigation (Fig. 1). If, instead, the value of the analyses was evaluated by estimating the predictive value of positive test:

$$PVP = \frac{TP}{TP + FP}$$

and the predictive value of negative test:

$$PVN = \frac{TN}{TN + FN}$$

an excess of "ill" (positive BP) in relation to "well" would result in too high PVP and too low PVN (Fig. 1), and vice versa as regards an excess of "well".

This would not have influenced the total assessment in our investigation as there were 47 positive BP (ill) and 46 negative BP (well). In the single allergen groups and different score limits the dispersion of positive and

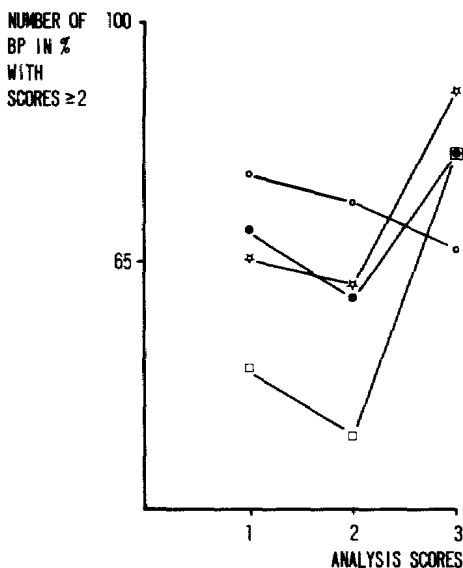


Fig. 4. Number of BP with scores 2 and 3 in % of all positive BP (ordinate) in relation to an increasing analysis score (abscissa) for the analyses: CH, $\circ$; ST, $\square$; RAST, $\bullet$; and HR, $\star$.

For details, see text.

negative BP varied, and this would affect the results if predictive values were used. Eriksson (11) has also taken the influence of the dispersion into consideration, whereas other investigations contain this error.

In general, HR appeared to be the best individual analysis with very few false positive and negative values, followed by RAST, ST, and CH. RAST was superior to HR in the pollen group and ST superior to RAST in the mould fungi group. CH was only satisfactory in animal and pollen allergy.

None of the analyses were satisfactory diagnosing feather and cotton allergy. BP was positive at HR and RAST scores 2 and 3 though not in RAST with allergy to animal dandruff. ST required a maximum score 3 in order to be entirely specific. In this case only CH could ensure a diagnosis in the pollen group.

ST was the most sensitive of the individual analyses. All patients with ST score 0 showed

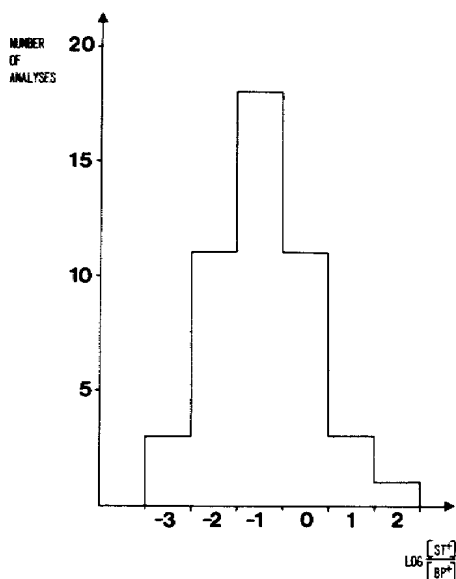


Fig. 5. The relation (log) of the allergen concentration resulting in positive ST and BP: $\log [ST+] / \log [BP+]$ (abscissa) in relation to the number of analyses (ordinate).

For details, see text.

negative BP to the allergen in question. We achieved a similar sensitivity with HR score 0 for animal dandruff, and RAST score 0 for pollen. In the mould fungi group, where the sensitivity was unsatisfactory with CH, RAST, and HR, an intracutaneous test for screening must be considered.

BP was always positive in analysis combinations with score 6 or more. BP was always negative in analysis combinations with score 0 with the exception of combined CH + RAST (8% FN).

In spite of the relatively satisfactory single analyses in this work, it is of great advantage to combine CH and ST with serological analyses, as this gives a double control, and in addition, the diagnostic reliability increases with an increasing number of combinations, for example, four analyses with only 1 1/2 score in each achieves a definite diagnosis, whilst ST with its high sensitivity eliminates most of the FN.

Unfortunately, HR is still too expensive and time-consuming to use in the daily routine and RAST is too expensive to use for screening. HR is a considerable aid to diagnosis, and efforts are being made to simplify and reduce the cost of this analysis.

With high and low scores BP can often be omitted in this investigation under optimal conditions. However, in many cases it will still be necessary to use BP if a completely reliable diagnosis is required.

As expected the sensitive intracutaneous test produced many FP. The prick test is considered approximately 100 times less sensitive, and the use of extracts at the highest concentrations (10^{-2} and 10^{-3}), to provoke a positive intracutaneous test, gave no positive reaction. Therefore the prick test with weak extracts will not seek out all the ill persons but there would be fewer FP.

The relation between the allergen concentration to which the patient reacted in ST and BP (47 positive) is shown in Fig. 5 with the number of analyses as ordinate and the $\log [ST+] / \log [BP+]$ as abscissa. On an average ST is 10 times more sensitive than BP, and only in four cases is BP more (10–100 times) sensitive than ST.

ACKNOWLEDGEMENTS

The investigations were supported by grants from the Dagmar Marshall Fund, the Merchant Einar Willumsen Fund, and The Danish Medical Research Foundation.

REFERENCES

1. Aas, K.: The bronchial provocation test. Charles C Thomas, Springfield, 40–47, 1975.
2. Aas, K. & Johansson, S. G. O.: The radioallergosorbent test in the *in vitro* diagnosis of multiple reaginic allergy. *J. Allergy Clin. Immunol.* **48**, 134–142, 1971.
3. Berg, T. L. O. & Johansson, S. G. O.: Allergy diagnosis with the radioallergosorbent test. *J. Allergy Clin. Immunol.* **54**, 209–221, 1974.
4. Berg, T. Bennich, H. & Johansson, S. G. O.: *In vitro* diagnosis of atopic allergy. *Int. Arch. Allergy* **40**, 770–778, 1971.

5. Bronsky, E. A. & Ellis, E. F.: Inhalation bronchial challenge testing in asthmatic children. *Pediat. Clin. N. Am.* 16, 85-94, 1969.
6. Bruce, C. A., Rosenthal, R. R., Lichtenstein, L. M., Norman, P. S.: Diagnostic tests in rag-weed-allergic asthma. *J. Allergy Clin. Immunol.* 53, 230-239, 1974.
7. Cheska, M., Eriksson, R. & Varga, J. M.: Radio-immunosorbent assay of allergens. *J. Allergy Clin. Immunol.* 49, 1-9, 1972.
8. Eriksson, N. E., Ahlstedt, S. & Belin, L.: Diagnosis of reaginic allergy with house dust, animal dander and pollen allergens in adult patients. *Int. Arch. Allergy Appl. Immunol.* 52, 335-346, 1976.
9. Eriksson, N. E.: Diagnosis of reaginic allergy with house dust, animal dander and pollen allergens in adult patients. *Int. Arch. Allergy Appl. Immunol.* 53, 341-348, 1977.
10. Eriksson, N. E.: Diagnosis of reaginic allergy with house dust, animal dander and pollen allergens in adult patients. *Int. Arch. Allergy Appl. Immunol.* 53, 441-449, 1977.
11. Eriksson, N. E.: Diagnosis of reaginic allergy with house dust, animal dander and pollen allergens in adult patients. *Int. Arch. Allergy Appl. Immunol.* 53, 450-458, 1977.
12. Malling, H.-J.: Histamine-release from basophil granulocytes. A comparative investigation between bronchial provocation and histamine-release in asthma patients. *Ugeskr. Læg.* 139, 2312-2316, 1977.
13. Ryssing, E.: The results of provocative tests with inhalant allergens in asthmatic children. *Acta Allergol.* 14, 433-444, 1959.
14. Weeke, E., Löwenstein, H., Prahl, P. & Weeke, B.: The value of a radioallergosorbent test (RAST) in the diagnosis of bronchial asthma in children. A comparative clinical trial of skin testing, RAST, and bronchial provocation test in 27 children with bronchial asthma. *Ugeskr. Læg.* 138, 2712-2717, 1976.

Address:
 Ole Østerballe
 Children's Allergy Clinic
 University Hospital (Rigshospitalet)
 Blegdamsvej 9
 DK-2100 Copenhagen Ø
 Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.