

Relationship between skin-prick tests, the multiple allergosorbent test and symptoms of allergic disease

J.P.FINNERTY, S.SUMMERELL and S.T.HOLGATE

Immunopharmacology Group, Southampton General Hospital, Southampton, U.K.

Summary

In 47 atopic subjects, skin-prick testing to 10 common allergens was performed, and specific IgE to the same allergens was assessed by the multi-allergosorbent chemiluminescent assay (MAST-CLA). Overall agreement between the tests was 66.4% for conventionally positive skin tests (weal diameter ≥ 3 mm), rising to 78.5% when a positive skin test was defined as having a weal diameter greater than or equal to 5 mm. Agreement between the tests was statistically significant for all allergens except *Alternaria*. A history was obtained from each subject of the presence or absence of allergic symptoms on exposure to cats, and whether there was a history of grass pollen allergy. MAST-CLA testing for specific IgE to cat dander predicted a history of cat allergy with an efficiency of 74.5%, while a positive MAST-CLA test for Cocksfoot grass predicted a history of grass pollen allergy with an efficiency of 85.1%. Similar results were obtained on skin testing for these allergens. We conclude that MAST-CLA gave results comparable to those obtained by skin-prick testing, and correlated equally well with the history of allergic symptoms.

Clinical and Experimental Allergy, Vol. 19, pp. 51–56. Submitted 12 May 1988; revised 22 July 1988; accepted 15 August 1988.

Introduction

The diagnosis of atopy in clinical practice is frequently based on skin tests using a combination of allergens. Usually this involves the application of single dilutions of the allergens to be tested, introduced epicutaneously by the method of Pepys [1]. A skin weal response greater than or equal to 2–3 mm in diameter is conventionally regarded as a positive reaction. Skin-prick tests thus performed give quick and reasonably reliable results, and are extremely safe. The drawbacks to the technique are the intrinsic variability of the weal response, with a coefficient of variation ranging from 105 to 40% depending on the skin area tested and the size of the weal response [2,3], and the need to discontinue antihistamine therapy. In addition the results are unreliable in the presence of dermatographism [4].

The measurement of circulating serum levels of specific immunoglobulin E (IgE) can be used as an alternative to skin prick testing. The use of the radio-allergosorbent test (RAST) for specific IgE has been shown to correlate with the results of skin-prick tests [5, 6], and accords at least as

well with patients' clinical history of allergic disease, and the results of direct allergen provocation [7].

A new method for the simultaneous measurement of total IgE and specific IgE directed against 35 different allergens has recently been developed, employing chemiluminescence rather than γ -isotope emission in the assay. The multiple allergosorbent chemiluminescent assay (MAST-CLA) requires the minimum of technical knowledge to perform, and a result can be obtained within 24 hr of analysis of a single venous blood sample [8]. Since this technique has potential as a diagnostic tool in allergic disease, we compared the results of immediate skin-prick testing and the MAST-CLA system applied to 10 aero-allergens in 47 known atopic subjects. Using the two tests, we also compared the results for allergy to cat dander and Cocksfoot grass to the clinical history relevant to each allergen.

Method

A total of 47 subjects were entered into the study. The mean age of our subjects was 21.4 years (s.d. 3.4), with a male to female ratio of 1:16:1. All were known to be atopic, either from previous skin tests performed in the

Correspondence: Dr J. P. Finnerty, Medicine 1, Level D, Centre Block, Southampton General Hospital, Southampton SO9 4XY, U.K.

department, or from their clinical history. Subjects with current eczema, or who were taking H1-antihistamine preparations were excluded. Verbal consent was obtained prior to entry, and the study was approved by the Southampton University and Hospitals Ethical Committee.

Immediate skin-prick testing to the following 10 aero-allergens was performed: beech, oak, Bermuda grass, Cocksfoot grass, rape pollen, nettle pollen, cat dander, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Alternaria*. Testing to all 10 allergens together with histamine and diluent controls was performed on both forearms. Skin-prick testing was performed according to the method of Pepys [1]. Single dilutions of the antigens were provided by Hollister-Stier Inc. (Strawberry Hill, Newbury), and were identical to those used in the MAST-CLA test chambers. The concentration of the allergens was 5000 protein nitrogen units per millilitre (PNU/ml), except for Bermuda grass, Cocksfoot grass, and *D. pteronyssinus*, where the strength was 400 AUR/ml (AUR stands for Activity Units by RAST, i.e. the allergen extract is RAST standardized). Skin test results were recorded both as the maximum diameter of the weal at 12 min, and the weal area at the same time. The weal area was determined by circumscribing the weal in ink, transferring the impression of the weal to adhesive tape, and determining the area subsequently by computer-linked planimetry using a HipadTM digitizer.

Subjects then completed a simple questionnaire to define their allergic status. Subjects were classified as grass pollen-allergic if they had ever developed streaming or inflamed eyes, sneezing and running nose, or wheezing and coughing while outdoors during the months May through July. If they suffered with these symptoms at other times, they were classified as grass pollen allergic only if they gave a history of exacerbation of these symptoms during the summer months. Similarly, they were classified as allergic to cats if they could recall ever having developed any of the symptoms mentioned in the presence of cats, or on entering households which kept cats.

A 10 ml sample of venous blood was obtained, and allowed to clot at room temperature for 2 hr. Serum for the MAST-CLA assay was obtained by centrifugation for 5 min at 2000 r.p.m. at room temperature. The methodology has been fully described elsewhere [8]. Briefly, the MAST-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 two threads act as positive and negative controls. Samples of the subject's serum (1.3 ml) are incubated in the test

chamber for 16–24 hr at room temperature during which time IgE specific for the allergens present binds to the appropriate cellulose thread. The serum is then allowed to drain from the chamber, which is then washed out with a phosphate buffer, pH 7.2. Goat anti-human myeloma IgE labelled with horseradish peroxidase and made up in the same phosphate buffer, is then added to the chamber, and incubated at room temperature for a further 4 hr. The horseradish peroxidase bound to each thread reflects the quantity of specific IgE in the serum.

To quantify this, the chamber is washed with phosphate buffer and a photoreagent mixture is added, which luminesces in the presence of horseradish peroxidase. The chemiluminescence is recorded on Polaroid high speed film, using an exposure time dependent on the batch of film (in our case, 10 min), followed by 30 sec of processing.

After a further 5 min drying, the developed photograph has a series of white bands where a thread has registered a positive response, and remains black where the result is negative. The MAST densitometer measures the transmittance level (in volts) of the band corresponding to each thread. To calculate the specific IgE level, it subtracts the transmittance level of the negative control band from the transmittance level for each allergen, to give a net voltage reading. It then classifies the allergen-specific IgE concentration as very low or undetectable (net voltage < 0.18 V), low (0.67–0.18 V), moderate (1.90–0.67 V), high (3.5–1.90 V) and very high (> 3.5 V). In our study, we interpreted a MAST-CLA test as positive if the net voltage reading was > 0.18 V.

Results

All of the subjects in the study were identified as atopic in having at least one positive skin-prick test. Ninety percent had an elevated total serum IgE as judged by a positive MAST-CLA IgE assay.

Relationship between SPT and MAST-CLA

Least squares linear regression analyses were performed between MAST-CLA voltage and greatest weal diameter, weal area, and the common logarithm of the weal area ($\log_{10}$ weal area) on skin testing for each allergen, using the SPT results from the right forearm. The greatest correlation coefficients were achieved with MAST-CLA voltage plotted against weal area, closely followed by MAST-CLA voltage against weal diameter (Table 1). A significant correlation at the 5% confidence level was obtained between MAST-CLA voltage and weal area for all allergens except *Alternaria*.

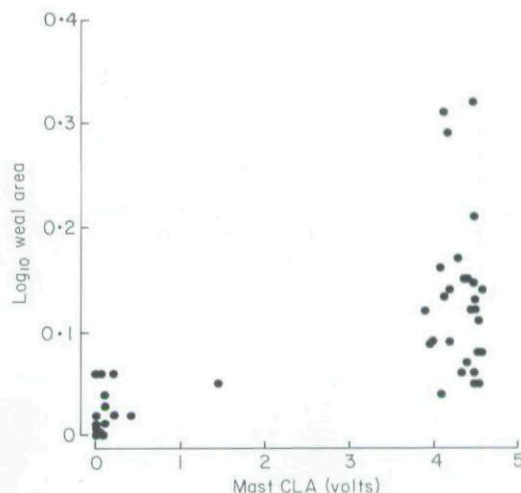
Correlation between MAST-CLA and skin test results

Table 1. Least squares linear regression analysis of MAST-CLA voltages against skin-test parameters

Allergen	Skin test parameter	<i>r</i> value	<i>P</i> value
Beech	Weal diameter	0.37	<0.01
	Weal area	0.49	<0.01
	Log ₁₀ weal area	0.47	<0.01
Oak	Weal diameter	0.51	<0.01
	Weal area	0.64	<0.01
	Log ₁₀ weal area	0.62	<0.01
Bermuda grass	Weal diameter	0.57	<0.01
	Weal area	0.68	<0.01
	Log ₁₀ weal area	0.67	<0.01
Cocksfoot grass	Weal diameter	0.71	<0.01
	Weal area	0.66	<0.01
	Log ₁₀ weal area	0.70	<0.01
Rape pollen	Weal diameter	0.37	0.01
	Weal area	0.41	<0.01
	Log ₁₀ weal area	0.40	<0.01
Nettle	Weal diameter	0.49	<0.01
	Weal area	0.65	<0.01
	Log ₁₀ weal area	0.62	<0.01
Cat dander	Weal diameter	0.69	<0.01
	Weal area	0.72	<0.01
	Log ₁₀ weal area	0.73	<0.01
<i>D. pteronyssinus</i>	Weal diameter	0.77	<0.01
	Weal area	0.64	<0.01
	Log ₁₀ weal area	0.69	<0.01
<i>D. farinae</i>	Weal diameter	0.77	<0.01
	Weal area	0.69	<0.01
	Log ₁₀ weal area	0.72	<0.01
<i>Alternaria</i>	Weal diameter	0.03	0.87
	Weal area	0.03	0.82
	Log ₁₀ weal area	0.03	0.85

was not improved by logarithmic or square root transformation of MAST-CLA voltage. Despite achieving significance by linear regression analysis, it was clear from examining the plots that the relationship was more complex than a straight line. As a typical example, the plot of MAST-CLA voltage against weal area for *D. pteronyssinus* is shown in Fig. 1. Thus in order to make a clinically useful comparison between the two techniques, it was necessary to categorize the readings obtained with each into absolute positive and negative results, and compare the agreement between the test results thus obtained.

The MAST-CLA voltage readings were categorized into positive and negative results, with a positive result representing a voltage reading of >0.18 V, and were analysed with respect to the corresponding SPT results (Fig. 2). Three classifications were used, with three arbitrary values used for a positive SPT as follows:

**Fig. 1.** Results for *D. pteronyssinus*: log₁₀ of the skin weal area of SPT in mm² plus one, plotted against the corresponding MAST-CLA values in V.

greatest diameter ≥ 3 mm; greatest diameter ≥ 5 mm; and area determined by planimetry ≥ 7 mm² (equivalent to a circular weal of diameter ≥ 3 mm). Figure 2 shows that for eight of the 10 allergens tested, agreement between MAST-CLA and skin tests is $>75\%$ where the criterion for skin test positivity is a weal of diameter ≥ 5 mm.

Overall agreement between SPT and MAST-CLA is shown in Table 2, where the results for all 10 allergens have been summed. It is seen that the greatest total agreement (agreed negative plus agreed positive results) between MAST-CLA and skin test results occurs when the criterion for skin test positivity is a weal diameter ≥ 5 mm, where the agreement reaches 78.5%. This is significantly greater than the agreement obtained using the other two criteria for skin test positivity employed ($P < 0.025$, Chi-squared).

Clinical correlation

The correlation between skin-prick tests and MAST-CLA results, and the history obtained of allergy to grass pollen and to cats was analysed. For grass pollen allergy, 36 subjects were positive and 11 negative. The results of skin testing and MAST-CLA for Cocksfoot grass were then compared, using a weal diameter of either ≥ 3 or ≥ 5 mm as the criterion of positivity for the skin tests (Table 3). Subjects were regarded as correctly classified if they had a positive skin test or MAST-CLA result and a positive history of allergy, or conversely a negative skin test or MAST-CLA result and a negative history of allergy. The percentage of subjects thus correctly classified is the efficiency of the test.

For grass allergy, MAST-CLA classified the subjects

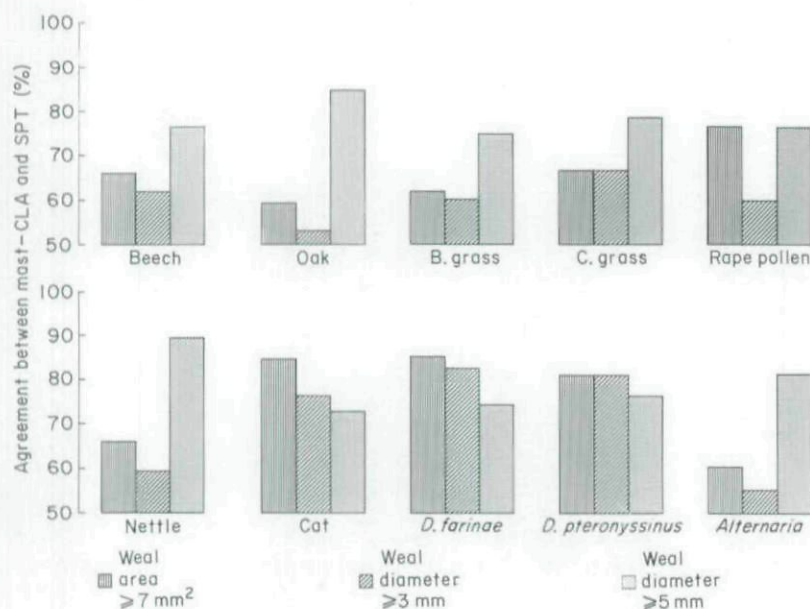


Fig. 2. Bar chart of agreement between MAST-CLA results and SPT results for all 10 allergens, using three criteria for SPT positivity.

Table 2. Relationship between MAST-CLA and skin tests: overall results

Result	Criteria for positive skin test		
	Area $\geq 7 \text{ mm}^2$	Diameter $\geq 3 \text{ mm}$	Diameter $\geq 5 \text{ mm}$
Total agreement	71.5	66.4	78.5
Agreed negative	41.7	36.6	57.7
MAST-CLA positive, SPT negative	7.0	6.8	15.7
MAST-CLA negative, SPT positive	21.5	26.8	5.7
Agreed positive	29.8	29.8	20.9

All figures are expressed as percentage values. 'Agreed negative' indicates results negative both on MAST-CLA testing and skin testing. 'Agreed positive' indicates results positive both on MAST-CLA testing and skin testing. 'Total agreement' is the sum of 'agreed negative' and 'agreed positive'. The three criteria for skin-test positivity given in the table are explained in the text.

Table 3. Relationship of history of grass pollen allergy with MAST-CLA and skin test results for Cocksfoot grass allergen

	History positive	History negative
MAST-CLA positive	29	0
MAST-CLA negative	7	11
Weal diameter $\geq 3 \text{ mm}$	33	5
Skin test negative	3	6
Weal diameter $\geq 5 \text{ mm}$	26	1
Skin test negative	10	10

The number in each cell represents directly the number of subjects. The criteria for skin test positivity are explained in the text.

according to their history with an efficiency of 85.1%. Using a 3 mm weal as the criterion for a positive skin test gave an efficiency for skin testing of 83.0%, while using a 5 mm weal gave an efficiency of 76.6%. The positive predictive value of MAST-CLA (that is, the percentage of positive MAST-CLA tests where there was also a positive history of allergy) was 100%. The corresponding values

Table 4. Relationship of history of cat allergy with MAST-CLA and skin test results to cat dander

	History positive	History negative
MAST-CLA positive	10	9
MAST-CLA negative	3	25
Weal diameter ≥ 3 mm	10	8
Skin test negative	3	26
Weal diameter ≥ 5 mm	6	3
Skin test negative	7	31

The number in each cell represents directly the number of subjects. The criteria for skin test positivity are explained in the text.

for skin-prick tests were 86.8% (weal ≥ 3 mm) and 96.3% (weal ≥ 5 mm).

In the same way, skin tests and MAST-CLA results for cat dander were correlated with the presence or absence of a clinical history of allergic symptoms on exposure to cats. Thirteen of our 47 subjects were classified as being positive for symptoms of cat allergy (Table 4). The efficiency of MAST-CLA in identifying a history of cat allergy was 74.5%. Using a 3 mm weal as the criterion for a positive skin test gave an efficiency for skin testing of 76.6%, while using a 5 mm weal as the criterion gave an efficiency of 78.7%. The positive predictive value of MAST-CLA was 52.6%, with values for skin-prick tests of 55.6% (weal ≥ 3 mm) and 66.7% (weal ≥ 5 mm).

Discussion

Skin-prick tests are widely used to help define allergic diseases, with the skin-prick method of Pepys being most commonly used [1]. Most authorities regard a weal > 3 mm in its greatest diameter as a positive result, although the Scandinavian Society of Allergology recommends the result be expressed as the ratio of the allergen-induced weal diameter to the control histamine-induced weal diameter [10]. In this paper we have regarded an allergen-induced weal of ≥ 3 mm in its greatest diameter as being a conventionally positive skin test, with a weal ≥ 5 mm in greatest diameter being a strongly positive skin test.

Several comparisons have been made between blood levels of specific IgE estimated by RAST and the results of skin-prick tests to the corresponding allergens. In a representative study, Collins-Williams and Bremner found 82.7% agreement between RAST and skin tests for 12 common allergens, but noted that positive skin tests were significantly commoner than positive RAST tests [5].

There is a good correlation between RAST estimations

and a history of allergic disease, although the absolute RAST titre correlates poorly with the severity of symptoms [14]. In a representative study, RAST gave 73% agreement with the results of direct allergen provocation testing, although there was marked variation between allergens [12]. A more recent study in children, looking at their history of allergic symptoms on exposure to cats and dogs showed RAST to have a positive predictive value between 53 and 76% [13].

Previous studies have established a strong agreement between RAST estimations and MAST testing using the radio-isotope MAST technique which is the precursor to the chemiluminescent assay used in our study. In their study of 20 atopic children, Ownby *et al.* found agreement between MAST and RAST for five common allergens to be 90% [15]. Brostoff *et al.* using sera from 75 atopic subjects showed MAST and RAST results to give either identical class results, or results differing by only one class in 80% of cases on testing for eight allergens [16]. Moreover, both tests have a similar agreement with clinical status. Emanuel compared both RAST and MAST in 54 patients suspected of nasal allergy, to a clinical standard based on skin-prick tests and nasal provocation tests for five allergens [17]. He found agreement with the clinical standard to be 93% for RAST and 86% for MAST.

Our results indicate strong agreement between conventionally positive skin-prick tests and MAST-CLA results for *D. farinae*, *D. pteronyssinus* and cat dander, while for the other seven allergens investigated, agreement was greatest between MAST-CLA and strongly positive skin tests (Fig. 2). These results are consistent with previous work comparing RAST and skin-prick tests [11]. Taking our results overall, MAST-CLA results correlated most closely with strongly positive skin tests, with agreement in 78.5% of tests (Table 2). Our finding of an absence of a linear relationship between skin-test parameters and MAST-CLA results despite logarithmic transformation of the data is consistent with previous work comparing skin tests with RAST [9].

Further, there was no significant difference between MAST-CLA and skin-prick testing in the ability to predict a history of grass pollen allergy or cat allergy (Tables 3 and 4). For grass pollen allergy, a positive MAST-CLA result had a 100% predictive value. For cat allergy, MAST-CLA had a positive predictive value of 52.6%, corresponding to the reported positive predictive value of RAST in this situation [13].

In conclusion, MAST-CLA testing in an atopic population gave results closely corresponding to strongly positive skin-prick tests for 10 common allergens, and correlated as well as skin-prick tests with a clinical history of allergy.

References

- 1 Pepys J. Skin testing. *Br J Hosp Med* 1975; 14:412-21.
- 2 Galant SP, Maibach HI. Reproducibility of allergy epicutaneous test techniques. *J Allergy Clin Immunol* 1973; 51:245-50.
- 3 Dreborg S, Holgersson M, Nilsson G, Zetterstrom O. Dose-response relationship of allergen, histamine, and histamine releaser in skin prick test and precision of the skin prick test method. *Allergy* 1987; 42:117-25.
- 4 Mygind N. Skin testing. In: *Essential allergy: an illustrated text for students and specialists*. Oxford: Blackwell Scientific 1986:119-23.
- 5 Collins-Williams C, Bremner K. Comparison of skin tests and RAST in the diagnosis of atopic hypersensitivity. *Ann Allergy* 1976; 36:161-4.
- 6 Brown WG, Halonen MJ, Kaltenborn WT, Barbee RA. The relationship of respiratory allergy, skin test reactivity, and serum IgE in a community population sample. *J Allergy Clin Immunol* 1979; 63:328-35.
- 7 Berg TLO, Johansson SGO. Allergy diagnosis with the radioallergosorbent test. A comparison with the results of skin and provocation tests in an unselected group of children with asthma and hayfever. *J Allergy Clin Immunol* 1974; 54:209-21.
- 8 Brown CR, Higgins KW, Frazer K, Schoelz LK, Dyminski JW, Marinkovich VA, Miller SP, Burd JF. Simultaneous determination of total IgE and allergen-specific IgE in serum by the MAST chemiluminescent assay system. *Clin Chem* 1985; 31:1500-5.
- 9 Perrin LF, Dechamp C, Deviller P, Joly P. Reproducibility of skin tests. A comparative study of the Pepys prick test and the Morrow-Brown needle and their correlation with the serum IgE level. *Clin Allergy* 1984; 14:581-8.
- 10 Aas K, Backman A, Belin L, Weeke B. Standardization of allergen extracts with appropriate methods. *Allergy* 1978; 33:130-7.
- 11 Paggiaro PL, Bacci E, Amran DL, Rossi O, Talini D. Skin reactivity and specific IgE levels in the evaluation of allergic sensitivity to common allergens for epidemiological purposes. *Clin Allergy* 1986; 16:49-55.
- 12 Aas K, Johansson SGO. The radioallergosorbent test in the in vitro diagnosis of multiple reagenic allergy. A comparison of diagnostic approaches. *J Allergy Clin Immunol* 1971; 48:134-42.
- 13 Ferguson AC, Murray AB. Predictive value of skin prick tests and radioallergosorbent tests for clinical allergy to dogs and cats. *Can Med Assoc J* 1986; 134:1365-8.
- 14 Nickelsen JA, Georgitis JW, Reisman RE. Lack of correlation between titers of serum allergen-specific IgE and symptoms in untreated patients with seasonal allergic rhinitis. *J Allergy Clin Immunol* 1986; 77:43-8.
- 15 Ownby DR, Bailey J. Comparison of MAST with radioallergosorbent and skin tests for diagnosis of allergy in children. *Am J Dis Child* 1986; 140:45-8.
- 16 Brostoff J, Pack S, Merrett T. New multiple specific IgE assay—MAST. *Lancet* 1984; ii:748-9.
- 17 Emanuel IA. Comparison of in vitro allergy diagnostic methods. *Immunol Allergy Practice* 1985; 7:10-16.

Copyright of Clinical & Experimental Allergy is the property of Blackwell Publishing Limited and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.