

Specific IgE antibodies in the diagnosis of atopic disease

Clinical evaluation of a new *in vitro* test system, UniCAP™, in six European allergy clinics

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A new immunoassay system utilizing new automatic instrumentation, new software for evaluation of data, and reagents updated for increased speed and accuracy was evaluated. Six clinical studies included 894 consecutive patients. Major symptoms were rhinoconjunctivitis, asthma, atopic dermatitis, and urticaria. The prevalence of inhalant allergy was 54–69%. Phadiatop®, detecting atopic sensitization to common inhalant allergens, agreed with clinical diagnosis in 764/836 cases (91.4%). The clinical sensitivity and specificity were 93% and 89%, respectively. The clinical sensitivity and specificity of UniCAP specific IgE derived from 5170 comparisons with clinical diagnosis were 89% and 91%, respectively. Specific IgE measurements in UniCAP and in the Pharmacia CAP System agreed in 266/274 cases (97%). A comparison of the sensitivity and specificity of Pharmacia CAP System RAST in 1987 and with UniCAP specific IgE in 1995 showed equivalent performance without change of efficacy or degradation of IgE antibodies after 8 years. The systems were equivalent also in terms of measured values ($r=0.96$, slope=1.12), confirming the standardization of allergens and of assay calibration. UniCAP is an efficient laboratory system for routine diagnostic testing of allergy and a valuable tool for basic studies on allergens and antibodies.

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The solid-phase sandwich immunoassay originally described by Wide et al. in 1967 (1) for the determination of allergen-specific IgE antibodies has proven to be a remarkably useful principle, which has since been applied to a variety of assays. Measurement of IgE antibodies against a wide range of allergens has become an established tool in the investigation of the cause of allergy-like disorders and for prediction and monitoring of the development of allergic diseases (2). The introduction of the high-capacity allergen solid phase, ImmunoCAP™ (3), improved the sensitivity (4–7), provided the basis for quantitative internationally standardized measurements, and simplified the laboratory work. Increasing demands of accuracy, reproducibility, speed, and cost-effectiveness from

clinics and laboratories have triggered the development of a fast, fully automated laboratory system based on ImmunoCAP technology. In addition to new instrument designs, the UniCAP system utilizes reagents updated to meet the requirements for increased speed and accuracy (8–10) and new software for evaluation and reporting of the data.

Although the system can be used for the measurement of allergen/antigen-specific antibodies of several immunoglobulin classes, as well as for assay of other analytes, this study is restricted to the investigation of the clinical efficiency of measurement of IgE antibodies for allergen-specific diagnosis (UniCAP specific IgE) and for differential diagnosis of atopic disease (UniCAP Phadiatop).

Material and methods

Study design

In this paper, we report results from six independent studies. Two of them were run in 1987 (5, 6) and followed up in 1995. The other four studies (1–4) were all run in 1995.

Study outline

The objective of these studies was to evaluate the diagnostic performance of UniCAP specific IgE and Phadiatop in patients with suspected allergy compared to the clinical diagnosis set by the physician.

Consecutive patients with suspected allergy visiting the clinic in 1995 were evaluated in studies 1–4. In studies 5 and 6, the patient sera collected and included in studies performed in 1987 (12, 13) were analyzed together with those of studies 1–4. Every patient was seen on one occasion by the physician. Case history was collected, and the patient was examined and evaluated according to the routine at the clinic. Blood samples were collected.

For each patient, the following judgments were made by the physician:

- 1) conclusion per allergen: clinical allergen-specific diagnosis (for one or more of the separate allergens listed) based on established procedures at the different clinics
- 2) final conclusion: the overall judgment of whether the patient had an atopic allergy involving common inhalants (atopy) or not (nonatopy).

All serum samples were analyzed for specific IgE, total IgE, and Phadiatop in accordance with "Directions for use" with UniCAP at Pharmacia & Upjohn Diagnostics AB in Uppsala, Sweden, when the clinical phase was ended.

Clinical sensitivity and specificity were calculated for UniCAP specific IgE and Phadiatop test results, the physician's diagnosis being used as the true status.

The studies were conducted in accordance with the principles enunciated in the World Medical Association Declaration of Helsinki.

Patients

A total of 894 consecutive patients, 6–81 years of age, with suspected allergy were included in the studies (Table 1).

Allergens

Allergens for testing were selected by the investigators to cover the majority of the specific

Table 1. Patient groups studied

Study no.	Patients	Age range (years)	n	Sex M/F
1. Italy	Atopic	6–56	97	49/48
	Nonatopic	12–64	45	18/27
	Uncertain	6–59	20	4/16
2. Spain	Atopic	11–65	79	36/43
	Nonatopic	11–64	66	24/42
	Uncertain	12–47	4	3/1
3. Germany	Atopic	11–64	109	41/68
	Nonatopic	13–64	53	13/40
	Uncertain	17–53	11	5/6
4. The Netherlands	Atopic	12–59	131	40/91
	Nonatopic	14–64	57	15/42
	Uncertain	18–55	9	4/5
5.* Sweden	Allergic	16–63	81	32/49
	Nonallergic	16–75	65	26/39
6.* UK	Allergic	18–73	36	14/22
	Nonallergic	19–81	31	8/23

*When investigated in 1987, categorized as allergic/nonallergic.

sensitivities represented among their patients. Eighteen allergens and Phadiatop were evaluated, as summarized in Table 2.

Clinical investigations

The physician's judgment of the diagnosis was based on the normal procedure for allergy classification at the respective clinic, such as case history, physical examination, skin prick test (SPT), intracutaneous test (ICT), total IgE, and specific IgE.

Table 2. Allergens included in clinical studies

Allergen	Study no.					
	1	2	3	4	5	6
d1 <i>D. pteronyssinus</i>	x	x	x	x	x	x
d2 <i>D. farinae</i>	x	x	x		x	
e1 Cat dander	x	x		x	x	x
e3 Horse dander				x	x	
e5 Dog dander		x		x	x	
g3 Cocksfoot grass					x	
g5 Perennial ryegrass	x	x				
g6 Timothy grass	x		x		x	x
gx1 Grass mix				x		
t3 Silver birch			x	x	x	
t9 Olive tree	x	x				
w6 Mugwort			x	x	x	
w9 English plantain	x					
w21 Wall pellitory	x					
m2 <i>Cladosporium</i>						x
m6 <i>Alternaria</i>	x	x		x		x
f13 Peanut		x				
f14 Soybean			x			
Phadiatop	x	x	x	x	x	x

The allergen extracts for skin testing were commercial preparations routinely used by the respective clinic.

Laboratory investigations

The samples collected were tested with UniCAP, a new automated laboratory system.

Summary of the UniCAP procedure. Serum aliquots of 40 µl were automatically dispensed to allergen ImmunoCAP according to patients' specific testing requests. The test procedure includes: incubation for 30 min at constant temperature (37°C), rinse and addition of β-galactosidase-labeled anti-IgE, incubation for 24 min, rinse and addition of development solution (the enzyme substrate methyl-umbelliferyl-β-D-galactoside), incubation for 9 min, stopping the enzyme reaction, and reading the fluorescence generated by the methyl-umbelliferon released. The concentrations of the patient samples are calculated from IgE calibration curves ranging from 0.35 to 100 kU/l, as defined by the WHO International Reference Preparation for human IgE, 75/502, and expressed in kU/l (total IgE) or kU_A/l (allergen-specific IgE antibody).

The instrument software utilizes five parameter Rodbard algorithms and prints reports showing data on calibrators, samples, and controls. The allergen ImmunoCAP of UniCAP is identical to the allergen ImmunoCAP used in the Pharmacia CAP System. Anti-IgE reagents have been redesigned to meet the requirements of the fast automated procedure. Calibrator curves can be stored for up to 4 weeks.

Pharmacia CAP System. The samples collected in 1987 and included in studies 5 and 6 were tested with the Pharmacia CAP System in 1987 (12, 13). The samples of study 3 were tested with the Pharmacia CAP System at the clinic as part of the routine investigation. All tests were performed according to the directions for use.

Statistical methods

A descriptive compilation of demographic variables was performed. The diagnostic performance of UniCAP was evaluated by calculating performance characteristics, including sensitivity and specificity (11), with 95% confidence intervals. Analyses were made for each allergen and each study separately as well as for all data combined. The conclusion from each separate allergen and the final conclusion of the atopic state were used to classify patients as atopic or nonatopic.

The correlation between methods was studied by linear regression analysis of actual measured concentrations. Scatter plots have logarithmic scales for clear demonstration of the distribution over the wide measuring ranges.

Results

Clinical performance of UniCAP specific IgE and Phadiatop

The six studies included 894 consecutive patients with allergy-like symptoms visiting the clinics at the time of the study. In the studies initiated in 1995 (studies 1–4, *n*=681), the reported prevalences of symptoms were, for asthma, 24.4%; for rhinoconjunctivitis, 67.7%; for atopic dermatitis, 21.4%; and, for urticaria, 13.7%.

The prevalence of inhalant allergy, as judged by the individual clinician, was in the range 54–69%. The average prevalence was 62%.

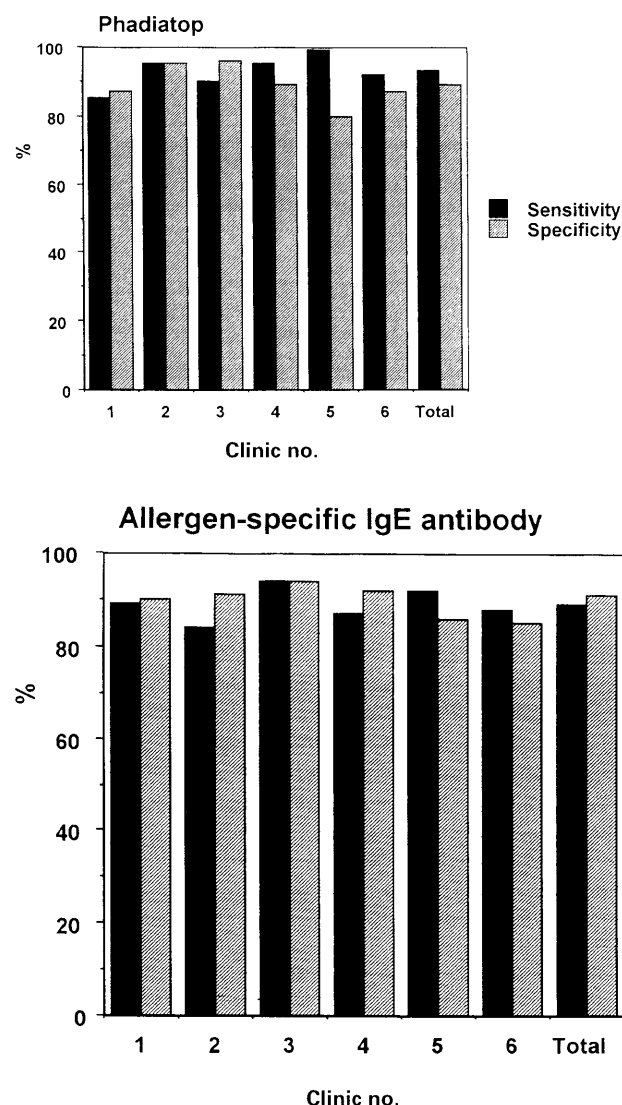
Phadiatop, a test designed to detect atopic sensitization to common inhalant allergens, was applied to UniCAP technology, and found to agree with clinical judgment in 764/836 cases (91.4%). Calculated as clinical sensitivity and specificity of Phadiatop, the figures were 93% and 89%, respectively. Data per study are reported in Fig. 1.

The prevalence of clinical sensitivity to individual allergens varied between the studies, obviously dependent on factors such as geographic location and specialization of the clinic. The reported prevalences of positive clinical conclusions for sensitivity to common allergens are summarized in Table 3.

Allergen-specific IgE antibodies were measured with UniCAP in all cases where the investigating physician was able to reach a conclusive positive or negative clinical diagnosis for the individual allergen. The concordance between presence/absence of IgE antibodies and positive/negative clinical diagnosis was calculated as clinical sensitivity and specificity of the assay, the clinical conclusion being accepted as the correct diagnosis. The results calculated from the 5170 comparisons summarized in Fig. 2 were 89% for sensitivity (1121/1265) and 91% for specificity (3545/3905).

It should also be remembered that for the group with positive test and negative clinical diagnosis it would be incorrect to assume that the test was wrong. This group may well have comprised mainly patients with specific IgE antibodies but no current symptoms. It is well known that a number of patients are atopic but do not necessarily develop clinical allergy.

The sensitivity and specificity data obtained in the individual clinics are in good agreement. The slight



Figs. 1 and 2. Clinical sensitivity and specificity of UniCAP calculated for individual clinics 1–6 and on all tests performed at all six clinics (total). Fig. 1 shows performance of Phadiatop as differential diagnostic test for atopic sensitization to common inhalants. Fig. 2 shows data on allergen-specific tests compared to positive or negative allergen-specific diagnosis.

variation observed (Figs. 1 and 2) was expected and can be ascribed to differences in the patient populations, in the prevalences of sensitivity to allergens, in the quality of allergen extracts used for skin test, and in the investigative routines of the clinics.

When calculated per allergen, the sensitivity and specificity also showed good agreement between clinics with some variation, as illustrated for UniCAP house-dust mite (d1 *Dermatophagoides pteronyssinus*) in Fig. 3.

Assay system comparisons

In one of the clinics, independent IgE antibody measurements were made with Pharmacia CAP

Table 3. Reported prevalences of clinical sensitivity (%)

Allergen	Study no.					
	1	2	3	4	5	6
d1 <i>D. pteronyssinus</i>	27.2	16.8	20.2	48.7	13.7	46.3
d2 <i>D. farinae</i>	25.3	16.8	17.3		12.3	
e1 Cat dander	9.3	18.1		38.6	33.6	14.9
e3 Horse dander				10.2	18.5	
e5 Dog dander		18.1		28.9	18.5	
g3 Cocksfoot grass					24.0	
g5 Perennial ryegrass	30.9	44.3				
g6 Timothy grass	21.6		37.0		24.0	26.9
gx1 Grass mix				33.5		
t3 Silver birch			30.1	31.0	28.1	
t9 Olive tree	15.4	34.9				
w6 Mugwort			12.1	12.2	4.1	
w9 English plantain	3.1					
w21 Wall pellitory	21.6					
m2 <i>Cladosporium</i>						3.0
m6 <i>Alternaria</i>	4.3	14.8		4.1		1.5
f13 Peanut		5.4				
f14 Soybean			4.0			

System RAST as part of the routine clinical investigation. These data agreed with the UniCAP measurements in 266/274 cases (97%) (Table 4).

Two studies, (12) and (13), were initiated in 1987 as part of an investigation of the clinical performance of the Pharmacia CAP System. Serum samples were stored at -20°C and retested with UniCAP 8 years later in 1995. In addition to the study on the agreement of UniCAP specific IgE and clinical diagnosis, these two studies allow comparisons between the Pharmacia CAP System data generated in 1987 and UniCAP data from measurements on the same samples in 1995.

Fig. 4 shows the direct comparison (12) of sensitivity and specificity of the two assay genera-

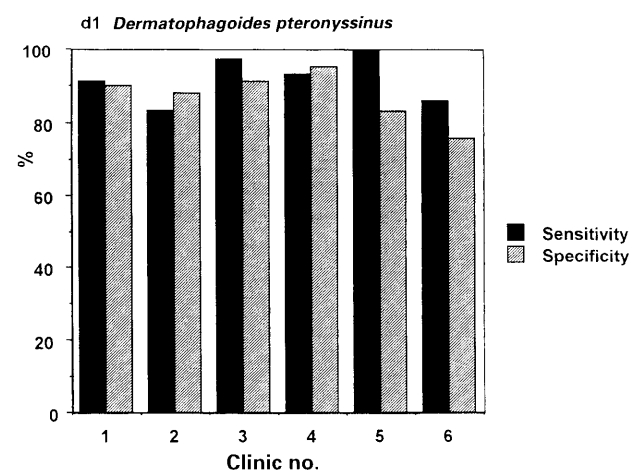


Fig. 3. UniCAP house-dust mite (d1). Clinical sensitivity and specificity.

Table 4. Agreement between data obtained in two laboratories with UniCAP and Pharmacia CAP System, respectively. Tests were performed in 1995

		UniCAP		
		Positive	Negative	Total
Pharmacia	Positive	162	5	167
CAP System	Negative	3	104	107
Total		165	109	274

Positive: ≥ 0.35 kU_A/l.

tions for the common allergens cat dander, timothy pollen, and birch pollen. Sensitivity and specificity were also calculated for all patients/all allergens (total) included in the study. The comparison included 1298 tests in UniCAP and 1260 tests in Pharmacia CAP System on nine allergens. The systems show equivalent performance. Data also support earlier statements on the stability of IgE antibodies in serum at -20°C . No degradation could be observed after 8 years.

Fig. 5 (13) shows that the systems are equivalent not only in terms of discrimination of positive and negative in relation to clinical diagnosis but also in terms of measured values. The correlation ($r=0.96$) and the slope ($b=1.12$) of the linear regression analysis of measured values confirm the high degree of standardization of the assay systems as well as of the allergens used over the 8-year period.

In conclusion, the studies verify the diagnostic efficiency of the new generation of ImmunoCAP technology and the automated UniCAP immunoassay system. The data also confirm the reproducibility of the quantitative measurements of IgE antibodies over the 8-year interval and between the

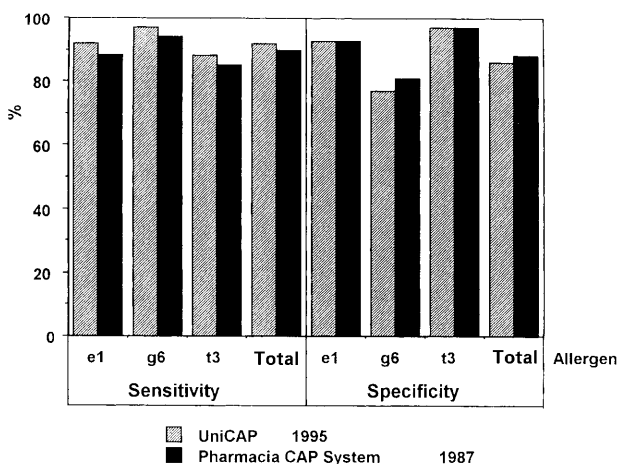


Fig. 4. Comparison of clinical sensitivity and specificity as calculated for Pharmacia CAP System (tested in 1987) and UniCAP (stored samples tested in 1995). Study 5.

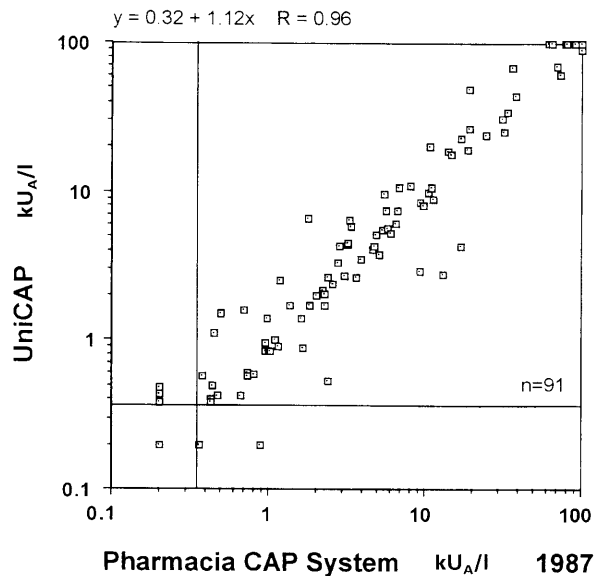


Fig. 5. Correlation of concentrations of allergen-specific IgE measured by Pharmacia CAP System in 1987 and by UniCAP in 1995 by retesting of same serum samples after storage at -20°C .

two assay systems, the UniCAP and the Pharmacia CAP System. The precise and reproducible measurements of specific antibodies allow further detailed studies on allergens as well as on the sensitization patterns of allergic patients.

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