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Evaluation of a capsulated hydrophilic carrier polymer (the ImmunoCAP) for measurement of specific IgE antibodies

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The Pharmacia CAP System is a new assay for serum specific IgE, utilising a solid phase capable of binding more antigen than conventional systems. The CAP System has been evaluated in 69 consecutive patients referred to one allergy clinic in relation to skin prick test (SPT), radioallergosorbent test (Phadebas RAST®) and specific allergy diagnosis for five inhalant allergens, *D.pteronyssinus,* timothy grass pollen, cat epithelium/dander, *Cladosporium* and *Alternaria.* Good correlation was obtained between RAST and CAP for all allergens, e.g. r = 0.974 for *D.pteronyssinus* and r = 0.964 for grass pollen. When sensitivity and specificity were examined for both CAP and RAST versus SPT, CAP was usually found to be of greater sensitivity than RAST, and of similar or slightly lower specificity. SPT gave more positive reactions than either *in vitro* test, but CAP gave more positives than RAST. Twenty-two of 336 (6.6%) tests were CAP positive/RAST negative, whereas a negative CAP with a positive RAST occurred in only 2/336 (0.6%) tests. Of patients with any test (SPT or RAST or CAP) for specific IgE positive, up to 20–30% did not have clinical allergy, confirming the importance of the history in interpreting these tests. Our results suggest that, for the allergens tested, the Pharmacia CAP System is more sensitive than the RAST, identifying more positive tests and approximating more closely to the SPT. It offers the additional advantages of speed and efficiency.

Key words: asthma, allergic, diagnosis; immunoglobulins; immunology; radioimmunoassay; rhinitis, skin tests.

The Pharmacia CAP System is a new test system for the measurement of total and specific IgE in serum or plasma. It can be performed either as a radioimmunassay or as a fluorometric test. The system is based on a new type of solid phase consisting of a flexible hydrophilic carrier polymer encased in a capsule, the ImmunoCAP. The carrier consists of a cyanogen bromide activated cellulose derivative which has two main advantages. It can bind at least three times more protein than the corresponding paper disc and up to 50 times more than can be adsorbed on a coated tube. The solid phase also provides favourable reaction conditions, with short diffusion distances, so that most of the reaction between IgE antibodies in the test sample and the bound antigens take place very quickly: most within 2 min of the initial incubation step with equilibrium being reached in less than 20 min (1, 2).

The system uses a mixture of polyclonal and monoclonal anti-IgE antibodies which are labelled either with ^{125}I or beta galactosidase generating fluorescence. Thus the tests can be performed as a radioimmunoassay or as as a fluorometric test. Both reagents have a high immunoreactivity and low background allowing a wider measuring range than existing tests for

IgE antibodies. The new test is quick to perform and is partly automated. It therefore appears to offer considerable potential advantages over the current radioallergosorbent test (RAST).

The aim of this study was to evaluate the Pharmacia CAP System, in patients referred to an allergy clinic, in relation to Phadebas RAST®, skin prick test and allergy diagnosis.

PATIENTS AND METHODS

Sixty-nine consecutive patients referred to one allergy clinic entered the study (age range 17–70 years; 46 females and 23 males). Not all patients had allergic disorders and as judged by skin prick tests to a range of common allergens, 45 patients were atopic (i.e. had one or more positive skin tests) and 24 were non-atopic.

The patients had a variety of disorders but asthma (21 subjects), rhinitis (40 subjects) and conjunctivitis (14 subjects) predominated. Individual patients often had more than one of these disorders. Some of the patients with these disorders were sensitive to several allergens (Table 1), while other patients had non-allergic rhinitis or asthma.

Responses to five inhalant allergens were assessed: *D.pteronyssinus* (d1), timothy grass pollen (g6), cat epithelium/dander (e1), *Cladosporium herbarum* (m2) and *Alternaria tenuis* (m6).

SPT were performed in duplicate to the five allergens and to phenolsaline (negative control) and histamine chloride 1 mg/ml (positive control). The aqueous allergen extracts used (Bencard) were not potent or standardised but are extracts widely used in allergy diagnosis, and therefore seemed appropriate for comparison with a new test for specific IgE. A wheal diameter ≥ 2 mm larger than the negative control was taken as a positive response.

A detailed history was taken from each patient and skin prick tests were performed. A specific allergy diagnosis for each of the inhalant allergens was made on the basis of the history and skin prick tests. Where patients were found to be allergic to more than one allergen, these were ranked in order of clinical importance. A simple scoring system was used to assess the severity of symptoms (mild, moderate, severe) and records were made of frequency of symptoms, frequency after contact with animals, and of drug use.

Blood was taken for assay of serum specific IgE by both the Pharmacia CAP System and Phadebas RAST. Undiluted sera were assayed blind, in duplicate, against each of the five allergens. The radioimmunoassay version of the Pharmacia CAP System was used (1, 2). In brief, test sera and standards are incubated at room temperature (approximately 20°C) with the ImmunoCAP for 30 min, then washed. IgE

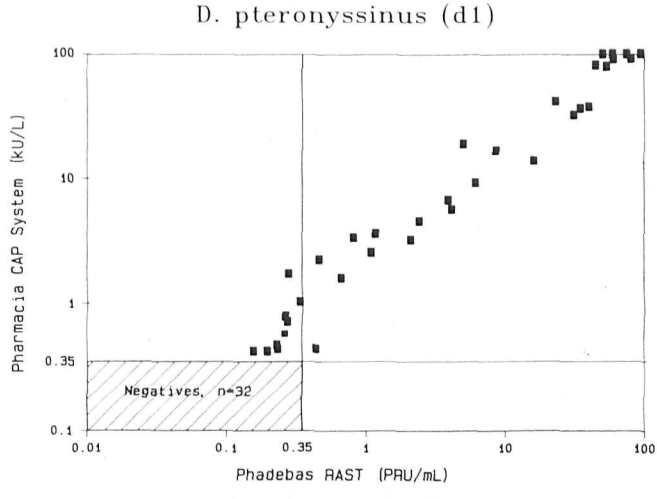

Fig. 1. Correlation between *D.pteronyssinus* IgE measured by Pharmacia CAP System and Phadebas RAST ($n = 35$).

Table 1

Symptoms due to different allergens

Allergen	Total	Asthma	Rhinitis	Conjunctivitis
D.Pter.	35	13	23	8
Grass pollen	27	13	22	9
Cat	13	5	12	4
Cladosporium	10	6	7	3
Alternaria	9	4	7	1

(Fc) specific combinations of polyclonal and monoclonal anti-IgE labelled with ^{125}I are added. After a second incubation step of 150 min, the ImmunoCAP is washed, and bound radioactivity counted in a gamma counter. The specific IgE assay is calibrated against a WHO standard for IgE, in the range 0.35–100 kU/l. Results are read from a standard curve. The upper and lower cut-off points are 100 kU/l and 0.35 kU/l as for the Phadebas RAST, and results can be expressed either as kU/l or as classes (0–6, as for RAST but where class 5 is equivalent to 50–100 kU/l and class 6 is > 100 kU/l). The solid phase allergens in the ImmunoCAP are optimised to give maximum binding with low background levels, even when undiluted test sera are used (2). The standard procedure was used for the Phadebas RAST (13) with the increased measuring range (0.35–52.5 PRU/ml).

Sensitivity and specificity

The terms sensitivity and specificity can cause confusion, unless clearly defined. When a test has a sensitivity of 100% for a particular disease it gives positive results in *all* patients with the disease (i.e. the number positive in the test divided by the number with the disease) (6). Specificity is used to characterise the true incidence of negative results obtained when a test is applied to subjects known to be *free* of the disease (i.e. the number negative in the test divided by the number of subjects without the disease). There is some difficulty in applying these terms, as defined above, to allergy since, for certain allergens, there is no unequivocal way of defining the presence of disease (i.e. clinical reactivity to a particular allergen). A clinical diagnosis of sensitivity to allergen(s) can be made in different ways, e.g. by taking together some of the following parameters: positive case history, positive skin test, positive RAST, or positive provocation test. (It is clear that a positive test for specific IgE in itself need not mean clinical allergy.)

In this paper sensitivity and specificity of the Pharmacia CAP System will be considered in

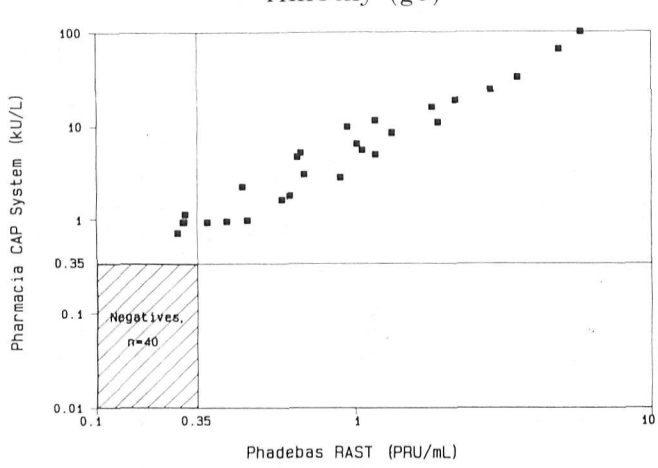

Fig. 2. Correlation between timothy grass pollen IgE measured by Pharmacia CAP System and Phadebas RAST (n = 27); two points are overlapping in the CAP positive/RAST negative area.

relation to the results of other tests for specific IgE, e.g. by comparison with the sensitivity and specificity of RAST versus the SPT. Thus sensitivity is defined as the number positive in the test under study divided by the number positive in the variable compared. Specificity is the number negative in the test under study divided by the number negative in the variable compared.

RESULTS

Correlation between Pharmacia CAP System and Phadebas RAST

Good correlation (Spearman rank correlation test) was obtained between the two techniques for measuring serum specific IgE: r = 0.974 for *D.pteronyssinus,* (n = 67, positive observations = 35); r = 0.964 for grass pollen (n = 66, positive observations = 26) and r = 0.926 for cat epithelium/dander (n = 67, positive observations = 14) (Figs 1-3). A similar pattern was also obtained for *Cladosporium* and *Alternaria* (Table 2) but the number of subjects with positive results (the majority of tests were negative for those allergens) were too small for statistical analysis.

Taking a value of 0.35 as the lower cut-off point or upper limit of normal for both tests, Figs 1-3 show a number of patients were positive by CAP but negative by RAST.

When sensitivity and specificity were examined for either the CAP System or RAST versus skin test, the new test was usually found to be of greater sensitivity than RAST (for 4 of 5 allergens), and of similar or slightly lower specificity (Table 3). A detailed breakdown of the data showed that for grass pollen: of 30 patients with positive skin prick tests, 24 were positive by CAP and 22 were positive by RAST, and for *D.pteronyssinus*: of 39 patients with positive skin prick tests, 30 were positive by the Pharmacia CAP System whereas 25 were positive by RAST. This suggests the CAP System was of greater sensitivity.

Comparison of CAP System and Phadebas RAST

Good concordance was found between CAP and RAST for all allergens (87-97%) (Table 2). Overall, a positive CAP System with a negative RAST was found in 22 of 336 (6.6%) tests of which 13 had a positive SPT and three were clinically allergic; whereas a negative CAP System with a positive RAST occurred in only two of 336 (0.6%) tests (Table 2). Both of these patients had positive skin prick tests to the allergen in question, but neither was considered to be clinically allergic to that allergen.

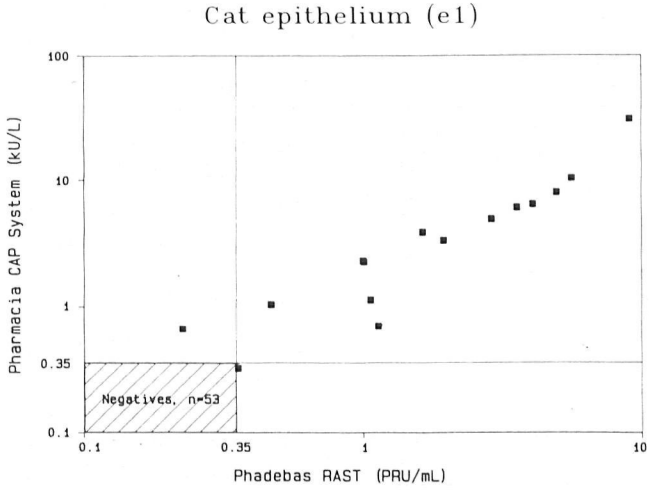

Fig. 3. Correlation between cat epithelium/dander IgE measured by Pharmacia CAP System and Phadebas RAST (n = 14).

Table 2

Comparison of results obtained by CAP System (C) and RAST (R)

Allergen	Concordant			Discordant		
	C+/R+	C−/R−	Total No. (%)	C+/R−	C−/R+	Total No. (%)
d1	26	32	58 (87)	9	0	9 (13)
g6	23	40	63 (94)	4	0	4 (6)
e1	12	53	65 (97)	1	1	2 (3)
m2	4	56	60 (91)	5	1	6 (9)
m6	6	58	64 (96)	3	0	3 (4)

Comparison of CAP System and skin test

The concordance varied with the allergen, but was close to 80% (range 73–87%) for all allergens except cat epithelium/dander, where the concordance was 64% (Table 4). Where the results were discordant, a negative CAP with positive SPT was more common (64/336 tests) than the reverse pattern (15/336 tests).

Comparison of CAP System or Phadebas RAST with skin test

SPT gave more positive reactions than the CAP System or RAST but the CAP System was closer to the SPT. The number of subjects positive in each test is shown in Fig. 4. These are not necessarily the same individuals for each test, because of discordance, hence the numbers differ from those quoted in the text above (grass pollen and *D.pteronyssinus*) where the same individuals are being compared.

Table 3

Sensitivity and specificity of CAP and RAST versus SPT for all allergens

Allergen	Test assessed	Sensitivity v SPT	Specificity v SPT (%)
D.pter.	CAP	77	82
	RAST	64	96
Grass pollen	CAP	80	92
	RAST	73	97
Cat	CAP	32	94
	RAST	35	94
Cladosporium	CAP	30	95
	RAST	22	100
Alternaria	CAP	35	94
	RAST	29	98

Specific IgE antibody level in relation to symptom score

When patients with grass pollen rhinitis were subgrouped according to severity of symptoms (none, mild, moderate or severe; $n = 18$) a trend was seen between symptom severity and level of specific IgE assayed by the Pharmacia CAP System, but the numbers were too small for statistical analysis (data not shown). This trend was not observed for rhinitis due to other allergens or for other diseases, e.g. asthma.

Evaluation of all tests for specific IgE in relation to allergy diagnosis

Of patients with any test (SPT, Phadebas RAST or Pharmacia CAP System) for specific IgE, positive, up to 20–30% did not have clinical allergy (data shown for *D.pteronyssinus* in Table 5), i.e. subjects are identified who are atopic, but do not have clinical allergy.

DISCUSSION

The Phadebas RAST is the main test used for *in vitro* measurement of specific IgE antibodies, while the SPT is the most widely used *in vivo* test. Any new *in vitro* test must therefore be compared with RAST, and ideally should offer advantages over RAST.

The position of the Phadebas RAST as a diagnostic test and its relationship to other tests for allergy is well established: there have been many studies comparing RAST with SPT, history and provocation tests. A number of studies find that the RAST is concordant with the SPT in about 80% of cases (4, 5, 11). For example, in asthmatics clinically allergic to house dust, 79–82% of patients with positive SPT to *D.culinae* or house dust had positive RAST (11). In another study of 118 children with rhinoconjunctivitis and/or asthma tested against at least six allergens (pollens and animal danders), the RAST was positive in 196 of 235 (80%) with positive SPT (4). Other studies find a lower correlation, and this seems to depend on both the nature of the allergen and the quality of that allergen extract (5, 13). Where SPT and RAST are discordant, the SPT is usually positive and the RAST negative.

The agreement between RAST and provocation tests is generally found to be higher than between RAST and SPT, e.g. 90% compared with 85% in Fagerberg & Wide's study of 35 adults with dog-induced asthma (5), and 90% compared with 80% in 118 children with rhinoconjunctivitis or asthma tested to several allergens (4). The correlation between RAST and provocation is better in very sensitive patients, compared with those patients who are less sensitive or non-reactive on challenge (3).

In Berg & Johansson's study (4), involving large numbers of patients, no negative skin test (to combined SPT and intradermal testing) with positive RAST was found, i.e. no false positive RAST was found. A positive SPT with negative RAST was found in 58/370 (16%), and about one third of these had positive provocation tests.

To summarise these observations, SPT is more often positive than RAST, and a proportion (but not all) of SPT positive/RAST negative subjects have positive provocation tests, but usually only to higher concentrations of allergen. A substantial number of SPT positive subjects do not have clinical allergy.

These conclusions fit with other observations: the prevalence of positive SPT (i.e. atopy) in unselected populations is 30–50% (7, 9) (e.g. of 708 teenagers tested against 12 allergens, 49% had at least one positive SPT, and 43% at least two positive reactions (8)) yet the prevalence of clinical allergy is much lower: estimates vary, but at best prevalence rates of up to 10–15% are reported (10–12).

This study shows good correlation between RAST and CAP. The CAP System was however of greater sensitivity than RAST, and allowed identification of an additional 22 (6.6%) positive tests. This is probably because more allergen is bound to the solid phase in the ImmunoCAP, and this is more accessible to the IgE antibodies. The use of undiluted sera is unlikely to have given false positives, since the solid phase in the ImmunoCAP is designed to give low background noise (2) and undiluted sera were used in both assays. Although SPT gave more positive results than either of the *in vitro* tests for specific IgE, the CAP System approximated more closely to the SPT than the RAST, again suggesting greater sensitivity. This was achieved with little or no reduction in specificity.

The quality of the extracts used for SPT, and the size of the wheal taken as positive will determine the incidence of positive reactions. In this study we used standard extracts (Bencard), provided at a single concentration, widely used

Table 4

Comparison of results obtained by CAP System (C) and skin prick test (S)

Allergen	Concordant			Discordant		
	C+/S+	C−/S−	Total No. (%)	C+/S−	C−/S+	Total No. (%)
d1	30	23	53 (79)	5	9	14 (21)
g6	24	34	58 (87)	3	6	9 (13)
e1	11	32	43 (64)	2	22	24 (36)
m2	7	41	48 (73)	2	16	18 (27)
m6	6	47	53 (79)	3	11	14 (21)

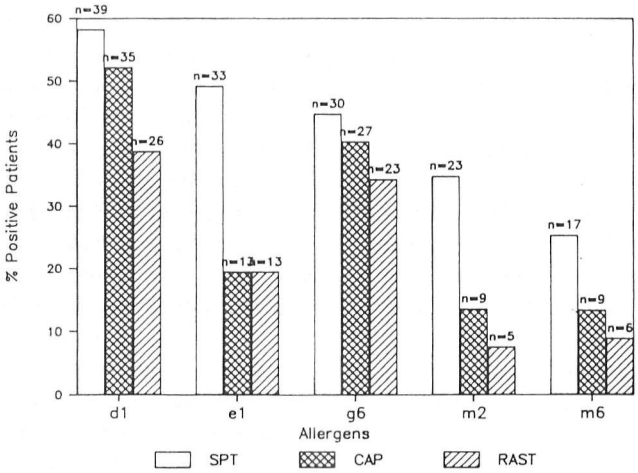

Fig. 4. Number and percent of patients with positive skin prick test (SPT) or Pharmacia CAP System (CAP) or radioallergosorbent test (RAST) to *D.pteronyssinus* (d1), cat dander (e1), timothy grass pollen (g6), *Cladosporium* (m2) and *Alternaria* (m6).

in allergy diagnosis. These are not potent, when compared with some of the more recently introduced lyophilised biologically standardised extracts which can be used at high concentration. However, the Bencard extracts are good quality and have been shown to give more positive reactions than other conventional extracts (8). Our cut-off point of wheal diameter ≥ 2 mm for a positive SPT was low, but this is a commonly used value. It is therefore not surprising the incidence of positive SPT reactions exceeded the incidence of positive CAP tests. One would not, in any case, expect the incidence of specific IgE antibodies to be the same when measured by an *in vivo* test (for skin-fixed antibody) or an *in vitro* test (for circulating antibody).

The sensitivity for CAP (and for RAST) versus the SPT varied greatly, depending on the allergen being tested. The best results were seen for grass pollen (80%) and *D.pteronyssinus* (77%) with lower values for the other allergens. This is likely to be a result of the relative qualities of allergen extracts used both for skin test and in the CAP System.

Depending on the tests used to detect specific IgE (SPT or CAP or RAST) a substantial number of patients were identified with specific IgE antibodies yet no clinical allergy, as judged by history. This applied to 20–30% of patients with *D.pteronyssinus* IgE antibodies. A further study including provocation tests would give a more accurate figure for "clinical allergy" and would help in the evaluation and interpretation of a positive CAP test. However, the substantial data comparing SPT, RAST and provocation tests, discussed above, suggests that there are a definite number of atopic subjects who have no evidence of clinical allergy to the allergen in question at the time of investigation.

Table 5

Allergy diagnosis compared with results of SPT; or RAST; or CAP for *D. pteronyssinus*

Diagnosis +ve in	28/39 +ve SPT (72%)	in 21/26 +ve RAST (81%)	in24/35 +ve CAP (69%)
Diagnosis +ve in	3/28 -ve SPT	in 10/41 -ve RAST	in 7/32 -ve CAP

This emphasises the importance of the history in the interpretation of any test for specific IgE.

Our results show that, for *D.pteronyssinus,* grass pollen, *Alternaria* and *Cladosporium,* the CAP System is more sensitive than the Phadebas RAST. It offers the additional advantages of speed, efficiency and automation, which may lead to reduced running costs if large numbers of samples are being tested. Thus, for the allergens studied, the CAP System appears to be an advance on the RAST.

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