

Comparison of CLA with BPT, SPT, and RAST in children with asthma

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Bronchial provocation tests (BPT) with allergen extracts were performed in 82 children with asthma, and the results were compared with the chemiluminescent assay (CLA), the radio-allergo-sorbent-test (RAST) and skin prick test (SPT). It was found that CLA matched BPT with a concordance of 87% (77–93%), RAST with a concordance of 74% (64–83%) and SPT with 80% (70–88%). Most often, CLA and RAST gave similar results, although their classes did not match precisely.

Key words: CLA; BPT; RAST; SPT.

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CLA (chemiluminescent assay, (Dome/Hollister-Stier) is a new semiquantitative method which in one assay determines specific IgE for 35 allergens (1, 2). The result can be obtained within 24 h, by analysis of a single venous blood sample.

We wanted to compare this new method of CLA with RAST and skin prick test (SPT) in children with extrinsic asthma, using bronchial provocation test (BPT) as the standard against which the others were judged.

Methods

The patient population was highly selected because all BPT were performed as a part of the investigations in other studies conducted in the clinic.

The patients were selected by a case history and clinical symptoms of extrinsic asthma and/or a positive SPT/RAST. Blood samples for CLA and RAST were collected prior to a new SPT. Finally, the BPT was performed with an allergen selected on the basis of the case history.

The study was approved by the local ethics committee, and all patients and their parents gave their informed consent.

CLA method. The CLA plastic test chamber has 38 cellulose threads suspended at right angles to its long axis. Each of 35 of these threads is coated with a specific antigen for which it tests. One thread is coated with IgG to human IgE to enable determination of total IgE, while the remaining 2 threads act as positive and negative controls.

An allergy profile is run by filling a CLA plastic test chamber with patient serum. As the serum incubates, IgE binds to the allergen-coated threads and to the total IgE thread. After the serum incubation, the test chamber is flushed with buffer to remove unbound serum components and is then filled with an enzyme-labeled anti-IgE. The enzyme-labeled antibody couples with the IgE bound to the threads. After a final buffer wash, the test chamber is filled with the photoreagent mixture and overlaid on Polaroid® film. Light emitted from the reaction of the labeled antibody with the photoreagent mixture exposes the film. On the resulting photograph, a white band corresponds to each positive reaction. The transmittance of each band is measured using a densitometer. The degree of transmittance is directly proportional to the allergen-specific IgE and the total IgE concentration in the patient's serum. The negative control thread measures the nonspecific binding associated with each patient sample. The patient's specific IgE and total IgE level minus the nonspecific binding is automatically calculated by the densitometer to determine the classification of each reaction. The allergen-specific IgE concentrations are classified as 0 (net voltage <0.18 v), 1 (0.67–0.18 v), 2 (1.9–0.67 v), 3 (3.5–1.9 v), 4 (>3.5 v) (2).

A class 2 or more in this study was considered positive.

RAST was performed as Phadebas RAST® (Pharmacia) and the results expressed in PRU/ml or in RAST classes 1–4.

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Table 1. Number (%) of tests matching BPT results

		BPT		
		Negative n=27	Positive n=55	
CLA	n	22	49	71
	%	81	89	87
	%	(62-94)	(78-96)	(77-93)
RAST	n	17	44	61
	%	63	80	74
	%	(42-80)	(67-90)	(64-83)
SPT	n	20	46	66
	%	74	84	80
	%	(54-89)	(71-92)	(70-88)

Values in parentheses are the 95% confidence limits.

Table 2. Comparison of RAST and CLA classes

		CLA					
		0	1	2	3	4	
RAST	0	5	0	0	0	0	5
	1	13	0	5	0	5	23
	2	5	4	4	3	4	20
	3	1	0	0	1	17	19
	4	0	0	0	0	15	15

Note: Equivocal taken as Class 0.

nique was carried out by using nebulized aqueous allergen extracts in increasing concentrations. Patients who reacted with a fall in FEV₁ after inhalation of saline were excluded from the study. Patients with suspected allergy to danders or moulds were challenged with allergen extracts obtained from ALK, Copenhagen, in concentrations from 200 to 100 000 SQ-U/ml. The remaining patients, suspected allergic to house dust mite, were challenged with extracts from Dome Hollister-Stier in concentrations 20, 40, 100, 200 and 400 allergy units by RAST(AUR)/ml. Challenges were stopped if the FEV₁ was 80% or less of the prechallenge value. The results were classed as positive or negative.

Statistics

Two by two contingency tables were established to compare the concordance between each test with the

Skin prick test was performed on the volar surface of the patients' forearms with Phazet® (Pharmacia), except for 5 who were tested with ALK extracts. The wheals were read after 15 min and compared with a positive (histamine) reference and a negative reference (diluent). The contours of the wheal were circled with a felt-tipped pen and transferred to paper with a strip of tape. The wheal areas were measured with a digitizer in mm². A reaction ≥ the histamine reference was considered positive.

Bronchial provocation test. Forced expiratory volume in 1 sec (FEV₁) was measured with a Vitalograph® and chest auscultation was performed. The tech-

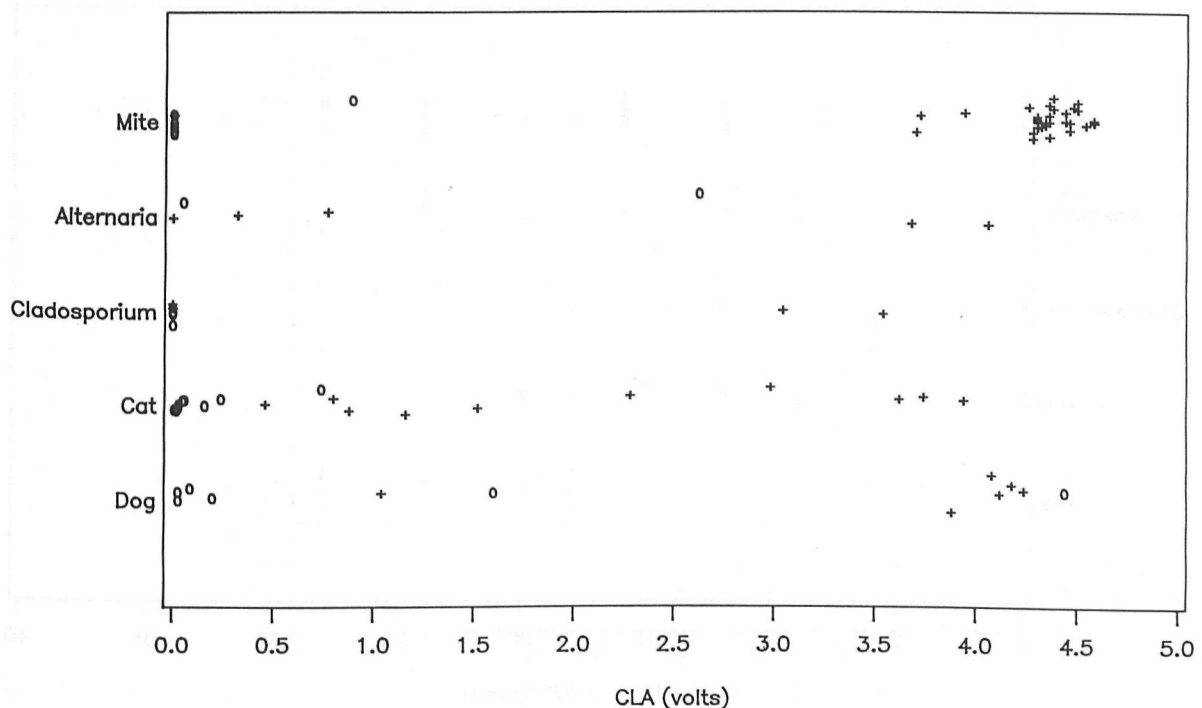


Fig. 1. CLA response with negative (O) and positive (+) response for each of the tested allergens.

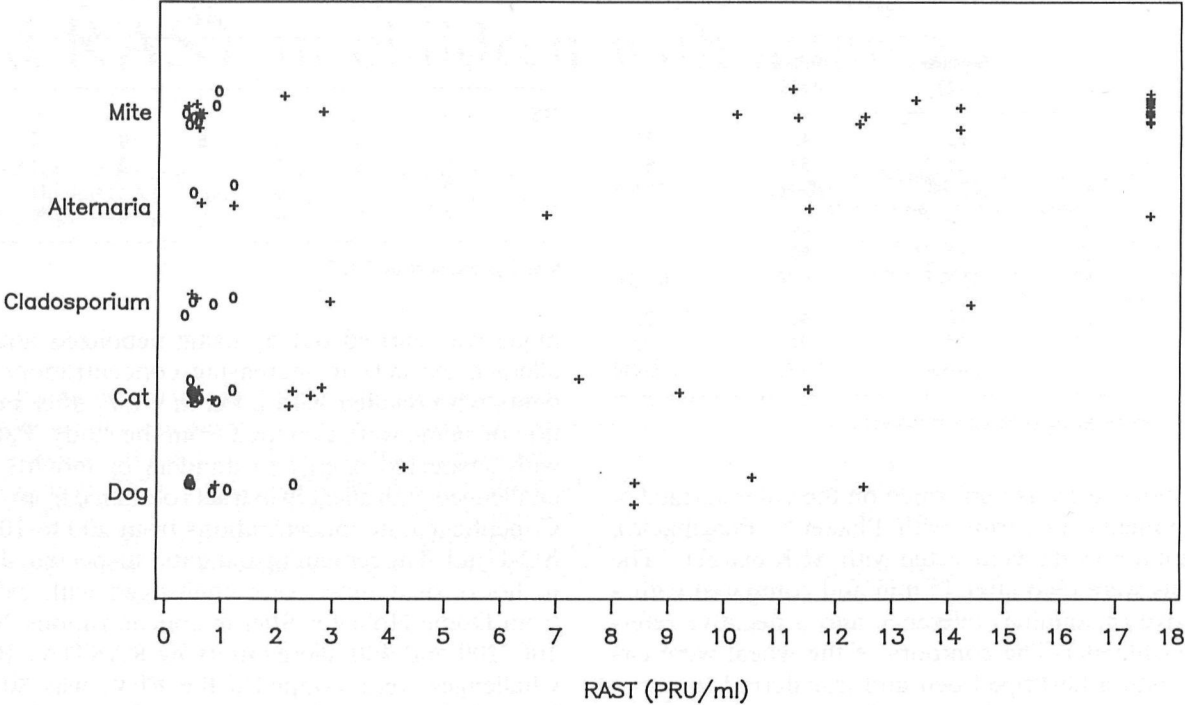


Fig. 2. RAST response with negative (O) and positive (+) BPT response for each of the tested allergens.

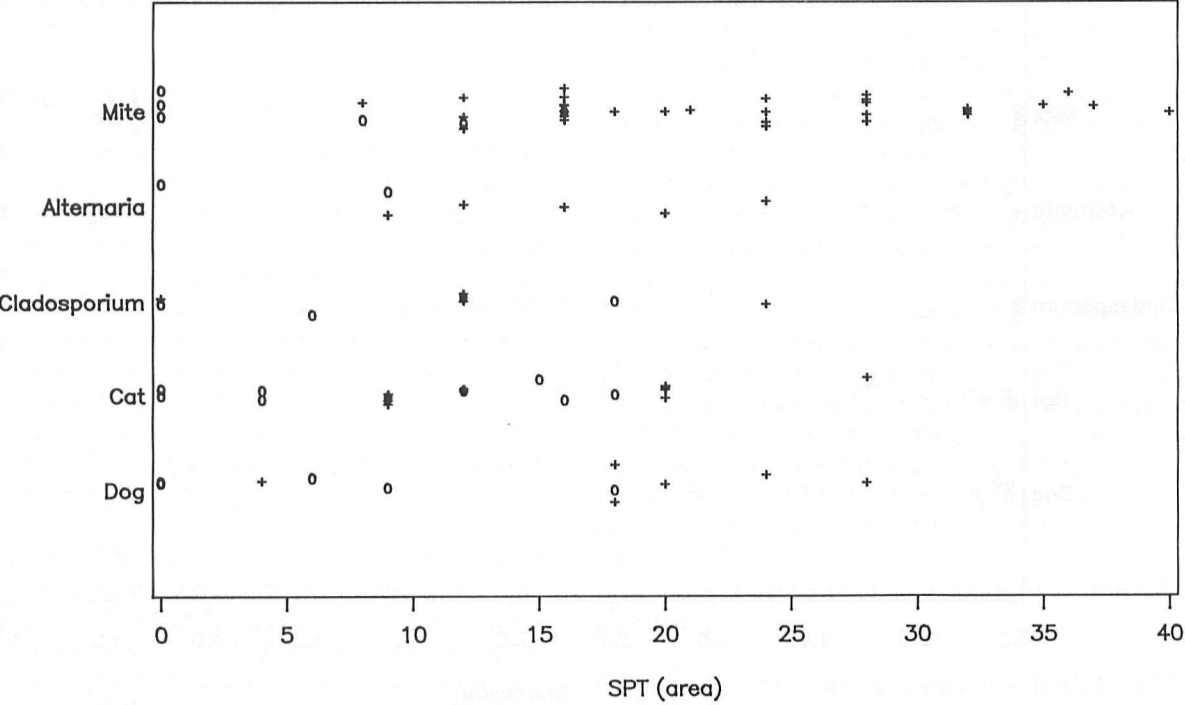


Fig. 3. Skin prick test (SPT) response with negative (O) and positive (+) BPT response for each of the tested allergens.

results expressed as positive and negative. The relevance of the differences was estimated on the basis of 95% confidence limits. As categorizing results as positive or negative, though useful, omits much information, the recorded data, uncategorised, were considered.

A way to display results where continuous data define a known binary outcome is a receiver operating characteristic (ROC) curve (4). Using different cutoffs to define positive and negative results gives different sensitivity and specificity, one improving at the expense of the other. This can be shown on a 2-dimensional plot. The closer the curve is to the top left hand side the better the test.

Results

A total of 82 patients aged between 9 and 15 years were selected and investigated with BPT with either dog dander (12 patients), cat dander (19), Cladosporium (8), Alternaria (7), or house dust mite (36). Nine patients did not respond to any of the tests, though it was not possible to know whether they were genuinely non-allergic.

There were insufficient data to enable detailed investigation of the separate allergens. However, the percentage of tests all positive or negative were sim-

ilar for Cladosporium and Alternaria (25% and 29% respectively), dog and cat agreed more frequently (58% and 53%) and mite most frequently (64%). As seen in Table 1 CLA matched BPT in 87% (77-93) of the tests, where RAST matched BPT in 74% (64-83) and SPT in 80% (70-88).

The CLA, RAST, and SPT data are shown in Figs. 1 to 3. It is seen in the figures, that though CLA had a few negative BPT results at high values, it had very few positive BPT at low values, whereas RAST had no negative values until quite low observations, but a great many positive BPT results at low values. CLA and RAST did not match exactly, and the classes were not necessarily equivalent (Table 2).

Using different cutoffs to define positive and negative results gave different sensitivity and specificity. This can be shown in a ROC curve (Fig. 4). Fig. 4 shows that CLA had the best overall performance though it was weakest at the tails.

Discussion

In earlier studies, the CLA test has been compared with traditional RAST, SPT and symptoms of allergic diseases and, usually, good agreement has been found (3, 5, 6, 7, 8).

To our knowledge, it is the first time the CLA test

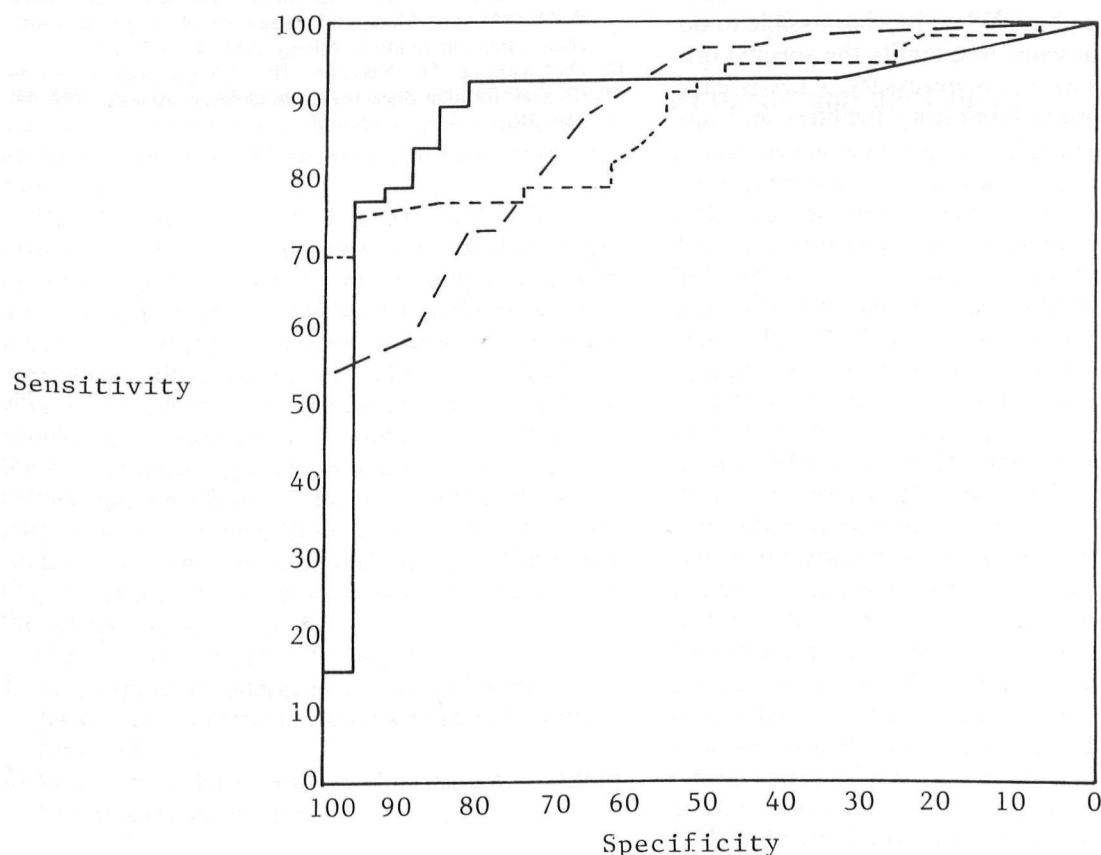


Fig. 4. Relationship between sensitivity and specificity in a ROC curve with BPT as standard. — CLA, ---- RAST, --- SPT.

has been compared with SPT, RAST, and BPT. The results obtained were that in these selected asthmatic children, CLA matched BPT more closely than RAST and to the same degree as SPT. The patients were selected by case history and/or positive SPT/RAST to a specific allergen. A selection on the basis of positive test results gives a risk for a high number of false positives. If patients had a positive SPT and negative RAST, or vice versa, and the patients were selected on the basis of the positive, then SPT and RAST were almost bound to show poorer sensitivity and/or specificity than CLA, since the results of CLA had no importance in the selection of patients.

In a controlled study of the sensitivity and specificity of different tests compared with BPT, Stafanger et al. concluded (9): "If BPT is not used, the various *in vivo* and *in vitro* tests can be used singly, or in combinations, to give approximate predictions of how the patient will react to inhalation of the allergen. The reliability of this approximation depends upon proper selection of allergen concentration, proper evaluation of the relationship between reactivity in the test or tests, and level of reactivity in BPT, i.e. "the true diagnosis".

In this study we have shown that CLA is comparable with RAST and SPT. Case history in combination with SPT is still first choice in the investigation of the allergic child. When it is impossible to do a SPT, or if it is of value to quantify the specific IgE response, an *in vitro* test is necessary. CLA is easy to do without special laboratory facilities and ap-

pears useful in situations where multiple allergen testing is required.

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