

RAST in the diagnosis of hypersensitivity to horse allergens.

A comparison with clinical history and *in vivo* tests

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Summary

Case history, skin tests and RAST were compared in a group ($n = 40$) of children with a history suggesting allergy to horses and in a group ($n = 43$) in whom there was no suspicion of hypersensitivity to horses.

There was an agreement of 91% between case history and prick test. The same magnitude of agreement was found between case history and RAST (89%), and the agreement between RAST and prick test was 90%.

The results of this investigation are clearly in contrast to earlier reports, in that there was a very good correlation between prick test, RAST and case history.

The results suggest that: (a) a proper evaluation of the skin test is of critical importance for the degree of correlation with other diagnostic tests; and (b) RAST is as reliable as carefully performed and evaluated skin tests.

Introduction

In the diagnostic routine, positive skin tests with animal epithelium extracts are frequently reported in patients with no clinical symptoms of animal allergy (Grow & Herman, 1936; Jarisch, 1976). Schultze-Werninghaus *et al.* (1976) demonstrated positive skin prick tests in as much as 51% of asthmatic patients exposed to animals but without symptoms of allergy to animals. Only 11% of the patients with a positive skin test to horse allergen extracts had symptoms suggesting hypersensitivity to horse. Eriksson (1977) found a poor agreement of about 50% between provocation tests and skin test results with horse allergen extracts, and also a low correlation between positive skin tests and the presence of circulating IgE antibodies to horse allergens as determined by RAST (Eriksson, Ahlstedt & Belin, 1976). Only 33% agreement between positive skin tests and RAST results to horse allergens was reported by Wüthrich & Kopper (1975).

Horse allergen extracts have been shown to demonstrate a considerable variability, both in allergenic composition and potency (Aronsson & Wide, 1974; Ponterius *et al.*, 1973; Löwenstein, Markussen & Weeke, 1976). The extracts also contain several components without allergenic activity. It has been suggested that non-specific

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skin reactions can be elicited by non-allergenic components in the extracts (Berrens, Hénoco & Koers, 1974; Brehm *et al.*, 1975).

In view of the disagreements between various diagnostic variables it was considered important to investigate the reliability of carefully performed skin tests and RAST in the diagnosis of allergy to horse epithelium.

Materials and methods

Patients

Two groups were selected from hospitalized patients and out-patients of the Allergy unit of the Paediatric clinic and the Allergy Institute, Voksentoppen, Oslo: (a) forty patients presenting a clear-cut clinical history of allergy to horse allergens, or with a history giving rise to a strong suspicion of horse allergy; and (b) forty-three patients in whom no suspicion of allergy to horses existed, and four patients with a questionable history of allergy to horse allergens.

The age and sex distribution of the groups is shown in Fig. 1.

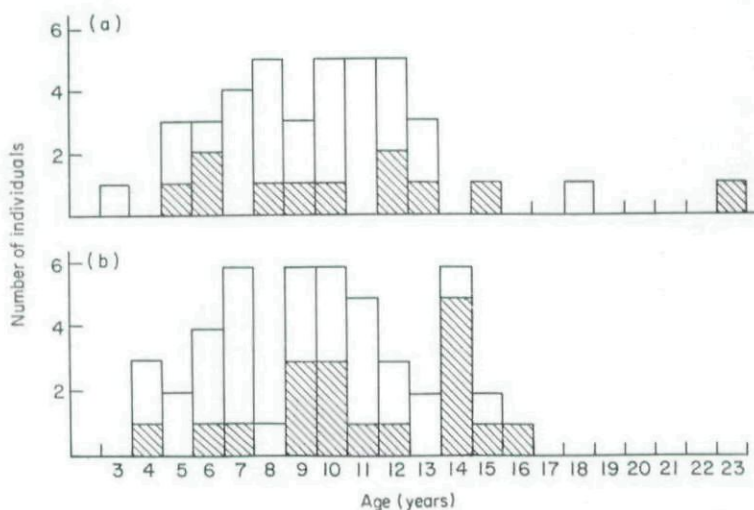


Fig. 1. Age and sex distribution of investigated patients. (a) group (a) ($n = 40$); (b) group (b) ($n = 47$). (□) boys; (▨) girls.

Case histories

A careful case history was taken from each participant. The case histories were scored from 0 to 2; the score 0 indicated that hypersensitivity to horse allergens was improbable, 1 that no adequate information could be obtained and 2 indicated that horse allergy was probable.

In vivo tests

Allergen extracts. An extract of horse dandruff (stock concentration 1:100 w/v) was supplied by Dr Lars Yman, Pharmacia Diagnostics AB, Uppsala, Sweden. The extract had been subjected to gel chromatography on Sephadex G-25 to remove small molecular weight components, and to sterility and acute toxicity tests. A commercial

horse allergen extract (stock concentration 1:10 w/v) from Nyegaard & Co. (Nyco, Oslo, Norway) was also applied in the skin prick tests.

Skin prick tests. The prick tests were carried out with increasing concentrations of the Pharmacia extract up to 1:100 w/v. The Nyco extract was applied only in the 1:10 concentration w/v. The tests were evaluated according to the recommendations from the Northern Society of Allergology (Aas & Belin, 1972), i.e. comparison with the reactions to a negative control solution (–) and a histamine control, histamine hydrochloride 1 mg/ml (+++).

Prick test reactions of +++ or more were considered significantly positive. The significance of lesser skin test reactions was also investigated.

Intracutaneous tests. In cases of positive prick tests no further skin test was generally performed. But if reactions of less than +++ were obtained, intracutaneous titration tests were usually carried out, starting with extract concentrations of 1:10⁷ or 1:10⁶ w/v of the Pharmacia extract. The strongest extract concentration used was 1:10³ w/v. The test results were evaluated as described for the prick test, with a positive (+++) histamine-HCl control, 0.1 mg/ml and a negative control (–).

Provocation tests. In eight patients bronchial provocation tests (BPT) were carried out with the Pharmacia horse dandruff extract as described by Aas (1975a). The procedure started with the application of a nebulized extract of concentration 1:10⁷. If no reaction occurred at this concentration, the test was repeated with increasing extract concentration with 10 min intervals up to 1:100 w/v. The results were evaluated by measuring the forced expiratory volume (FEV₁) and the peak expiratory flow (PEF) 10 min after application of each extract concentration. A decrease of at least 25% was regarded significantly positive (Aas, 1975a).

RAST

Prior to the *in vivo* testing procedures a blood sample was drawn from each patient. Serum was prepared and analysed with RAST for IgE antibodies against horse allergens. Three allergen preparations were used: Phadebas RAST® horse dander and horse dandruff (identical with the extract for *in vivo* tests), and horse serum albumin (supplied by Dr Lars Yman).

Duplicate RAST assays were performed and evaluated according to the instructions for use of the Phadebas RAST (Pharmacia Diagnostics AB, Uppsala, Sweden), and the test reagents employed were the same as those commercially available. RAST class 0 indicates no detectable IgE antibodies in the serum. Classes 1–4 represent increasing concentrations of IgE antibodies. RAST class 1 representing a borderline class and classes 2–4 were regarded as positive results.

Results

Comparison of RAST results

Phadebas horse allergen and horse dandruff. Expressed in Phadebas RAST classes there was agreement in the results with the two horse allergen preparations in seventy-nine out of the eighty-seven serum samples analysed (Fig. 2). Furthermore, in all the eight cases not in full agreement, one or both results represented borderline values close to the class borders in question (Fig. 3). Consequently there was, in fact, a very good agreement between the two sets of RAST results.

Horse dandruff and horse serum albumin. IgE antibodies against horse serum

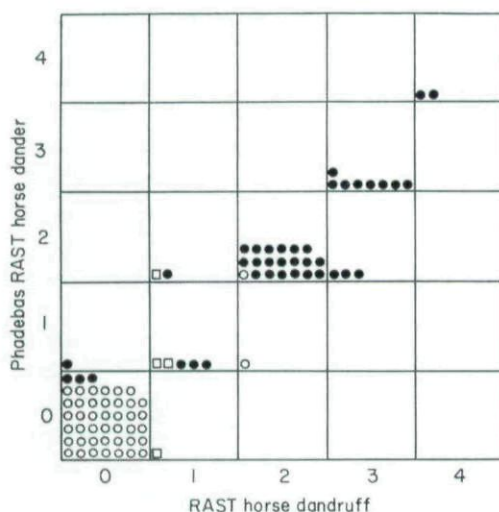


Fig. 2. Comparison of RAST results obtained with horse dander of Phadebas RAST and horse dandruff. Results expressed in Phadebas RAST classes. (○) Case history score 0; (□) case history score 1; (●) case history score 2.

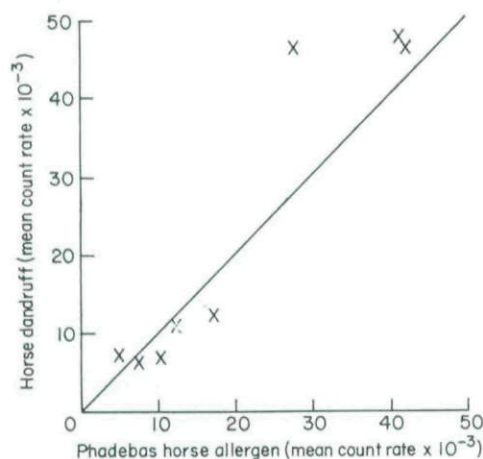


Fig. 3. Comparison of RAST results (expressed in mean count rates) for eight cases with different RAST class results for Phadebas horse allergen and horse dandruff.

albumin were detected by RAST in only three serum samples. One of them was in borderline class 1. All three also had rather high titres of IgE antibodies against horse dandruff (classes 2, 3 and 4).

Comparison of prick test results

Two cases were recorded positive in the prick tests performed with the Nyco extract but were inconclusive with the Pharmacia extract (Fig. 4). In both cases positive intracutaneous tests were obtained with the latter extract preparation.

In seven cases inconclusive prick tests (< +++ with extract concentration of 1:10 w/v) were obtained with the Nyco extract yet the tests were positive (≥ +++

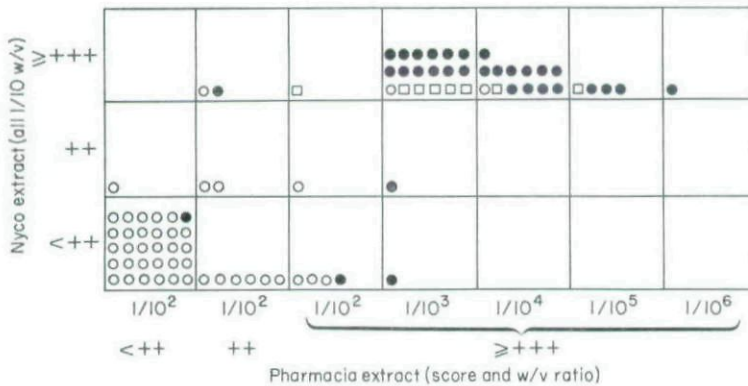


Fig. 4. Comparison of prick test results obtained with Nyco and Pharmacia horse allergen extracts. Symbols as in Fig. 2.

with an extract concentration of 1:100 and 1:1000 w/v) with the Pharmacia dandruff extract. Intracutaneous tests were carried out in three of these cases and were all recorded positive ($\geq +++$). RAST was positive in three and negative in four of the seven cases.

RAST was positive in one case where the prick tests were negative with both extract preparations. However, the intracutaneous test was positive at a concentration of 1:10⁴ w/v.

Agreement between history, prick test and RAST

There was an overall agreement of 91% (79/87) between a prick test of $\geq +++$, using the Pharmacia extract at a maximum concentration of 1:100 w/v and the case history. The same degree of agreement was found between RAST and the case history (88.5%), when identical extracts were applied in the two test systems. Prick test and RAST agreed in 90% (71/79) of the cases in this study (Table 1).

Table 1. Agreement between case history, prick test (horse dandruff) and RAST (horse dandruff)

Test		No.	Case history	Prick test (%)	Prick test (%)
			% Ratio	(horse dandruff, positive $\geq +++$ at max. concentration: 1/100 w/v)	(horse dandruff, positive $\geq +++$ at max. concentration: 1/100 w/v)
RAST	Negative	45	91.1 (41/45)	86.7 (39/45)	66.7 (30/45)
	Borderline (class 1)	8	50.0 (4/8)	—	—
	Positive	34	94.1 (32/34)	94.1 (32/34)	97.1 (33/34)
	Total	87	88.5 (77/87)	89.9 (71/79)	79.8 (63/79)
Prick test (positive $\geq +++$)	Negative	41	97.6 (40/41)	—	—
	Positive	46	84.8 (39/46)	—	—
	Total	87	90.8 (79/87)	—	—
Prick test (positive $\geq +++$)	Negative	31	100 (31/31)	—	—
	Positive	56	71.4 (40/56)	—	—
	Total	87	81.6 (71/87)	—	—

When skin reactions of +++ and larger were regarded as positive in the prick test at a concentration of 1:100 w/v, 13% (6/45) of the positive cases were found to be negative in RAST (Table 1 and Fig. 5). There was a history of horse allergy in four of these cases, and the positive prick test was confirmed by intracutaneous test of +++ in three of the cases. In one patient with a negative RAST result and a negative case history, but with a positive prick test, a BPT was carried out and was found to be negative (Table 2, case no. 8).

In two cases inconclusive prick test results (++) were combined with positive RAST results of class 2 and 3 respectively (Fig. 5). The history suggested horse allergy in one of the cases, and the presence of specific IgE against horse allergens was also indicated by positive intracutaneous tests in both cases.

If prick test reactions of ++ with the Pharmacia extract had also been regarded

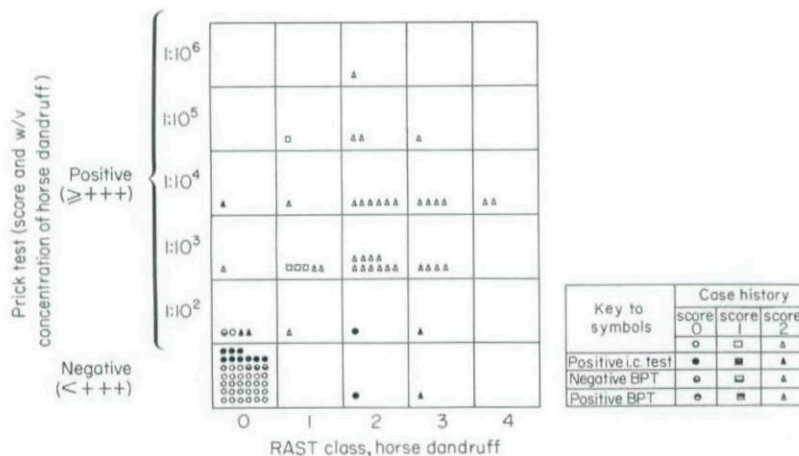


Fig. 5. Comparison of RAST and prick test results.

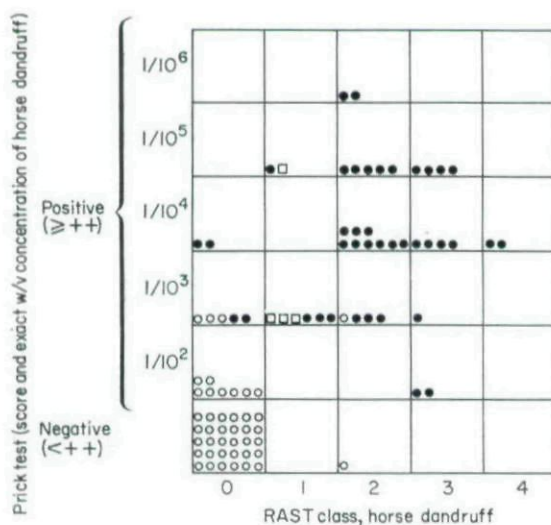


Fig. 6. Comparison of RAST and prick test results. Symbols as in Fig. 2.

Table 2. Cases of agreement case history, skin prick test and RAST

Patient no.	Case history score	Prick test, horse dandruff		RAST (classes)			Intracutaneous test, horse dandruff		BPT, horse dandruff (and w/v)
		Score	w/v	Horse dandruff	Phadebas horse allergen	Horse serum albumin	Score	w/v	
17	0	++	1/100	2	1	2	++	1/10 000	n.d.
8	0	+++	1/100	0	0	0	n.d.	n.d.	Neg. (1/100)
57	0	+++	1/100	0	0	0	n.d.	n.d.	n.d.
4	0	+	1/1000	2	2	0	++	1/10 000	n.d.
12	2	++	1/100	3	2	0	++	1/100 000	n.d.
3	2	++	1/10 000	0	0	0	++	1/100 000	n.d.
6	2	+++	1/100	0	0	0	+++	1/10 000	n.d.
22	2	+++	1/100	0	0	0	++	1/1000	n.d.
44	2	+++	1/1000	0	1	0	++	n.d.	n.d.

n.d. = Not done.

as positive, the agreement with the RAST results would have been lower, but even then with a quite acceptable correlation of 80% (Table 1 and Fig. 6). A number of cases with no history of horse allergy and with class 0 in RAST would then have been interpreted as positive in the prick test. 29% (16/56) of the positive prick tests did not correlate with a positive case history. This suggests that some of the skin reactions elicited at the ++ level by the 1:100 w/v concentration of the Pharmacia extract were non-specific. The same observation was made in intracutaneous tests, which were positive in a number of cases where none of the other parameters indicated hypersensitivity to horse allergens (Fig. 5).

The cases with obvious disagreement between history, skin tests and/or RAST are accounted for in detail in Table 2. RAST seems to be falsely negative in three patients (patients 3, 6 and 22). The prick test apparently gave misleading positive results in two cases (patients 8 and 57). Both RAST and skin tests were positive in one case with a negative case history (patient 17).

Discussion

In clinical allergy, hypersensitivity to animal danders is often evident from the case history, and the demonstration of immunologically specific reactions in the skin and/or serum will often confirm the diagnosis.

The results of our investigation are in clear contrast to those of Schultze-Werninghaus *et al.* (1976), Eriksson *et al.* (1976) and others in that we found a very good correlation between history, prick testing and RAST results with the diagnostic criteria to be described.

The value of any diagnostic test in clinical allergy depends on its immunological specificity. This in turn depends on several variables—namely, the quality of the allergen extract (Aas & Belin, 1972; Aas, 1975b), the concentration used in the test and the techniques and the criteria for accepting a reaction as distinctly positive. Biological variables are decisive for the skin test results and also the concentration of IgE antibodies in the serum for RAST (Foucard, 1973).

Allergen extracts from horse dander may contain irritants, and this material should therefore be used with much care in the *in vivo* diagnostic work, and subsequently combined with a critical appraisal of the results in order to obtain an immunologically specific diagnosis.

The poor correlation between positive results in skin testing, history and RAST results reported from several investigations (Eriksson, 1977; Eriksson *et al.*, 1976; Grow & Herman, 1936; Jarisch, 1976; Schultze-Werninghaus *et al.*, 1976; Wüthrich & Kopper, 1975) may be due to one or more of several factors, for example the use of too high extract concentrations in the skin testing.

Intracutaneous skin testing is 10–100 times more sensitive than prick testing, and unless the correct concentration of allergen extract is used, skin testing will give a poorer correlation with history and RAST than prick testing (Stenius *et al.*, 1971). Despite careful test evaluation, in the present study positive intracutaneous tests were also demonstrated in several cases where history, prick tests and RAST were negative.

The concentration of allergen extract used in prick testing is also critical, too strong concentrations giving many false positive results. The same situation will arise if the evaluation criteria of the skin test reactions are not set rigidly enough.

In this investigation, considerable time and effort were spent on formulating the

case history, and a definite history was obtained in almost all cases (Table 1). Even so, in one of the four patients (patient 17), in whom no adequate information could be obtained at the start of the investigation, further interviews confirmed the test results. The girl did, in fact, react on contact with horse dander.

Nevertheless, the diagnosis of clinical allergy to horse dandruff was confirmed in 97.5% of the patients when a properly performed and evaluated prick test was correlated with the history. The same degree of immunological significance was reached in patients with a negative history, and in whom a negative prick test was obtained (93%).

A higher degree of immunological specificity was obtained when prick tests of $\geq +++$ at an allergen extract concentration of 1:100 w/v were regarded as positive, rather than if $\geq ++$ reactions at the same allergen extract concentration were considered positive (Fig. 5 and 6).

RAST results gave about the same high level of agreement as the prick test when correlated with the case history. Both carefully evaluated prick test and RAST results were thus equally effective in establishing the presence or absence of clinical allergy to horse dander in the majority of the patients tested.

As several investigators have shown (Aronsson & Wide, 1974; Lowenstein *et al.*, 1976; Ponterius *et al.*, 1973), horse dandruff contains several allergenic fractions, amongst them horse serum albumin. In our study, only three patients showed IgE antibodies to the latter allergen. They also showed high titres of IgE antibodies to horse dandruff.

In nine cases there was disagreement between case history and the results of skin tests and RAST (Table 2). In such situations a challenge test would be of great value to reach a definite and immunologically specific diagnosis. This was needed only in one patient who had an apparently positive prick test without clinical significance.

In conclusion, a carefully taken case history combined with RAST or a carefully performed and evaluated prick test, with due consideration to correct extract concentrations, will give a very good correlation and a high degree of immunological specificity. In this respect, the future use of allergen extracts containing a minimum of non-specific irritants will be extremely important for *in vivo* tests (Aas, 1975b).

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