

***In Vitro* Diagnostic Evaluation of Patients with Inhalant Allergies: Summary of Probability Outcomes Comparing Results of CLA- and CAP-Specific Immunoglobulin E Test Systems**

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ABSTRACT

For the diagnosis of allergy, presence of allergen-specific immunoglobulin E (IgE) usually is established either by allergen skin tests or by in vitro allergen-specific IgE measurements. However, in vitro assays of specific IgE often are modified as manufacturers improve allergens or change reagents to optimize test performance, affecting the diagnostic performance of in vitro allergen-specific IgE assays. This investigation compares the diagnostic outcomes of the Hitachi Chemical Diagnostics chemiluminescent assay (CLA) and Pharmacia, capsulated hydrophilic carrier polymer (CAP) in vitro allergen-specific IgE test methods in patients with inhalant allergy to a panel of selected allergens. Sera were obtained from 60 consecutive patients who had a clinical history suggesting inhalant allergy and were evaluated by allergen skin-prick test (SPT). Only patients with clinical findings of allergic asthma or rhinoconjunctivitis were included. Sera from patients with at least one positive SPT, which clinically correlated with the

case history, were used for specific IgE measurements. Sensitivity and specificity were defined as conditional probabilities describing performances of the CAP system and the CLA system in reference to a standard composed of a combination of allergen-specific symptoms and a positive SPT. A test concordance of 79% was found between the CLA and CAP test results with a correlation coefficient of 0.8. Allergen-specific IgE assay sensitivity of the CLA and CAP systems was similar and allergen dependent, ranging from 67 to 100%. Assay specificity ranged from 39 to 86% for the CLA system and from 36 to 81% for the CAP system. When comparing the specific IgE results with allergen SPTs, 75% ($\pm 3\%$) of CLA^{positive} patients had a positive SPT, and 92% ($\pm 4\%$) of CAP^{positive} patients had a positive SPT. Eighty-four percent ($\pm 4\%$) of CLA^{negative} patients had a negative SPT, whereas 69% ($\pm 5\%$) of CAP^{negative} patients had a negative SPT. The overall concordance between skin tests and in vitro tests was 76% for CLA and 67% for CAP. CLA and CAP score values showed good correlation and both tests may be useful when skin tests cannot be performed to identify subjects with IgE-mediated allergy. The CLA and CAP assays for allergen-specific IgE may be useful as part of an initial allergy evaluation because of the high negative predictive value of negative test results. For the majority of allergens the sensitivity was high. However, the specificity of both in vitro tests was low, indicating that positive in vitro test results should be evaluated carefully in conjunction with clinical symptoms and allergen-specific skin tests to determine the clinical relevance of the allergen sensitization. (Allergy and Asthma Proc 24:253–258, 2003)

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The chemiluminescent immunoassay system (CLA) from Hitachi Chemical Diagnostics (Mountain View, CA) is a partly automated semiquantitative allergen-specific immunoglobulin E (IgE) measuring system. Recently, the assay has undergone several changes with respect to its analytes and detection device. Attempts have been made to optimize the clinical correlation with skin tests and Pharmacia, capsulated hydrophilic carrier polymer (CAP) (Pharmacia Diagnostics, Piscataway, NJ) by using well-characterized allergenic extracts. Allergen source material and extracts have been characterized by protein content, immunochemistry, and major allergen content when possible. Based on such evaluations, the extracts used in the assay have been adjusted in amount and content of allergenic proteins to provide better clinical performance.¹ This study evaluates the performance of the CLA system with reference to a clinical standard established by an allergen-specific case history and physical examination followed by allergen skin-prick testing. All patient sera were analyzed for the presence of allergen-specific IgE to a panel of 10 inhalant allergens using the CLA system and compared with another *in vitro* allergen-specific IgE assay, the Pharmacia CAP system.

MATERIALS AND METHODS

Patient Serum Samples

Only sera from patients who showed at least one positive skin-prick test (SPT) and who were considered to be clinically allergic by case history and physical examination were selected for inclusion. Allergen-specific IgE tests were performed in duplicate, using two different commercial reference laboratories. *In vitro* allergen-specific IgE test results were scored and reviewed and blinded from the physician who did the patient evaluation until the study period was finished and the code was broken.

Allergens for the *In Vitro* Assays

Source materials used as allergens were supplied to the individual manufacturers by various raw material companies. The batch and extract composition are unique in each *in vitro* allergen-specific IgE assay and were optimized according to current internal standards of the *in vitro* test manufacturer. The following allergens were included: birch pollen, timothy grass pollen, mugwort pollen, horse epithelium/dander, dog epithelium/dander, cat epithelium, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Cladosporium*, and *Alternaria*.

Allergen-Specific Case History

Each patient was interviewed carefully by the same physician about severity, timing, and duration symptoms of asthma and rhinoconjunctivitis when exposed to each of the allergens included in this study. Results were recorded in a detailed patient symptom chart and later compared with individual allergen SPT results. Clinical

evaluation of inhalant allergy was assessed according to the international guidelines on asthma and rhinitis.^{2,3}

SPT

SPT was performed in duplicate with an ALK-Abello lancet (ALK-Abello, Horsholm, Denmark) with a 1-mm tip according to the European Academy of Allergy and Clinical Immunology recommendation.⁴ ALK-Abello supplied 10 histamine-equivalent prick (HEP) glycerinated allergen extracts for SPTs and patients were tested with birch pollen, timothy grass pollen, mugwort pollen, horse epithelium/dander, dog epithelium/dander, cat epithelium, *D. pteronyssinus*, *D. farinae*, *Cladosporium*, and *Alternaria* applied to the volar surface of the distal forearm, including a 10-mg/mL histamine dihydrochloride standard and the diluent as a negative control. Wheals were read after 10 minutes, wheal reactions were outlined with a fine marker and transferred to paper with transparent tape. A cutoff value of a 5-mm wheal response to the allergen was used. The mean value of duplicate tests was used. Skin testing and phlebotomy were performed after each patient had reviewed and signed an informed consent form approved by the local ethics committee.

CLA System

The hospital's reference laboratory performed the test according to manufacturer's instructions, and the comparison test was sent to a commercial laboratory for Pharmacia CAP. Briefly, 10 allergen-coated cellulose threads (CLA-Top, Hitachi Chemical Diagnostics) suspended in a plastic frame were incubated with serum overnight followed by removal of nonspecific serum proteins by buffer washing. Then, the frames were incubated for 4 hours with anti-IgE enzyme labeled with horseradish peroxidase and subsequently washed. After the addition of substrate (luminol), generated light was detected by a luminometer set at 425–426 nm. The specific IgE assay required 18 hours of total incubation time. Positive and negative control threads were included in each frame. The results were read in a luminometer and reported in both luminescence units and by a six-class (class 0, 0/1, 1, 2, 3, and 4) score system provided by the manufacturer. A test score result >0/1 was considered positive.

Pharmacia CAP

The fluorescent version of this assay was used, the methodology of which has been described in detail previously.⁵ The sera were sent for measurement to another commercial reference laboratory and scored using a seven-class system (class score, 0–6). Results were compared with a standard curve using upper and lower limits of 100 and 0.35 kU/L, respectively. A test result of >0.35 kU/L was considered positive. CAP classes are scored as follows: 0.35–0.70 kU/L, class 1; 0.71–3.5 kU/L, class 2; 3.6–7.5

kU/L, class 3; 7.6–17.5 kU/L, class 4; 17.6–50 kU/L, class 5; >50 kU/L, class 6.

Statistics and Data Handling

When calculating the outcome probabilities, the diagnosis of allergy was established by the presence of symptoms when exposed to the allergen in question in conjunction with a positive skin test response to the same allergen. Those subjects meeting these criteria were considered true positives. Patients with latent allergy having no symptoms and only showing a positive skin test were considered nonallergic, and the sensitivity and specificity of the *in vitro* methods were established based on the true positive cohort results. To calculate the 95% confidence interval as a measure of the precision, sensitivity, and specificity estimate, the standard error of the proportion was computed by

$$\sqrt{s(p(1-p)/n) \times 1.96},$$

where p is the proportion and n is the number of cases.

Efficiency expresses the likelihood of the test detecting all true positives and true negatives. Positive and negative predictive value is the likelihood that a positive and negative IgE test result reflects presence or absence of a positive skin test in relation to allergen-specific case history. Sensitivity and specificity pairs may vary considerably with the cutoff value established for a test. Initially, threshold values for each *in vitro* test were chosen according to the manufacturers' specifications. However, in this study, two sets of sensitivity/specificity values were calculated and evaluated based on 0.35 kU/L (CAP class 1) or 0.70 kU/L (CAP class 2 for positivity) and multiple allergen screening test CLA class score 0/1 or class score 1 cutoff for positivity. The quality of the sensitivity/specificity pairs depends on where it lies within its effective range, known as "quality indices" or κ -coefficients.⁶ The two indices $k(1, 0)$ and $k(0, 0)$ were calculated as

$$k(1, 0) = \frac{\text{sensitivity-proportion of positive tests}}{1 - \text{proportion of positive tests}}$$

$$k(0, 0) = \frac{\text{specificity-proportion of negative tests}}{1 - \text{proportion of negative tests}}$$

To compare further the concordance of the test results when evaluating the presence of allergen sensitization, the SPT threshold value was decreased to a 3-mm wheal size and consequently compared with either an allergen-specific *in vitro* IgE class score of >0/1 or >1.

RESULTS

Patient Characteristics

Sixty adult patients gave informed consent to participate in the study. Sixty patients had a >3-mm skin test wheal response to one of the aforementioned allergens and the frequency decreased to 49 when applying a 5-mm cutoff

TABLE I

Overall Test Responses in 60 Study Patients

Diagnostic Method	Positive	Negative	Total
Physician diagnosis of allergy	41	19	60
Allergen skin test	49 (60)	36 (0)	60
CAP	35	25	60
CLA	32	28	60

This table reports the frequency numbers in 60 patients with at least one positive allergen skin test and evaluated by allergen-specific case history, allergen skin test (values in parentheses are the frequency when using a 3-mm wheal size cutoff value), CAP, and CLA IgE tests. The physician diagnosis of allergy was based on a skin test response of a 5-mm wheal reaction and presence of allergen-specific symptoms when exposed to the appropriate allergen.

(Table I). The physician diagnosed 41 patients as allergic combining the skin test data with the allergen-specific case history and physical examination. The two allergen-specific IgE tests reported a similar number of positives; however, the frequencies were lower compared with the skin test (Table I). The majority of the patients (59%) reported only symptoms of rhinoconjunctivitis when exposed to the allergen in question and the remainder of patients noticed symptoms of allergic rhinitis and asthma. Dust mites (33%), cats (27%), and grass (11%) were the allergens most frequently associated with asthma symptoms. In allergic patients, the average allergen and histamine standard induced wheal size was 4.0 and 4.6 mm, respectively. No nonspecific skin test reactions were recorded. Most patients were sensitized to pollens (58%) and dander (53%). None of the patients revealed positive skin tests to molds. The outcome probabilities of the mold data cannot be evaluated because of the lack of true positives. However, the mold data were used in computing the total probability values.

Outcome Probabilities

Table II shows the outcome probabilities for each allergen-specific IgE assay based on a reference set by allergen-specific case history and SPT results using a 5-mm cutoff for positivity. The sensitivity of the CLA test ranged from 0.67 to 1.00, horse allergen yielding the lowest sensitivity and grass, mugwort, and dog allergens yielding the highest sensitivity of 1.00. Specificity ranged from 0.39 (birch) to 0.86 (horse). The percentage of CLA^{positive} patients with a positive SPT was 75% with an SE of 3%. The percentage of CAP^{positive} patients with a positive SPT was 92% with an SE of 4%, this difference being statistically significant ($p < 0.05$). The percentage of CLA^{negative} patients with a negative SPT was 84% (SE, 4%), and 69% (SE, 5%) CAP^{negative} patients had a negative skin-prick test; this difference was statistically significant ($p < 0.05$). A greater number of clinically false positive CAP results were ob-

TABLE II

Summary of Outcome Probabilities

Test	Allergen	<i>p</i>	SE	SP	EFF	PVP	PVN
CAP	Birch	14	1.00	0.48	0.60	0.37	1.00
CLA	Birch	14	0.93	0.39	0.52	0.32	0.95
CAP	Grass	18	1.00	0.36	0.55	0.40	1.00
CLA	Grass	18	1.00	0.50	0.65	0.46	1.00
CAP	Mugwort	3	1.00	0.81	0.82	0.21	1.00
CLA	Mugwort	3	1.00	0.81	0.82	0.21	1.00
CAP	Cat	23	1.00	0.62	0.77	0.62	1.00
CLA	Cat	23	0.74	0.65	0.68	0.57	0.80
CAP	<i>D. pteronyssinus</i>	10	1.00	0.42	0.52	0.26	1.00
CLA	<i>D. pteronyssinus</i>	10	0.90	0.78	0.80	0.45	0.98
CAP	<i>D. farinae</i>	6	1.00	0.48	0.53	0.18	1.00
CLA	<i>D. farinae</i>	6	0.83	0.74	0.75	0.26	0.98
CAP	Dog	6	0.67	0.57	0.58	0.15	0.94
CLA	Dog	6	1.00	0.54	0.58	0.19	1.00
CAP	Horse	3	0.67	0.56	0.57	0.07	0.97
CLA	Horse	3	0.67	0.86	0.85	0.20	0.98
CAP	All	83	0.96	0.60	0.65	0.28	0.99
CLA	All	83	0.88	0.73	0.75	0.34	0.97

Sensitivity (SE), specificity (SP), efficiency (EFF), predictive value positive (PVP), and predictive value negative (PVN) by recording true negative, false negative, false positive, true positive results based on a clinical diagnosis and SPT results using a positive cutoff value of a 5-mm wheal diameter. The number of diseased (*p*) subjects is also reported. The IgE tests were evaluated based on a cutoff value of class score 1 or greater.

served with grass, birch, and dust-mite allergens. CLA showed low specificity for birch and grass allergens.

Generally, good concordance (test efficiency >87%) was found between the CAP and the CLA for all pollen allergens. In contrast, major discordance between the CAP and the CLA was noted with nonpollen allergens such as dust mites and dander allergens, which showed test efficiencies of 65–72% for mites and 72–82% for dander allergens. The overall *in vitro* test concordance was 79%. When comparing the *in vitro* tests to skin tests, it was found that the skin test concordances with CLA and CAP were 76% and 67%, respectively.

A positive CAP and negative CLA were observed in 109 of the test results among which 39 had a positive SPT (cutoff ≥ 3 mm). A false CLA^{negative} test was most often related to dander allergens. In 12 instances, mite allergen results were associated with CLA false negatives. For 70 (12%) positive CAP test results, skin test or clinical symptoms did not confirm the positive CAP result. This occurred most frequently with the mite allergens. A negative CAP with a positive CLA result was noted in 17 instances, in which the skin tests were negative in 13 cases. The negative predictive value of both *in vitro* tests was excellent. In contrast, a positive test result was not reliable, having a positive predictive value <50% for all allergens.

When assessing the specificity and sensitivity data pairs in Table II, it is possible that the *in vitro* assay cutoff value was set too low, resulting in false positives. Therefore, the

data were reevaluated by increasing the *in vitro* cutoff value for positivity to a threshold value of greater than class score 1. Using this criteria, the specificity increased ~5–10% (data not shown). An exception was dog allergen, which showed an increase of 20% in specificity with no significant change in sensitivity. Additional increases in the threshold value using a cutoff value of greater than an IgE class score of 2 resulted in an unacceptable decline (<50%) in assay sensitivity. The establishment of the assay cutoff for positivity was confirmed by calculating the *k* indices (data not shown). Only the dog allergen *k* indices indicated that the assay cutoff value should be set at a value > 0.35 kU/L. Both *in vitro* tests showed comparable quality indices having statistical significance ($p < 0.05$).

Table III reports the concordance in test score results. The correlation coefficient was 0.8 ($p < 0.05$). A class discrepancy of one score value occurred typically in the results of lower class scores. However, CAP class 2 results were associated with negative CLA test scores in 50 cases, related to detection of allergen-specific IgE to dust-mite and dander allergens.

DISCUSSION

A number of studies^{7–9} have reported good correlation between *in vitro*-specific IgE tests and skin tests and allergen bronchial challenges. However, the CLA test has not been compared with the newer IgE assays. Recently,

TABLE III

In Vitro Score Values: Intersystem Correlations

CLA Score	CAP Score					
	0	1	2	3	4	5
0	278	40	50	15	1	3
1	12	5	20	7	2	
2	4	4	18	7	3	
3	1	1	12	11		
4			3	56	28	14
						5

The concordance of individual CLA and CAP class score values.

this assay has been optimized by modifying analytes and reagents necessitating reevaluation of the CLA test performance.

Generally, the degree of test score correlation in the measurement of allergen-specific IgE was acceptable considering that the allergen extract composition differs between manufacturers. The correlation between the assay systems considered in this study showed a concordance of 79% between CAP and CLA test results. When discordance was noted, it was related to dust-mite and dog allergens. Increasing the dog allergen class score cutoff by one class, this discrepancy was minimized. A previous study comparing outcome probabilities of the Pharmacia CAP and HYTEC enzyme IgE immunoassay reported the lowest specificity (0.66) for dog allergen when using the same 10 inhalant allergens as in this study, suggesting that in some IgE assays, dog allergens may require a higher cutoff value for allergen-specific IgE determination when compared with other allergens for good correlation with clinical symptoms.¹⁰ We believe this study further substantiates this observation. A similar improvement in allergen-specific IgE assay performance was not obtained when changing the positive cutoff for dust mites. Both *in vitro* systems reported a greater number of positive responses to dust-mite allergen than positive responses to SPT, the CAP system recording the greater number of dust mite-positive responses. This finding is in accordance with a recent study comparing CAP with another specific IgE assay system.¹⁰ Possible explanations for the observed discordance between the assay performance for determination of allergen-specific IgE toward dust-mite allergens may be (1) a variability in the composition of mite allergen extracts on the solid phases of the *in vitro* test; (2) a lower sensitivity of the mite allergen used for skin-prick testing, or difficulties in assessing the correct clinical diagnosis by case history and physical examination. It is not possible to determine from this study whether the high number of clinically false positives recorded by CAP reflect latent dust-mite allergy or nonspecific analytical assay performance.

Generally, the reported outcome probabilities were typical for a study of this size,^{10–12} although this investigation also seemed to indicate a lower specificity of the allergen-

specific IgE assays than usually reported. Possible explanations for the positive *in vitro* results for allergen-specific IgE despite a negative skin test may be the use of an SPT device with a 1-mm tip and applying a conservative threshold value of 5 mm for skin test positivity. However, lack of skin-prick sensitivity is not very likely because biological standardized extracts with a skin test potency value of 10 HEP were used, providing an allergen exposure generally appropriate for diagnosing clinical allergy. Because of the study design and the lack of a “gold standard” reference test in allergy diagnostic testing, the diagnostic precision of either the CLA or the CAP systems with respect to clinical outcomes cannot be determined fully. Ultimately, the present investigation did not find a significant difference between the outcome probabilities of CAP and CLA allergen-specific IgE assessments.

The CLA assay for allergen-specific IgE offers reliable results concordant with skin test and the Pharmacia CAP assay. Potential advantages of *in vitro* assays include diagnosing IgE antibodies to inhalant allergens in selected patients in whom skin tests cannot be performed. The CLA and CAP assays for allergen-specific IgE may be useful as a part of an initial allergy evaluation because of high negative predictive value of negative test results. This means that when patients test negative by CLA and CAP *in vitro* allergen-specific IgE assays, inhalant allergy to the allergens tested is unlikely. If patients are history and skin test positive (5-mm wheal response) but have negative *in vitro* tests, the conclusion is that this probably reflects a difference in allergen composition and pertains only to a few allergens, *i.e.*, dog and horse extracts. For the majority of allergens, the sensitivity was high. However, the specificity of both *in vitro* tests was low, indicating that positive *in vitro* test results should be evaluated carefully in conjunction with clinical symptoms and allergen-specific skin tests to determine the clinical relevance of the allergen sensitization.

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