

Comparative evaluation of RAST and MAST-CLA for six allergens for the diagnosis of inhalant allergic disease in 232 patients

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Summary

The aim of this study was to compare a recent multiple allergosorbent chemiluminescent assay (MAST-CLA) with the RAST for the diagnosis of inhalant allergic disease in 232 patients with rhinitis and/or bronchial asthma. As judged by concordance of clinical history, skin prick tests to a range of six allergens common to our geographic area, and by nasal provocation tests, 70 patients were non-allergic and 162 allergic: 70 to grasses, 46 to mites, four to mugwort, eight to pellitory, and 34 were sensitive to several allergens. In our patient sample that, among other things, comprises subjects with fairly rare monoallergies, MAST-CLA testing gave results which closely corresponded to positive RAST for the allergens studied, and demonstrated a close correlation with the diagnosis of inhalant-specific allergy. Our results showed that, for overall allergens, MAST-CLA was lightly less sensitive and more specific than RAST (the two *in vitro* tests gave an identical overall efficiency).

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Introduction

The diagnosis of inhalant allergy in clinical practice is based on case history, skin tests using a combination of allergens and/or on the demonstration of serum allergen-specific IgE, and is confirmed in doubtful cases by performing provocation tests with inhalants. Serum specific IgE are usually detected by the RAST, a radio-allergo-sorbent test, that until recently has been the only *in vitro* method available to biologists and clinicians [1,2]. The RAST has been widely used by allergologists as an alternative or as an adjunct to skin tests for diagnostic purpose. For the detection of specific IgE to inhalant allergens, the RAST is reproducible, specific and sensitive when compared with clinical history, skin test, and provocation test [3]. More recently developed methods for the measurement of specific IgE are radioimmunoassays, immunoenzymatic or immunofluorimetric assays [4-7] and the multiple allergosorbent chemiluminescent

assay (MAST-CLA), which avoids the use of gamma-isotopes used in other tests. It is a multiple allergosorbent chemiluminescent assay in which allergens are bound to 35 threads; these are incubated first with the patient's serum, then with an enzyme-labelled anti-IgE and finally with a photoreagent mixture [8]. The chemiluminescence which occurs where specific IgE is bound to the thread is measured by exposing a Polaroid® film and is then semiquantitatively assessed by means of a densitometer. The technical data concerning this assay are described in detail elsewhere [8].

In recent studies of Scolozzi *et al.* [9] and of Finnerty *et al.* [10] the MAST-CLA has been compared with RAST and skin prick tests (SPT) for the diagnosis of inhalant allergic disease. These papers showed that MAST-CLA, RAST and SPT gave similar results, also as far as the diagnostic sensitivity, specificity and efficiency are concerned, but that no technique was as sensitive as skin tests for allergen-specific diagnosis [9].

The aim of this study was to verify the results of our previous report by comparative evaluation between RAST and MAST-CLA (applied to six aeroallergens) in a

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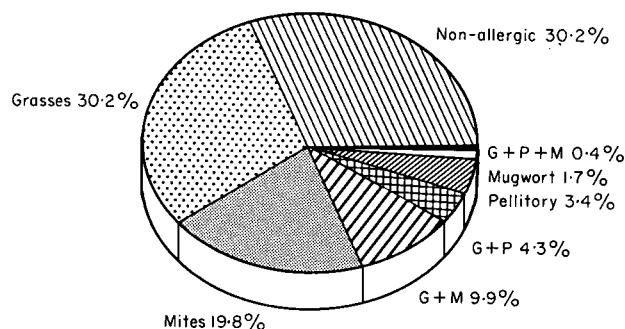


Fig. 1. Allergic or non-allergic patient sample. G, grasses. P, pellitory. M, Mites.

number of 232 adult patients suffering from rhinitis and/or bronchial asthma, identified by concordance of clinical history, SPT and nasal provocation test (NPT). In other words, we have compared RAST with MAST 'face to face' in a patient sample in which the allergen specific diagnosis was assessed by taking together the history, positive SPT and NPT.

Materials and methods

Patients

A total of 232 patients with rhinitis and/or bronchial asthma were entered into the study. The mean age of the subjects was 30.6 (range: 11–69) years, with a male to female ratio of 0.8.

As judged by history, SPT to a range of six aeroallergens common to our geographic area, and NPT, 162 patients (mean age: 29.7 years; range: 11–69; M/F ratio: 0.93) were allergic, and 70 (mean age: 32.9 years; range: 16–64; M/F ratio: 0.55) non-allergic. For the aim of this study allergic patients were defined as having symptoms of rhinitis and/or asthma which either showed seasonal variation or were provoked by household dust, in agreement with positive SPT and NPT. Of our patients, 70 were allergic to grasses, 46 to mites, four to mugwort, eight to pellitory, and 34 were sensitive to several allergens (Fig. 1).

Venous blood samples were taken from all the 232 patients and the separated serum stored for less than 2 months at -20°C and assayed the same week by RAST and MAST-CLA methods.

Skin prick tests

SPT were performed in duplicate on the volar side of one forearm according to the recommendations of the Subcommittee on Skin Tests of the European Academy of Allergy and Clinical Immunology. The grading sys-

tem was based on the relation between the reactions induced by allergen and the histamine reference [11]. The allergens used together with histamine dihydrochloride (1 mg/ml) and glycerol diluent controls were provided by Bayropharm, Dome/Hollister-Stier Inc., at concentrations of 10 000 UP/ml (*Phleum pratense*, *Dactylis glomerata*, *Artemisia vulgaris*, *Parietaria officinalis*), and of 5000 PNU/ml (*Dermatophagoides pteronyssinus* and *D. farinae*).

Nasal provocation test

NPT was performed in order to confirm the diagnosis of inhalant-specific allergic disease according to a technique described in detail elsewhere [12]. The allergen extracts were provided by Pharmacia (Uppsala/ALK, Sweden). Briefly, 0.2 ml of the 'ALK Diluent' followed by 10-fold increasing concentrations of the allergen extracts were nebulized in both nostrils, every 15 min, by means of a vaporizator. The test was stopped and considered positive when the following two criteria were met; (a) at least 0.5 ml of nasal secretion; and (b) at least five sneezes.

Serum specific IgE

The following tests were performed: RAST for determination of specific IgE antibodies against grass pollen, mugwort, pellitory, and house dust mites. The tests were carried out with reagents provided by Pharmacia Diagnostics, Sweden; MAST-CLA using the following allergens: three trees (silver birch, hazel, olive), six grasses (Bermuda, Cocksfoot, Kentucky blue, perennial and cultivated rye, timothy), two weeds (mugwort, pellitory), three moulds (*Alternaria*, *Aspergillus*, *Penicillium*), house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*) and cat dander (Bayropharm, Dome/Hollister-Stier Inc.).

The tests were performed according to the manufacturer's instructions and interpretation was made in four positive classes. It was assumed that scores 0, 1 represented a negative response, and scores 2, 3, 4 a positive response.

Statistical analysis

Correlations between RAST and MAST-CLA were determined by linear regression analysis (Pearson coefficient). The diagnostic sensitivity, specificity, and efficiency were calculated for each group of allergens by standard methods [13]. In this study sensitivity was the percentage of allergic patients identified by concordance of history, SPT and NPT who actually have the symptoms caused by the specific allergen (i.e. the number

Table 1. Relationship between diagnosis of allergy to grass pollen, mugwort, pellitory and dust mites, and positive RAST and MAST-CLA

Allergen	Allergen-specific diagnosis	Positive RAST (%)	Positive MAST-CLA (%)	<i>r</i> RAST vs MAST
Grasses	104	94.2	85.5	0.93
Mugwort	8	100	75	0.86
Pellitory	19	84.2	100	0.91
Dust mites	64	68.7	70	0.98

r = Pearson correlation coefficient.

positive in the test divided by the number with the symptoms caused by the specific allergen), and the specificity was the percentage of patients with negative RAST or CLA test reactions to a particular allergen who were not allergic to that allergen on the basis of history, SPT and NPT, divided by the total number who were actually unaffected by that allergen. The efficiency of the test was the percentage of the patients correctly identified as having or not having the allergic symptoms caused by the specific allergen by the test, of all the patients studied.

It is clear that there is some difficulty in applying these definitions to allergy. In fact a clinical diagnosis of allergy to a particular allergen can be made only by taking together the case history, positive skin tests, and/or positive determination of serum specific IgE antibodies and/or positive provocation tests. In this paper the sensitivity and the specificity of RAST and MAST-CLA were considered as relating to SPT and NPT, the combination of which is considered the most important part of clinical diagnosis of specific allergy (to a certain allergen) [11,14].

Results

Comparison with diagnosis of inhalant-specific allergic disease (positive clinical history, SPT and NPT).

The relationship between the results obtained with the different tests and the diagnosis of allergy is shown in Table 1 (in the non-allergic subjects there were absolutely no positive RAST and MAST-CLA results).

A good agreement was found between diagnosis of allergy to grasses, or pellitory and the two tests (for pellitory MAST-CLA was positive in 100%). RAST gave a totality of positive tests with mugwort, but lower frequencies were obtained with MAST-CLA (75%). For patients with house dust-associated symptoms a smaller proportion had a positive RAST or MAST-CLA. The

correlation coefficient for RAST with MAST-CLA was 0.89. Individual *r* values were 0.93 for grasses, mugwort 0.86, pellitory 0.91, and dust mites 0.98.

Clinical correlation

The diagnostic sensitivity, specificity, and efficiency of MAST-CLA and RAST for grass pollen, mugwort, pellitory and mites are shown in Table 2 and Fig. 2. The concordance between MAST-CLA and RAST was close for all allergens studied.

Discussion

The MAST-CLA has recently been compared with RAST and with skin tests [9,10] for the diagnosis of allergy to inhalants. Usually there was good agreement between the results of MAST-CLA, RAST, and skin tests.

In a representative study, Finnerty, Summerell and Holgate [10] found strong agreement between conventionally positive SPT and MAST-CLA results for house-dust mites and cat dander, while for the other allergens studied, agreement was greatest between MAST-CLA and strongly positive skin tests. Their results were in accordance with a previous paper comparing RAST and SPT [15].

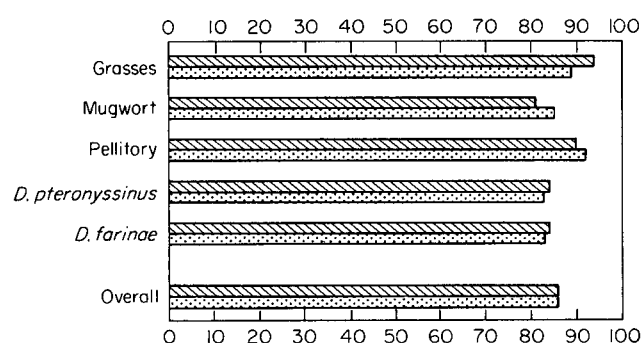
Strong correlation between positive MAST and positive RAST has been reported with agreement of 80% to 90% for common allergens [9,15-17], even if a correct comparison should require the use of reagents with the same allergens. Also in this study that, among other things, comprises patients with fairly rare monoallergies, close correlation was found between RAST and MAST, although the test allergens were not the same. In fact, these derived from different sources and at different strengths. The strong correlation between the tests might be due to the large number of 'very important' epitopes shared among the allergens.

MAST-CLA showed a sensitivity of 81.8%, a specificity of 89.5%, and an efficiency of 86.4%, compared with 85.8% 87.5%, and 86.2%, respectively, for the RAST analysis. When we considered the question of sensitivity *versus* specificity for the different test methods, overall agreement was good. The most striking discrepancies were seen in the tests for allergy to mugwort and pellitory (Table 2). RAST gave 100% of positive results for mugwort in those patients who were shown to be allergic to mugwort but it also gave more positive results in those who were supposedly non-allergic. The high sensitivity of the RAST thus appeared to be accompanied by some reduction in specificity. All patients who reported pellitory-associated symptoms had a positive test with MAST-CLA, but only 83% had a positive RAST to

Table 2. The diagnostic sensitivity, specificity and efficiency of MAST-CLA and RAST for grass pollen, mugwort, pellitory and mites according to clinical history, SPT and NPT results

Allergen	Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)		Efficiency	
	RAST	MAST-CLA	RAST	MAST-CLA	RAST	MAST-CLA	RAST	MAST-CLA	RAST	MAST-CLA
Grass	96.2	88.7	87.3	88.6	91.1	91.3	94.5	85.3	92.4	88.7
Mugwort	100	80	78.5	85.7	35.7	40	100	97	80.8	85
Pellitory	83	100	91.7	90.4	71.4	72	95.7	100	90	92.3
<i>D. pteronyssinus</i>	77.4	70.9	88.4	91.5	81.3	84.6	85.7	82.8	84	83.4
<i>D. farinae</i>	72.5	69.3	91.5	91.5	84.9	84.3	83.6	82	84	82.8
Overall	85.8	81.8	87.5	89.5	72.9	74.4	91.9	89.4	86.2	86.4

PPV, positive predictive value. NPV, negative predictive value.

**Fig. 2.** RAST versus MAST: efficiency for the diagnosis of allergy. ■, RAST. ▨, MAST.

pellitory. In this case, however, the high sensitivity was not accompanied by a striking lower specificity. Further, there was no significant difference between MAST-CLA and RAST in the ability to predict a diagnosis of allergy to tested allergens. But, for overall allergens, a positive MAST-CLA result had a 74.4% predictive value, corresponding to a 72.9% positive predictive value of RAST in this situation.

In conclusion, MAST-CLA testing in our allergic population gave results closely corresponding to positive RAST for six common allergens, and demonstrated a close correlation with the diagnosis of inhalant-specific allergy. Our results have shown that, for overall allergens, MAST-CLA was slightly less sensitive and more specific than RAST (the two *in vitro* tests gave an identical overall efficiency).

MAST-CLA is another non-radioactive immunoassay that can be an alternative to RIA methods. Therefore it can be performed in the physician's office as well as in laboratories that obviate the need for radioactive reagents. Further, when several allergens are being

detected, as a rule, MAST-CLA is cheaper than RAST because it permits the simultaneous semiquantitative measurement of specific IgE directed against 35 different allergens.

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