

Evaluation of a multiple food specific IgE antibody test compared to parental perception, allergy skin tests and RAST

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Summary

This study was set up to evaluate the food panel of a multiple specific IgE antibody assay in 67 atopic asthmatic children by comparing it to the conventional radioallergosorbent test (RAST) and skin-prick tests (SPT) and then comparing the results of these investigations with the parents' perceptions of food related problems.

Fifteen food specific IgE antibodies were measured using the multiple chemiluminescence assay (MAST-CLA). IgE antibodies to five of these food allergens were also measured by conventional RAST and SPTs were performed in 43 using 11 standardized food extracts matched to the multiple allergosorbent chemiluminescent assay (MAST-CLA) profile. SPT and MAST-CLA results showed good agreement with one another, range 68.8–96.7% (average 87%), with significant correlation for most allergens tested. MAST-CLA was discrepant with RAST and/or SPTs in 58/210 (27.6%).

A questionnaire was sent to the parents to determine their perception of food related symptoms. Sixty-two (92%) questionnaire replies were received, of which 56% reported symptoms with food. The most frequent symptom perceived to be due to food intolerance was behavioural disturbance. The commonest foods implicated were additives (39%), egg (27%), milk (26%), chocolate (23%) and orange (15%). History, SPT, MAST-CLA and RAST were compared for five allergens in 42 patients (210 values). In 14/210 (6.7%), all the tests were negative despite reported symptoms. Conversely in 49/210 (23.3%) at least one test was positive without symptoms.

This study did not support a benefit of multiple antibody testing instead of individually selected RASTs or SPTs. The frequent perception of food related symptoms in these asthmatic children was often not supported by SPT, RAST and/or MAST-CLA. This may be a reflection of the current public concern about food, or of non-IgE mechanisms. These uncertainties can only be resolved by double blind placebo controlled food challenge. The inclusion of food specific IgE antibodies together with inhalant antibodies in a multiple test system for use in atopic asthmatics may be misleading.

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Introduction

The suggested role of foods in provoking atopic symptoms and particularly asthma, remains controversial. Specific foods are frequently suspected, but it is difficult to prove their contribution to symptoms. A cause and effect

relationship can only be confirmed by elimination followed by repeated double-blind challenge tests which are not always practical, or acceptable to parents and patients [1,2].

In atopic individuals, IgE mediated reactions are still considered to be the principal mechanism involved in food induced symptoms although alternative mechanisms may sometimes be involved [2,3]. Investigation of

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food allergy has therefore generally involved a clinical history and examination, supplemented by allergy skin tests and specific IgE antibodies to relevant foods [1,4-6]. The value of specific allergy tests has been questioned and the quality of the tests depends appreciably on the quality of the allergen extracts [7,8].

The conventional technique for measuring IgE antibodies has been the isotopic radioallergosorbent test (RAST), which is still regarded as the gold standard. It is labour intensive and because each allergen must be tested individually, it is expensive and time-consuming to perform several tests for one patient. This has led to the use of a single 'mixed foods' RAST as a screening test which if positive, can be followed by RASTs to individual foods.

Several alternative non-isotopic methods have recently been developed which are able to test multiple allergens [9-12] but some have been marketed before undergoing full evaluation. It is important to test each component of a multiple assay to establish its reliability and to check that its inclusion in the system offers useful additional information to the clinician.

We have previously investigated the value of one such test: the multiple allergosorbent test (MAST-CLA, Alpha Laboratories, Eastleigh, U.K.) for the diagnosis of inhalant allergies and found that it correlated well with skin-prick tests (SPT), RAST and clinical history although measurement of total IgE on the same system was unreliable [13]. The panel of allergens in this system included 15 foods which have not been similarly evaluated. We therefore investigated the MAST-CLA measurements of food specific IgE antibodies in a group of asthmatic children, to determine whether they were useful as a predictor of food-related symptoms and to compare the results with parental perceptions, SPTs, and RAST results.

Method

Sixty-seven asthmatic children aged between 3 and 13 years, were studied (43 males and 24 females). They were randomly selected from the Paediatric Asthma clinic and had moderately severe asthma requiring prophylactic therapy as well as bronchodilators. All had evidence of atopy with positive skin tests for some aero-allergens routinely tested in clinic. Their food history was completely unknown at the time of recruitment.

A postal questionnaire was sent to the parents of these children to determine their beliefs about food as a cause of their child's symptoms. Parents were first asked to comment on symptoms in their children and whether they felt foods were the cause. If so, they were asked to identify foods which were to blame. They were asked if they had previously tried special diets. The questionnaire was

validated for its ability to predict food allergy in 50 other patients attending clinics, comparing the replies to the questionnaire with history and ultimate clinical assessment in the notes. All those with food related symptoms were correctly identified by the questionnaire although eight of the 50 reported symptoms with foods which were not included in the MAST.

Responders to the postal survey were asked to bring their children for food SPTs. The survey replies were compared with the food antibody levels measured by the MAST-CLA chemiluminescent method (15 foods), SPTs (11 foods), and RASTs (five of these food allergens).

MAST-CLA

The multiple allergosorbent chemiluminescent assay (MAST-CLA Allergy Profile, Alpha Laboratories) can simultaneously measure 35 specific IgE antibodies. The allergens chosen for the Brompton Paediatric Panel were common aero-allergens and food allergens frequently causing problems for children in the United Kingdom. The foods incorporated in this system were egg, milk, wheat, soybean, orange, pork, beef, codfish, shellfish, almond, peanut, walnut, hazelnut, chocolate and yeast.

The assay has been fully described elsewhere [11] and is summarized below. For each test 1.3 ml of serum is required and it is complete within 24 hr. The system consists of a long pipette with 38 parallel cellulose threads strung ladder-fashion from top to bottom. Each rung has a different allergenic extract covalently bound to it. The system includes positive and negative controls, a thread for total IgE and 35 other threads for individual allergens.

Assay method. The patient's serum is drawn up into the pipette and incubated overnight. After washing, the pipette is filled with a solution of anti-IgE labelled with a luminescent substance and incubated for a further 4 hr. Wherever patient's IgE is bound to an allergen coated thread, anti-IgE will also bind. After washing and addition of a photoreagent mixture, a band of light is emitted from threads where IgE has bound. The pipettes are then exposed onto a polaroid film and the degree of light emitted is measured by densitometry and expressed in volts. The quantity of IgE present is proportional to this voltage minus the voltage of the negative control. The result of each thread is finally expressed as a class value from 0 to 4 equivalent to RAST: 0 = non-detectable, = = very low, 1 = low, 2 = moderate, 3 = high, 4 = very high.

For this study a positive MAST-CLA was defined as greater than or equal to Class 2.

Skin-prick tests

The SPTs were performed by the prick method using

Table 1. Correlation between MAST-CLA and SPT results ($n=43$)

Allergen	Correlation coefficient (r)	Significance (P)
Milk	0.39	<0.01*
Egg	0.74	<0.001*
Wheat	0.362	<0.05
Soybean	0.138	<0.1
Orange	0.178	<0.1
Codfish	0.638	<0.001*
Almond	0.574	<0.001*
Peanut	0.894	<0.001*
Walnut	0.79	<0.001*
Hazelnut	0.727	<0.001*
Chocolate	†	

* Significant correlation.

† All values negative.

Dome-Hollister-Stier solutions (Bridgend, U.K.). Glycerol/phenol was used as a negative control and 1% histamine dihydrochloride as a positive control. The solutions used were: cow's milk (1:20 w/v), egg (1:20 w/v), soybean (1:10 w/v), whitefish (1:10 w/v), wheat (1:10 w/v), peanut (1: w/v), hazelnut (1:10 w/v), almond (1:10 w/v), walnut (1: w/v), chocolate (1:10 w/v) and orange (1:10 w/v). Weal diameters were measured after 15 min. A positive SPT was defined as a weal greater than or equal to 3 mm with a positive histamine control and a negative glycerol/phenol control. If it was not possible to stop anti-histamine medication, SPTs were not performed.

RAST

RASTs to milk, egg, soybean, fish and peanut were performed using commercially produced food RAST discs (Pharmacia, Milton Keynes, U.K.) with the technique recommended by the manufacturers. A positive RAST was defined as greater than or equal to Class 2.

Statistics

Correlation coefficients were determined by linear regression analysis.

Results

MAST-CLA compared to SPT

The correlation of SPTs (weal diameter) with MAST-CLA (grade) for each allergen is shown in Table 1. There was significant correlation for all foods except soybean

Table 2. Correlation between MAST-CLA and RAST for five test allergens ($n=51$)

Allergen	% agreement	Correlation coefficient (r)	Significance (P)
Milk	82.4	0.507	<0.001*
Egg	90.2	0.706	<0.001*
Soybean	64.7	0.18	<0.1
Fish	82.3	0.055	<0.1
Peanut	66.7	0.074	<0.1

* Significant correlation.

Table 3. Correlation between SPT and RAST results ($n=42$)

Allergen	Correlation coefficient (r)	Significance (P)
Milk	0.408	<0.01
Egg	0.458	<0.01
Soybean	0.131	<0.1
Fish	0.159	<0.1
Peanut	0.171	<0.1

and orange but some of the correlations were weak as for milk and wheat.

MAST-CLA compared to RAST

The results for MAST-CLA compared to RAST (51 cases) for milk, egg, soybean, fish and peanut are shown in Table 2. Only milk and egg showed significant correlation.

SPTs compared to RAST

There was poor correlation between SPT and RASTs as shown in Table 3, perhaps because of failure to ensure that the materials for each test came from the same allergenic source.

Questionnaire

Sixty-two questionnaire replies were received, which is a 92% response rate; 48 on the first request and 14 after a second request. Of the 62 responders, 61 had MAST-CLA tests but there was adequate serum in only 51 for RASTs. Forty-three children attended for SPTs. Some problem with food was reported in 34/62 (55%) of whom 28 (82%) had tried elimination diets. The foods repeatedly incriminated

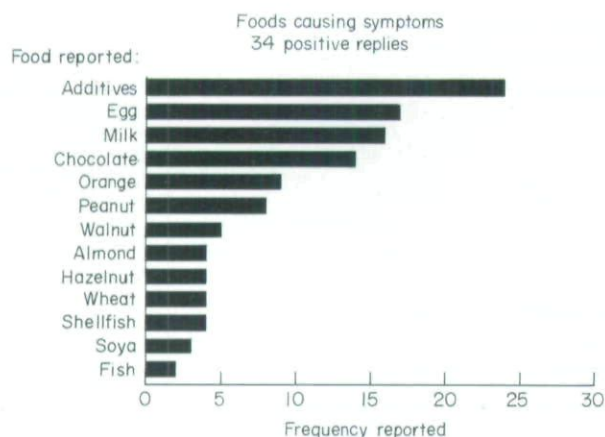


Fig. 1. Questionnaire results.

nated are shown in Figure 1. Thirty-one per cent were quite certain that food caused symptoms, 9% thought it probably did and 15% thought it might cause problems but were unsure.

Twenty-five parents associated food with atopic symptoms: eczema 11, gastrointestinal effects 5, urticaria/angio-neurotic oedema 12 and wheeze 9, i.e. some had more than one manifestation. Six parents mentioned additives as a cause of wheeze and four of these specifically mentioned that fizzy drinks produced wheeziness. Thirty-one (50%) parents reported behavioural disturbance in their children. Twenty-five (80%) of these blamed food for this: additives and preservatives (24), chocolate (2), and one both of these. There is no evidence that this type or reaction is IgE mediated and the two patients who reported behavioural disturbance from chocolate had negative SPTs and no detectable specific IgE antibodies detected by the MAST-CLA assay. Six of the 24 reports of adverse behaviour with additives were also associated with other symptoms: eczema in 4, asthma in 2.

Sixty-one per cent of parents whose children had SPTs perceived food as a cause of their child's symptoms. This is not significantly different from the perception of parents whose children did not have SPTs. The questionnaire data and investigation results were compared below. Twenty-five replies reported a positive history to at least one of the test foods and 37 were completely negative. (Non-test foods such as additives were excluded.)

MAST-CLA compared to clinical history

Both the history and *all* food MAST-CLA results were negative in 32 (52%). Thirteen children had matched positive MAST-CLA and symptoms from at least one test

Table 4. Comparison of parental history and CLA results ($n = 61$)

History test	+ve +ve	+ve -ve	-ve -ve	-ve +ve	Percentage agreement	Positive predictive value (PPV)
Milk	3	15	39	4	68.8	0.43
Egg	6	10	41	4	77	0.6
Wheat	3	2	50	6	86.9	0.33
Soybean	3	1	52	5	90.2	0.375
Orange	1	5	52	3	86.9	0.25
Codfish	2	1	57	1	96.7	0.67
Almond	3	3	51	4	88.5	0.43
Peanut	7	2	51	1	95	0.875
Walnut	4	2	54	1	95	0.8
Hazelnut	5	2	51	3	91.8	0.625
Chocolate	0	12	49	0	80.3	0

Table 5. Comparison of history, MAST-CLA, RAST and SPTs for five allergens (42 patients, 210 values)

All results positive	8 (3.8)*
All results negative	126 (60)*
History positive <i>all</i> tests negative	14 (6.7)*
History negative <i>any</i> or <i>all</i> tests positive	49 (23.3)*
MAST-CLA discrepant with RAST and/or SPTs	58 (27.6)*

*Numbers in parentheses are percentages.

food. Of these, five had symptoms and positive results from four or more test foods. However, five children had at least one positive MAST-CLA food result *without* symptoms and two of these had four or more positive results without recognizable symptoms. Twelve children had symptoms from one or more test foods but no positive MAST-CLA test result.

MAST-CLA was compared with the parental clinical history for each individual allergen in 61 cases (see Table 4). Of all results, 87% were concordant and 13% were discordant. For each food most results were in negative agreement and only a few results were in positive agreement. The sensitivities and positive predictive values were generally low and suggested that the test was not a good predictor of perceived food reactions.

MAST-CLA compared to SPT, RAST and history

The results of SPT, MAST-CLA, RAST and history were all compared for five allergens: milk, egg, soybean, fish and peanut (42 patients, 210 separate values, see Table 5). Agreement of all parameters was seen in 134 (63.8%)

results. In three instances MAST-CLA was positive but all other results were negative, and in four MAST-CLA was negative with all other results positive.

Discussion

This study showed that measurement of multiple food specific IgE antibodies did not give accurate or useful information in a group of atopic asthmatic children. Despite using a standard SPT method and ensuring that the same allergen source was used for both MAST-CLA and SPTs the results did not correlate well for milk, wheat and soybean although there was significant correlation for eggs, fish and nuts. The poor correlation between our RAST and MAST-CLA results and the RASTs and SPTs was probably due to the different origins of the allergen extracts used for each test. These poor results also illustrate that even the conventional SPTs and RASTs may have shortcomings unless the allergenic extracts for each individual test are carefully prepared. Aas found that SPT and RAST were diagnostically reliable for egg-white, peas, nuts, peanuts and codfish but not for other allergens and suggested that better purification and separation techniques were required for the preparation of allergen extracts for diagnostic tests [8].

The MAST-CLA results were not generally a good predictor of food-related symptoms as perceived by parents indicating either that the test was poor or that the mechanism was not IgE mediated. Poor stability of some allergens on the cellulose threads of the MAST-CLA pipette could contribute to poor test sensitivity. The test showed poor predictability apart from fish and nuts which are highly allergenic, causing immediate IgE mediated reactions which are so obvious that they have usually been correctly identified by patients. We have also looked at the MAST-CLA method in a number of children presenting with specific food-related symptoms and found better correlation (unpublished data). However, if it is actually necessary at all in such a case to perform a test, individual RASTs are both cheaper and have been validated.

Reactions to milk and egg were frequently reported by parents but were often associated with negative test results. Milk intolerance may have been due to non-IgE mechanisms [14]. Some children may have had allergic reactions during infancy which they had outgrown, a well recognized sequence in atopic children [15]. Demonstration of a corresponding fall in food specific IgE antibody may then be clinically useful to indicate the likelihood of further reactions on re-introduction but this again could be more economically done with a skin test and conventional RAST.

Non-specific binding to the cellulose threads of the pipette may explain the positive MAST-CLA results in patients without symptoms or positive SPT and RAST. Atopic individuals sometimes demonstrate IgE sensitization without ever having had symptoms and others whose symptoms have resolved may retain detectable IgE antibodies [15]. It is because of this variability that investigators have argued for and against any predictive value of any food allergy tests in eczema and other atopic problems [6,7,15,16]. Pike *et al.* could not assess the predictability of SPTs and RAST tests in identifying exacerbating foods in childhood eczema because no significant difference in symptom score was demonstrated on double-blind challenge [17].

The role of food in exacerbating atopic disease remains controversial. Our questionnaire replies show that parents of atopic asthmatics frequently perceive food as a cause of symptoms in their children and many parents had themselves initiated exclusion diets. In a postal survey of an unselected population in High Wycombe, 7.4% of respondents thought that additives caused problems and 15.6% thought that other foods were involved [18]. Behaviour was frequently linked with specific food substances especially additives. Other studies have suggested that food intolerance is generally overperceived and that reported symptoms are not necessarily reproduced by clinical challenge [1,5]. Some of the reports of food related symptoms in our survey may therefore reflect current beliefs in the general population and may not be specific to atopic children. Rona *et al.* found that parental suspicion of food intolerance was associated with higher social class and a higher level of education attained by the mother which may also be relevant to our results as our asthma clinic has a bias towards upper social class [19].

Many of our atopic asthmatics also have eczema and a few have immediate gastro-intestinal symptoms, urticaria or angio-oedema following specific foods. Not surprisingly parents reported the highly allergenic foods associated with this type of reaction more frequently than in a non-atopic population.

Asthma was only linked to food in nine (14.5%) questionnaire replies. Six of these identified additives and/or fizzy drinks as a cause of wheezing. This association has been previously described. Wilson *et al.* demonstrated increased bronchial hyper-reactivity following ingestion of cola drinks and orange squash and believe this may be respectively sulphite and tartrazine induced [20,21]. Supramaniam and Warner investigated the role of additives in urticaria and angio-oedema using elimination diets followed by double blind placebo controlled additive challenge [22]. In a few cases a small but significant fall in peak flow was observed following active

additive but not placebo. The mechanism by which additives induce asthma remains uncertain but it is not IgE mediated and therefore not predictable from SPTs or measurement of IgE antibody levels. In contrast only three (4.8%) parents linked asthma with foods where IgE mediated mechanisms may have been involved. This parental observation corresponds with the findings of Novembre *et al.* and Onorato *et al.* who demonstrated food-induced asthma in 5.2% and 2% of patients followed in pulmonary clinics [23,24].

Knowledge of multiple food specific IgE antibodies measured by the MAST-CLA method in our atopic asthmatic study group did not reliably add to our knowledge about their reactions to food. This suggests that routine inclusion of multiple food allergens together with aero-allergens in the MAST-CLA profile is misleading. Commercially produced allergy screening tests such as these are increasingly being marketed for general use but our observations show that the results must be interpreted with caution or they could be the basis for an erroneous diagnosis of food allergy and unnecessary dietary restrictions. Interpretation of the results of this type of multiple test are particularly difficult in atopic children whose parents not infrequently seek an 'allergy test' and ask for advice on the use of exclusion diets. In this particular study we did not investigate the validity of parental reports with elimination diet and subsequent double-blind placebo controlled food challenge. However, the limitations of the tests which were performed support the view that this remains the best method of confirming or refuting the validity of parental reporting in dubious cases [1,2].

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