

***In vitro* diagnosis of atopic allergy**

**The occurrence and clustering of positive RAST results
as a function of age and total IgE concentration**

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Summary

Specific IgE antibodies were determined on a routine basis in patient sera using the radioallergosorbent test (RAST) with a panel of ten common allergens. Total IgE was also assayed. Of all positive RAST reactions observed, 74% were against the following allergens: horse and cat epithelium, birch and timothy pollen, and house dust. Clustering (occurrence together with significantly elevated frequencies) accompanied both positive reactions to some animal danders and food allergens. Children in all age groups had significantly more positive food RAST results than adults, and this explains the clustering among food allergen results. Children aged 0-4 years had few positive pollen results. Among those with at least one positive RAST result, the mean number of positive results was 3.3, a figure which did not vary with age. In the present patient population 37% had a total IgE above the upper reference (normal) limit, and of these 95% had at least one positive RAST result. A normal or low IgE value was found to be less informative.

Introduction

The development of suitable techniques for *in vitro* diagnosis of atopic allergy became possible after the discovery of immunoglobulin E (Ishizaka, Ishizaka & Hornbrook, 1966; Johansson & Bennich, 1967). Several methods for the estimation of allergen-specific IgE antibody have been reported (Coombs, Howard & Mynors, 1953; Ishizaka, Ishizaka & Hornbrook, 1967; Wide, Bennich & Johansson, 1967; Zeiss *et al.*, 1973). When results obtained with the radioallergosorbent test (RAST) and provocation tests have been compared, the results have been found identical, i.e. both positive or both negative in 75-100% of the cases. Correlations with skin tests have been less good (10-40%) (Wide, 1973; Berg & Johansson, 1974).

A high percentage of patients with allergic asthma (Johansson, 1967) and allergic rhinitis and atopic eczema (Berg & Johansson, 1969) have increased serum total IgE levels. IgE assay thus has potential value for allergy diagnosis (Johansson, Bennich & Berg, 1972). RAST analysis and determination of total IgE have been in routine use

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at the Blood Centre in Uppsala for more than 6 years. The purpose of the present paper is to evaluate some of the experience from this laboratory service. We will also describe the clustering, occurrence together with significantly elevated frequencies, of some positive RAST reactions.

Materials and methods

Selection of patients

Serum samples sent to the Clinical Immunology Division, The Blood Centre, University Hospital, Uppsala for allergy diagnosis during a 5-month period were studied. RAST analysis was requested for a total of 1083 patients. Due to a shortage of personnel, requests could not be fulfilled in eighty-nine cases (9%). Samples from the University Hospital and its regional affiliates had priority for the RAST analysis. Rejection of a request for RAST was based mainly on the geographical origin of the sample, but also to a certain extent on the total IgE concentration found. The mean and standard deviation of the IgE values for the rejected samples were found to be similar to those values obtained for universally negative RAST. Another group of patients, not included in this study, consisted of 658 persons for whom only one or a few allergens were analysed by RAST. Thus, the data analysed in this study are for the remaining 336 patients, on whose sera RAST analyses were performed using a standard panel of ten allergens. The total serum IgE concentration was also determined. The age distribution of the patients is given in Table 1.

RAST and total IgE assay

The following allergen extracts were included in the RAST panel: epithelium from horse, dog, cat and cow; birch and timothy pollen; mixed fish, cows' milk and egg white; and house dust. For details on the nature of the allergen extracts see Foucard (1973). The allergen extracts were coupled to CNBr-activated paper discs. Sera, to be analysed, were diluted 1:3 with 0.9% saline, and a 0.1 ml aliquot was incubated with an allergen disc in a disposable polystyrene test tube for 3 hr at room temperature and then washed. A volume of 0.1 ml of anti-IgE labelled with ^{125}I having an activity of approximately 100,000 counts per minute was added to each tube. The discs were then incubated over-night at room temperature and washed again. The radioactivity bound to the discs was measured in a gamma scintillation counter.

The count rates for the patient samples were compared with a 'RAST score' scale for evaluation. A standard curve was constructed using a serial dilution of a birch positive serum on which RAST analyses were performed using birch allergen discs. The RAST score 0 indicated that no IgE specific for the allergen in question was found

Table 1. Age distribution for patients with negative and positive RAST

Results of RAST	Age groups (years)				
	0-4	5-9	10-14	15-	All
Negative reactions to all ten allergens	22	29	13	46	110
At least one positive reaction	39	72	49	66	226
All patients	61	101	62	112	336

and RAST scores of 1-7 corresponded to the presence of increasing concentrations of specific IgE. For further details see Johansson, Bennich & Berg (1971).

Total serum IgE was determined using the Phadebas® IgE Test (Pharmacia Diagnostics, Uppsala). The results were expressed in units/ml using the W.H.O. preparation 68/341 as a reference. One unit equals 2-3 nanograms.

Statistical methods

The IgE values do not conform to a Gaussian distribution but can be brought nearer this state by means of a logarithmic transformation (Johansson, 1967). Therefore the logarithms of the IgE values in units/ml were calculated. The IgE levels in a normal population vary with age, and for this reason a further conversion of the log IgE values into s.d. units was made. The s.d. units give the distance from the reference log IgE mean with the reference log IgE standard deviation as the measure, enabling simultaneous and identical treatment of data for all age groups (Gullick & Schauble, 1972).

Necessary IgE reference data were obtained by retesting sera obtained from healthy, non-atopic children and adults from a previous study (Johansson, 1968). For children below 5 years, however, new samples were obtained at 'Baby Welfare Centres' in Uppsala.

A χ^2 test was used to determine whether positive RAST results to any allergen (Table 1) or to certain allergens (Table 2) occur at significantly different frequencies in different age groups (Natrella, 1963, pp. 6-9). A Student's *t*-test was used to test the differences in the mean number of positive RAST results in different age groups (Table 2) (Natrella, 1963, pp. 3-40). A one-sided *t*-test was used when total IgE means in groups characterized by a different number of reagins detected by RAST were compared (Fig. 4) (Natrella, 1963, pp. 3-36).

The frequency f_{ij} with which patients positive to a RAST allergen *i* were also

Table 2. Average number of positive RAST tests for the different allergens in various age groups. Only patients with at least one positive test are included

Allergen	Age groups (years)				All ages
	0-4	5-9	10-14	15-	
Horse	0.64	0.57	0.55	0.56	0.58
Dog	0.05	0.17	0.27	0.18	0.17
Cat	0.56	0.53	0.63	0.50	0.55
Cow	0.15	0.17	0.16	0.18	0.17
Birch	0.36	0.58	0.59	0.50	0.52
Timothy	0.18	0.50	0.49	0.49	0.44
House dust	0.26	0.35	0.41	0.38	0.35
Fish	0.26	0.14	0.06	0.12	0.14
Milk	0.31	0.14	0.14	0.11	0.16
Egg-white	0.54	0.19	0.25	0.08	0.23
All allergens	3.31	3.33	3.55	3.09	3.29

positive to another RAST allergen j was calculated, in order to examine the co-variation of positive RAST reactions. The following equation was used:

$$f_{ij} = \frac{a_{ij}}{t_i}$$

where the ten allergens examined are designated 1, 2, ..., i , j , ..., 10, a_{ij} is the number of combinations found of a positive test for allergen i together with a positive test for allergen j ; and t_i is the total number of positive RAST reactions to allergen i .

Frequencies ($f_{any, j}$) for the combination of the second allergen j with any first allergen were calculated with the equation

$$f_{any, j} = \frac{\sum_{i=1}^i a_{ij} + \sum_{i=k}^{10} a_{ij}}{\sum_{i=1}^i t_i + \sum_{i=k}^{10} t_i}$$

No significant difference between the frequency f_{ij} and $f_{any, j}$ would be expected if positive RAST results to two allergens (i and j) occurred together in the same patient only as often as predicted by chance. The significance of the difference was tested with a χ^2 test (Natrella, 1963, pp. 6-9). This treatment is not rigorous, but significant differences observed can very probably be regarded as real.

As a further test for possible clustering and in order to get a visual representation of the interrelations between RAST-reactions to the ten allergens, the method of multidimensional scaling as evolved by Kruskal (1964a, b) was applied. Essentially, this is a procedure to obtain a picture, usually in two dimensions, of a number of multidimensional vectors (here allergens) in such a way that the 'distances' between vectors (allergens) are distorted as little as possible. As a measure of distance (or rather in this case similarity) the coefficient of correlation was computed for RAST results to the ten allergens from the 225 persons in the material. The actual computations were performed at Uppsala University Data Center on an IBM 370/155 by the program MDSCAL 5 MS (Kruskal & Carmone, 1969).

Results

One third of the patients had negative scores for all RAST tests (Table 1). There was a significant difference ($P < 0.05$) between the age groups, with the lowest proportion, 21%, of all-negative results in the age group 10-14 years, and the highest proportion, 41%, in the group aged 15 and above. Among those who had at least one positive RAST result, the frequency (i.e. the mean number per person) of positive results was 3.3 (Table 2). In this respect no significant difference between age groups was found.

The frequency of positive RAST reactions obtained with individual allergens varied with the age of the patient and the nature of the allergen (Table 2). Children aged 0-4 years tended to have less positive results to pollen than other age groups. In the case of timothy, the difference between the age groups was significant ($P < 0.01$). Children in all age groups had a higher frequency of positive RAST results to cow's milk and egg white than adults, and the differences between the age groups were significant ($P < 0.05$ and $P < 0.01$, respectively).

Of all positive RAST reactions observed, 74% were against the following five allergens: horse and cat epithelium, birch and timothy pollen and house dust (Fig. 1).

Table 3. Frequency (f_{1j} or $f_{any,j}$) at which a second positive RAST reaction was seen in patient groups positive to various (first) allergens. Data are for patients aged 5 years and above with at least one positive RAST result. For further details see Materials and methods

Second allergen (j)	First allergen (i)										Frequency ($f_{any,j}$)
	Horse	Dog	Cat	Cow	Birch	Timothy	House dust	Fish	Milk	Egg white	Any of ten
	Frequency (f_{1j})										
Horse		0.906*	0.693	0.912*	0.580†	0.648	0.814	0.619	0.739	0.897	0.719
Dog	0.276		0.277	0.529*	0.200*	0.253	0.414	0.333	0.391	0.414	0.305
Cat	0.667	0.875		0.735	0.630	0.615	0.814	0.762	0.783	0.793	0.705
Cow	0.295	0.563†	0.248		0.240	0.297	0.400	0.333	0.435	0.448	0.320
Birch	0.552	0.625	0.624	0.706		0.747	0.671	0.810	0.522	0.655	0.648
Timothy	0.562	0.719	0.554	0.794	0.680		0.629	0.667	0.609	0.690	0.631
House dust	0.543	0.906†	0.564	0.824†	0.470	0.484		0.571	0.609	0.724	0.576
Fish	0.124	0.219	0.158	0.206	0.170	0.154	0.171		0.348	0.345*	0.178
Milk	0.162	0.281	0.178	0.294	0.120	0.154	0.200	0.381		0.345	0.192
Egg white	0.248	0.375	0.228	0.382	0.190	0.220	0.300	0.476	0.435		0.267
Any of ten	0.857	0.969	0.911	1.000	0.830*	0.890	0.971	0.905	1.000	0.931	0.904

* Significantly different from $f_{any,j}$ ($P < 0.05$).

† Significantly different from $f_{any,j}$ ($P < 0.01$).

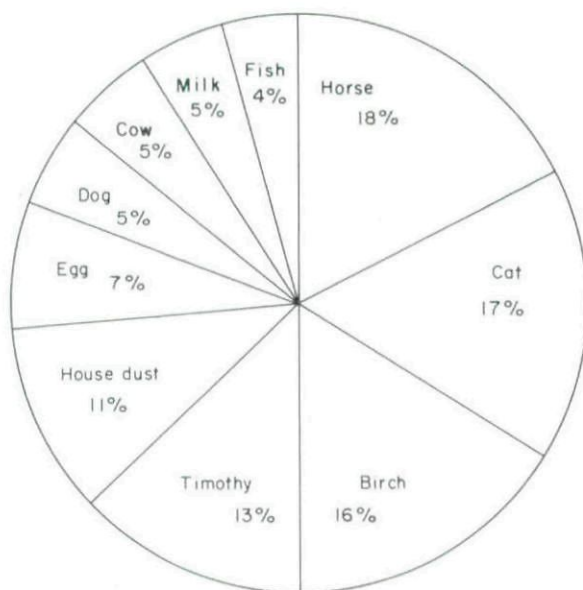


Fig. 1. Specific IgE against ten different allergens was sought by the RAST assay. Altogether 743 positive results were obtained. The distribution of the positive results among the different allergens is shown.

The remaining 26% positive reactions were rather evenly distributed between the food allergens and dog and cow epithelium.

Table 3 describes the frequencies at which various combinations of positive RAST reactions were observed. In order to eliminate most of the age-determined covariation the age group 0–4 years was excluded (*cf.* Table 2). As can be seen, positive horse, dog, cow and house dust reactions occurred together significantly more often than

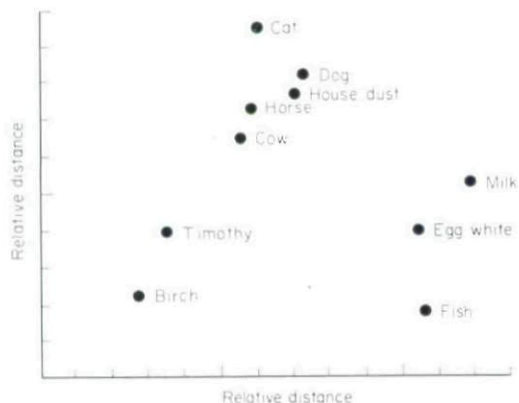


Fig. 2. A two-dimensional picture of the clustering of RAST reactions to the ten allergens used as obtained by multidimensional scaling (for details, see Statistical methods). Two allergens are close together in the picture when the RAST results to these two allergens were identical in many individuals. The points are far apart for the allergens that gave the same reactions in RAST in very few samples.

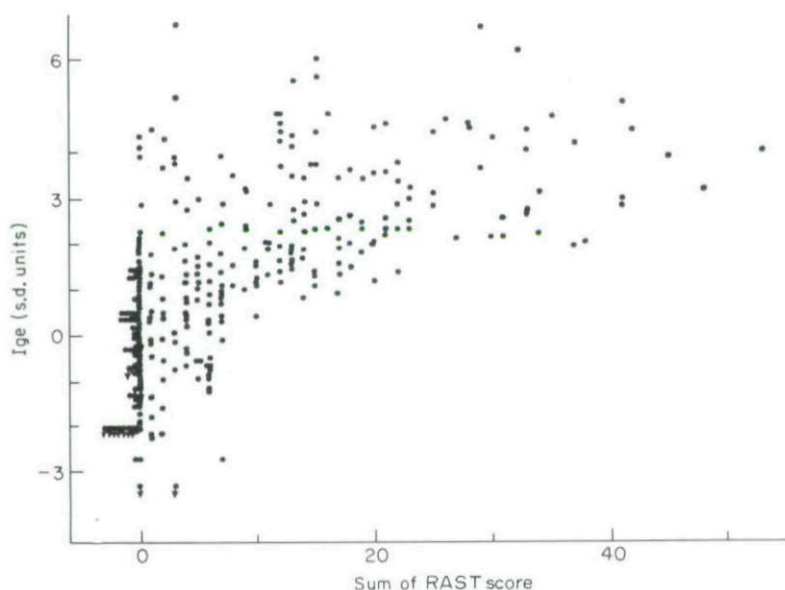


Fig. 3. The serum IgE concentration as a function of the sum of patients' RAST scores. In each serum specific IgE against ten allergens was sought, and each positive finding gave a RAST score between 1 and 7, depending on the concentration.

expected. Positive results for food allergens also tended to occur together. Positive birch reactions were significantly less frequently accompanied by other positive reactions than were positive reactions in general. The clustering of positive results is illustrated also in Fig. 2, and in this case data for all age groups are included. The allergens appear to occur in three clusters: animal danders, pollens and food antigens. House dust was found in close association to the danders.

Each positive RAST reaction was given a score of 1–7, and since every patient was tested against ten allergens, the maximal score sum was 70. The highest observed score sum was 53. Figure 3 shows the relationship between the total IgE concentration (log IgE) and the RAST score sum. Not surprisingly, the IgE level is seen to rise with the score sum.

A similar relationship between the total IgE concentration and RAST results can be seen from Fig. 4, which shows how IgE (log IgE) values increase with the number of positive RAST tests. It can also be seen that the patients with no positive RAST result had a log IgE mean 0.122 s.d. units lower and a log IgE standard deviation 0.419 s.d. units higher than our non-atopic reference population. The groups with 1, 2, 3, 4, 5, 6 and 7–10 positive RAST results all had significantly ($P < 0.005$ in each case) higher mean log IgE values than the group with no positive result.

Figure 5 shows the frequency distributions for log IgE values of patients with and without positive RAST results. Of the 123 patients with values above the reference range (above the mean +2 s.d. units), 95% had at least one positive RAST result. There were ten patients with a value below the reference range (below the mean -2 s.d. units), and among these four persons (40%) had at least one positive RAST result. When the forty-one patients with log IgE values below the mean -1 s.d. unit were examined, 27% was found with positive RAST results.

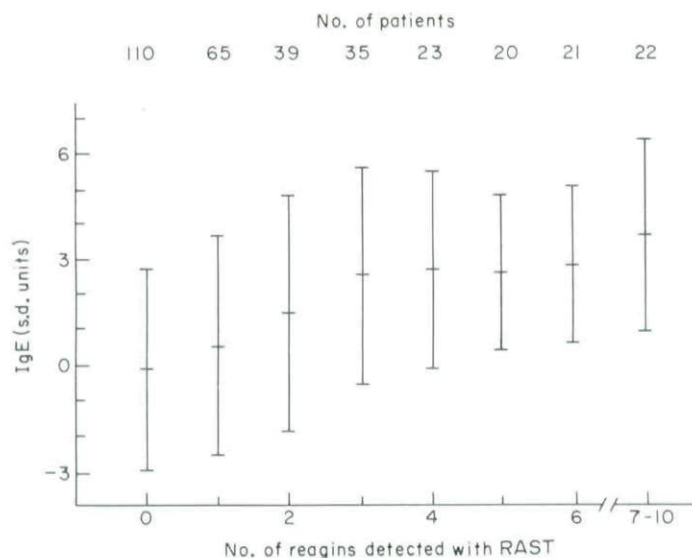


Fig. 4. The serum IgE concentration as a function of the number of reagins (positive RAST results) against different allergens detected in patients' sera. The mean ± 2 times the observed standard deviation is given. Reagins against ten allergens were sought in each serum.

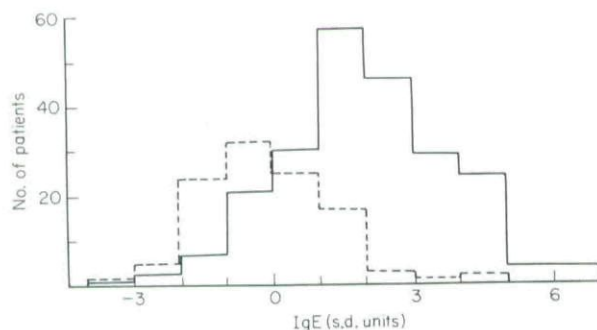


Fig. 5. Frequency distribution of IgE concentrations for patients with and without specific IgE detected by the RAST assay. —, Specific IgE found; ----, no specific IgE found.

Discussion

The composition of the present RAST panel was arrived at following discussions between the laboratory and clinicians. Birch pollen allergen is a suitable representative for wind pollinated trees in Sweden. It is easy to obtain potent extracts, and patients sensitive to other trees appear also to have IgE antibodies against birch with a high frequency (Zetterström, Fagerberg & Wide, 1972). Allergen extracts of various grasses cross-react in a similar manner (Milner & Tees, 1972; Berglund & Lundkvist, 1973), and timothy was chosen as a representative for the grasses present in Sweden. Food allergens were included in the panel due to their importance for children. Although house dust is a poorly defined allergen it was included in the RAST panel since it was considered to be of clinical importance. House dust mite (*D. pteronyssinus*) was a major allergen constituent in the house dust preparation, but it would probably be advantageous to test this allergen separately since it is available for hyposensitization

treatment. The four animal epithelia were also considered useful since reactions to them are frequent and they do not cross-react.

The house dust allergen extract used was known to contain significant concentrations of dog epithelium allergen (Johansson, unpublished observation), which explains the covariation between positive dog dander and house dust results (Table 3 and Fig. 2). The tendency towards co-variation among horse, dog and cow results cannot be explained by such contaminations. Cross-absorption experiments (e.g. addition of cow dander extract when IgE specific to horse dander was determined) excluded both contamination and cross-reaction as the main explanations. We can only conclude that atopic individuals seem to have a tendency to develop allergies to animal dander allergens as a group. A similar tendency was found for pollen and food allergens respectively. The co-variation among positive food results (Table 3) is related to their more frequent occurrence among children (Table 2).

The RAST-positive patients had on the average a little over three positive RAST-results (Table 2). This number is rather high, and to some extent it reflects the co-variation of positive tests described above. However, it may also be due to our selection procedure, which eliminated patients for whom RAST analyses against only one or a few allergens were requested. This procedure may have excluded from the study some patients with monovalent allergies; however, most of the samples were obtained from the hospital, and on these samples the RAST panel was always performed.

The distribution of positive RAST results by age and type of allergen reported here is similar to that described by Foucard (1973). Food allergies are most frequent among children 0-4 years, while reactions against epithelia and pollen become apparent later (Table 2). The highest frequency of positive results was obtained with horse dander, a finding which agrees with the results of conventional allergy diagnosis. The low frequency of positive tests to dog dander might to some degree be explained by difficulties in getting representative extracts (Fagerberg & Wide, 1970).

For one third of the patients, negative RAST results were obtained for all allergens in the panel (Table 1). Altogether 3360 RASTs were performed and of these 78% gave a negative result. The rather high frequency of serum samples without any positive test suggests the potential usefulness of a screening test for atopic allergy. It is known that patients with allergic asthma, rhinitis and eczema frequently have increased total serum IgE concentrations (Johansson, 1967; Berg & Johansson, 1969). The present study shows that a determination of the total IgE can be used as a screening test for allergy. In our study 37% of the patients had an IgE value above the upper reference limit (log IgE mean + 2 s.d. of the age group) (Fig. 5). Among the patients with a high IgE value only 5% were negative in all RAST tests. Presumably, some of these were allergic to less common allergens not included in our panel.

Normal or low IgE values are less informative than high ones. Even when the log IgE value was below the normal reference limit, there was a chance of obtaining a positive RAST reaction to one or more of the allergens used (Fig. 4). However, the RAST reactions were usually weak; the score sum was only 1-2 in seven of the ten RAST-positive patients with a log IgE value below the mean minus 1 s.d. (Fig. 3). Studies in progress (Johansson, 1975), using an improved technique for IgE quantitation (Ceska & Lundkvist, 1972), which gives a better discrimination in the low level range, indicate that a low IgE value is rarely found when a clinically significant reaginic allergy is present.

It has previously been shown that the serum IgE level rises as a result of the

allergenic stimulation during the pollen season (Berg & Johansson, 1971b) or at the beginning of a period of hyposensitization (Berg & Johansson, 1971a). The present study confirms the finding of Hogarth-Scott *et al.* (1973) that the total IgE is dependent upon the level of individual allergen-specific IgE species (Fig. 3). In addition, this study has shown a positive correlation between the total IgE and the number of positive RAST tests (Fig. 4).

Acknowledgments

The skilful technical assistance of Miss Ingegerd Ågren, Miss Marian Andersson, Miss Elisabeth Paulsson and Miss Lilian Posibiloch is gratefully appreciated. A travel grant from the Finnish Academy to F.B. is acknowledged.

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