

Clinical evaluation of CAP System and RAST in the measurement of specific IgE

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We investigated the diagnostic value of a new *in vitro* test, Pharmacia CAP System (Pharmacia Diagnostics AB, Uppsala, Sweden), for the quantitative measurement of allergen-specific IgE antibodies by comparison with RAST in 2 groups of patients, 71 atopic and 48 non-atopic. In the last 20 years RAST has supplied a good diagnostic tool, but this test presents some problems, the main one being sensitivity. The new test has a solid phase able to bind even very small amounts of specific IgE and an anti-IgE tracer with very low cross-reactivity with other immunoglobulins, thus presenting more favourable conditions. From the analysis of our results, Pharmacia CAP System gave higher sensitivity (94% compared to 88% of RAST) with no loss of specificity (96% for both tests). The reliability of these results is ensured by the proper selection of patients who were all suffering from pollinosis and were clinically diagnosed as certainly hypersensitive to a single pollen. A positive trend was found between severity of asthma and levels of specific IgE for timothy. Pharmacia CAP System appears to identify a larger number of atopic patients than RAST.

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The IgE antibody was discovered in 1967 (9, 10) and the radioallergosorbent test (RAST) was described by Wide et al. (17) in the same year. The commercial availability of this test (as Phadebas RAST) supplied a good diagnostic tool, still widely used, for the diagnosis of allergic diseases.

Nevertheless, RAST still presents some disadvantages; for example, it is less sensitive than *in vivo* tests (11), and in atopic patients with low-grade allergy Berg & Johansson (2) in 1974 found RAST was less sensitive than specific provocation tests for several inhalant allergens. Various modifications of the RAST technology have been proposed and tested but efforts to increase sensitivity result in a loss of specificity (8, 15, 18).

Recently, a new *in vitro* test (Pharmacia CAP System) has been proposed to measure IgE antibodies in a quantitative way. This test is based on a porous hydrophilic carrier polymer encased in a capsule (1, 3). The carrier can bind several times more allergen-protein in the CNBr (cyanogen bromide) activated form than the RAST paper disc. The Pharmacia CAP System uses a mixture of polyclonal and monoclonal second antibody which results in higher immunoreactivity and low background noise. Equilibrium is reached in less than 20 min and cross-reactivity with other immunoglobulin classes is minimal.

The aim of this investigation was to evaluate the sensitivity and specificity of the Pharmacia CAP System in comparison with Phadebas RAST results from the same patients. The allergens tested were timothy, birch, mugwort and wall pellitory.

Patients and methods

Patients

We studied 71 atopic subjects (40 males, 31 females, mean age 33.4 years, range 10-63) and 48 non-atopic subjects (20 males, 28 females, mean age 34.6 years, range 15-70). The former were selected among the outpatients referred to the Allergy Center of the 1st Department of Internal Medicine at the University of Milan from December 1987 to January 1988, and the latter were healthy Department staff members.

The atopic patients were selected on the basis of the following criteria: 1) presence of seasonal rhinitis and/or asthma; 2) positive skin prick tests (SPT) for pollens present in their area of residence in the period of symptoms; 3) no specific immunotherapy in the previous 2 years.

The clinical history was collected for each patient so as to define the kind and severity of symptoms (assessing a score of 0 to slight, 1 to moderate and 2 to severe symptoms) and the relationship between period and duration of symptoms and pollen season

of the most common allergenic species in northern Italy (grasses, wall pellitory, birch, mugwort). In the same period the pollen concentration in the atmosphere was determined using a Burkard spore trap.

In vivo tests

SPT were done with standardized Pharmacia extracts (Pharmalgen 100 000 BU/ml) of timothy grass, wall pellitory, birch and mugwort. Histamine hydrochloride, 10 mg/ml, served as positive control, and a glycerine solution as negative control. A wheal at least 3 mm larger than the negative control was considered positive. The reaction was read after 15 min and interpreted in relation to the size of the positive control. A reaction equal to that produced by histamine was graded 3 plus, one twice its diameter as 4 plus, one half its diameter as 2 plus, and one a quarter the diameter as 1 plus. We considered only the pollens whose extract gave a reaction of 3 plus or more.

The allergen identified as responsible for each atopic patient on the basis of clinical history and SPT was chosen for testing RAST and CAP System. In the group of non-atopic patients RAST and CAP System were run for all 4 allergens.

In vitro tests

RAST was done using commercially available material, Phadebas RAST (Pharmacia Diagnostics, Uppsala, Sweden), and results were expressed in PRU/ml (Phadebas RAST Units).

The CAP System was employed as follows: undiluted sera were incubated with an allergen-coated solid phase consisting of a hydrophilic carrier polymer encased in a capsule, the immuno-CAP. The test was run in microplates. After incubation and washing, an anti-IgE consisting of human IgE (Fc) specific combinations of polyclonal and monoclonal anti-IgE antibodies labelled with ^{125}I was added.

After incubation and washing, the immuno-CAPs are transferred to test tubes for measurement in a gamma counter. Results are then expressed in kU/l with calibration against the World Health Organi-

Table 1. CAP System results in patients with negative RAST

Allergen	Pt No.	CAP	RAST
Timothy	31	0.513	<0.35
	35	<0.35	<0.35
	38	0.399	<0.35
	49	0.388	<0.35
	50	<0.35	<0.35
Pellitory	65	<0.35	<0.35
Mugwort	69	<0.35	<0.35
Birch	54	0.443	<0.35

zation standard for IgE. The range is from 0.35 to 100 kU/l.

Assessment of the results

The RAST and CAP System results for each patient were matched to verify which of the 2 tests correlated better with the diagnosis based on clinical history and SPT. The RAST and CAP System responses for the allergen identified by clinical history as surely causing symptoms in every single patient were compared and used to establish sensitivity, specificity, positive predictive value, negative predictive value and efficiency of the 2 tests. On the basis of their main symptom we divided atopic patients into 2 groups, rhinitic and asthmatic, to assess whether the 2 tests differed significantly in relation to the symptoms.

Results

Of the 71 patients with seasonal symptoms, 42 suffered only from rhinitis and 29 suffered from asthma as well.

The Pharmacia CAP System gave 67 positive (94.4%) and 4 negative (5.6%) results in the atopic group. Phadebas RAST was positive in 63 cases (88.7%) and negative in 8 (11.3%) in the same group.

Of the 8 atopic patients giving negative RAST, 4 (50%) gave a weak positive response in the Pharmacia CAP System with timothy and birch. Detailed data are presented in Table 1.

The 71 atopic patients were grouped according to

Table 2. Mean value, positive responses and percentages for CAP System and RAST

Allergen	No. pts	CAP			RAST		
		mean value KU/1	No. pos.	%	mean value PRU/ml	No. pos.	%
Timothy	51	15	49	96.1	6.7	46	90.2
Pellitory	13	16	12	92.3	5.1	12	92.3
Mugwort	4	1.3	3	75	1.1	3	75
Birch	3	3.3	3	100	1.7	2	66.7

Table 3. Results in percentage of the diagnostic accuracy of the CAP System and RAST

Sensitivity	94	87
Specificity	96	96
Pos. predictive value	97	97
Neg. predictive value	92	84
Efficiency	95	91

the pollen responsible for the symptoms. Table 2 summarizes the mean values in each group for Pharmacia CAP System and Phadebas RAST (as kU/l and PRU/ml respectively). Table 3 reports the diagnostic accuracy of the 2 tests. The results related to investigators' clinical judgements based on SPT and clinical history show an increase in sensitivity, negative predictive value and efficiency in the Pharmacia CAP System, as compared with Phadebas RAST, without any loss of specificity or positive predictive value.

When atopic patients were divided according to symptoms (rhinitis and asthma or rhinitis only) asthmatic patients had a mean CAP value of 14.9 kU/l and rhinitic patients 12.3 kU/l. These differences were not significant. Mean RAST value was 7.2 PRU/ml for asthmatic patients and 5.02 PRU/ml for rhinitic patients.

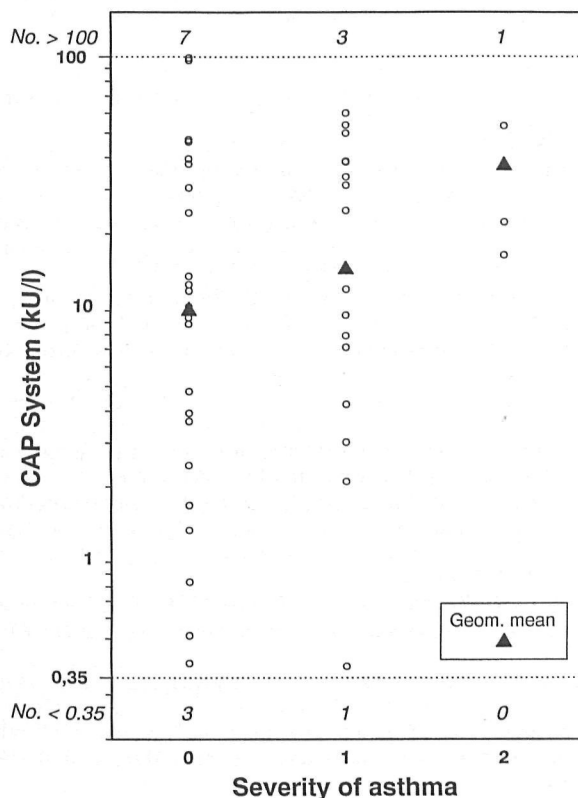


Fig. 1. Results from atopic patients with symptoms to timothy grass, obtained with the Pharmacia CAP System, plotted against severity of asthma.

The severity of each symptom (asthma, rhinitis) was also compared to the amount of specific IgE measured by the Pharmacia CAP System for each allergen. The correlation between severity of asthma and specific IgE to timothy grass measured by the Pharmacia CAP System showed a positive trend, illustrated in Fig. 1. No other such correlations could be detected between specific allergen and the kind of symptoms, but in any event this was not originally one of the aims of the study.

In the 48 non-atopic patients with negative SPT for the 4 allergens, only 2 were positive to both the CAP System and RAST. One patient showed a weak positive level of specific IgE to birch (CAP 1.26, RAST 0.39) and one showed a more pronounced IgE-titre to mugwort (CAP 8.18, RAST 11.4).

Discussion

In this study a new diagnostic *in vitro* test was used for measuring specific IgE. This test was compared regarding its diagnostic accuracy to the RAST principle, which has been extensively used for *in vitro* diagnosis of allergic diseases for over 20 years. A possible limitation of current RAST technology is its sensitivity, especially in the low titre region. It is generally considered that 10–25% of patients presenting a positive challenge test to specific allergens will give a negative RAST (11). A part of these are probably false-positive because of non-specific reactivity, but another part may be those with allergy to minor allergens or relatively low IgE titres to major/intermediate allergens.

Several attempts have been made to improve the sensitivity of IgE antibody measurements. Larger amounts of labelled antibody were tried but failed to improve the assay system, possibly because of the limited affinity of the anti-IgE antibody (7). The use of monoclonal anti-IgE antibody with higher specific radioactivity proved unacceptable for safety reasons (7). Another attempt to improve RAST sensitivity consisted in lowering the cut-off level of positivity from 0.35 to 0.14 PRU/ml, but the higher sensitivity resulted in lower specificity (8). Prolonging the incubation time gave a slight but inadequate gain in sensitivity (18).

Our results show the Pharmacia CAP System offers higher sensitivity with no loss of specificity. Specificity was 96% both for RAST and CAP System, while sensitivity was 94% for CAP System, as compared with 88% for RAST. These results seem reliable as the RAST values we obtained are consistent with those reported in the literature (5) and our patients were clinically diagnosed as certainly allergic to the investigated allergen.

CAP System was superior to RAST as regards positive and negative predictive value and efficiency.

No statistical significance could be detected, but it was theoretically unreachable because of the good RAST results for the allergens considered. With these pollen allergens most available tests for specific IgE already ensure good results. The CAP system could be more useful for other allergens such as mites, animal danders or moulds, for which the first studies reported more marked differences in sensitivity compared to RAST (4, 6, 14).

The good performance of the CAP System seems related to 2 main factors, the first being the high binding capacity of the solid phase which binds even very small amounts of IgE, including those against minor allergenic components. The other is the use of a mixture of polyclonal and monoclonal antibodies as radiolabeled tracer. Some studies have shown that polyclonal antibodies provide a more sensitive assay, but only monoclonal antibodies provide a homogeneous reagent recognizing all IgE present in serum and give linear IgE dilution curves over a wider range (7, 16).

This precision led us to use the CAP System to assess possible differences in specific IgE levels among patients with symptoms of different severity. A positive trend has been found in the correlation between asthma severity (as scored by the clinician) and the quantitative test results as kU/l in asthmatic patients sensitive to timothy grass pollen.

This finding, to be confirmed in studies in which the severity of symptoms is assessed more precisely and prospectively, might support Lindholm's report of a correlation between CAP System values and severity of birch allergy (12).

It is important to underline that these results were obtained with standardized allergens. Only the presence in the allergenic extract of all major, intermediate and minor allergens can ensure the detection of all specific IgE antibodies in serum. For instance, with the same standardized extracts of timothy grass pollen, we observed a correlation between levels of specific IgE measured by RAST and areas of wheals elicited by timothy extract (13).

Progress in the standardization of allergens together with the new technology in measuring IgE antibodies appear not only to achieve improvements in diagnosis, but also to provide valuable tools to assess the severity of the allergic disease.

In conclusion, this study shows the good performance of the Pharmacia CAP System. More symptomatic patients give positive test results with the new test. The standardization and the extended measurement range (0.35–100 kU/l) in combination with a higher capacity without any loss of accuracy indicated this test is a real improvement in specific IgE determination.

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