

Chemiluminescent Assay Versus Immunoblotting for Detection of Positive Reaction to Allergens

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Abstract

Background: The multiple allergen simultaneous test-chemiluminescent assay (MAST-CLA) has been widely used in South Korea to detect allergen-specific immunoglobulin E (IgE). However, MAST-CLA frequently shows concurrent positivity for immune-system cells that fight multiple allergens.

Objective: To compare the results of MAST-CLA with those of the new MAST-immunoblot assay, a potential alternative method for concurrently testing for IgE and positivity for multiple allergens.

Methods: A total of 55 patients with positive results in MAST-CLA were tested using the

MAST-immunoblot system. A total of 133 allergens that displayed discrepancies in MAST-CLA and MAST-immunoblot results were examined with the ImmunoCAP test.

Results: The positivity rates for 20 common allergens, as measured by MAST-CLA and MAST-immunoblot, were 53.4% and 19.5%, respectively. The agreement rate of MAST-CLA and ImmunoCAP (75.9%) results was higher than MAST-CLA and MAST-immunoblot (62.4%) results. The mean number of positive results for specific allergens per patient as measured by MAST-immunoblot and MAST-CLA were 5.6 and 15.9, respectively.

Conclusions: The higher agreement rate between ImmunoCAP and MAST-CLA results compared with MAST-CLA and MAST-immunoblot results was caused by frequently occurring concurrent positive reactions to multiple allergens. The frequency of concurrent positive results and the number of allergens showing concurrent positive results was lower in MAST-immunoblot than in MAST-CLA. Therefore, MAST-immunoblot may be helpful in the diagnosis of allergic disease and will decrease any confusion arising from concurrent positivity for multiple allergens.

Keywords: allergen-specific IgE; MAST-CLA; immunoblotting; ImmunoCAP

In most clinics, physicians encounter many patients with allergic symptoms; thus, clinical evidence of the presence of specific allergens that trigger allergic disease is crucial. The skin prick test (SPT) is regarded as the criterion standard for determining specific allergens and has been widely used for diagnosis;¹ however, this test has several limitations, including interference by medications such as antihistamines and an invasive nature that makes it difficult to perform on young children or patients with skin disease.¹⁻³ Hence, various in vitro tests measuring serum allergen-specific immunoglobulin E (IgE) antibody levels have been introduced. These tests are divided into two types, namely, an individualized test for each allergen or a single test for multiple allergens (eg, the multiple allergen simultaneous test [MAST]). The former includes the radioallergosorbent test (RAST) and the ImmunoCAP system; the latter includes the MAST chemiluminescent assay (CLA) and the MAST-immunoblot method. The most frequent combination of measurements used in South Korea is the MAST-CLA (CLA Allergen-specific IgE assay, Hitachi Chemical Diagnostics, Mountain View, CA) plus the ImmunoCAP Allergen Component Tests (Phadia AB, Uppsala,

Sweden).⁴ The use of MAST-immunoblot instead of MAST-CLA is, however, increasing.

The MAST-CLA has been widely used in South Korea because it does not require a radioactive isotope or costly equipment; it enables the simultaneous examination of 35 allergens at a relatively low cost; and it is highly sensitive and specific. In addition, MAST-CLA results correlate with the SPT.^{1,5} However, the MAST-CLA frequently shows concurrent positive results for multiple allergens in a single panel test; hence, health care professionals are often confused as to whether the results should be interpreted as multiple allergens and multiple allergic reactions, cross-reactivity, or false positives. In our experience, approximately 10% to 20% of MAST-CLA results showed concurrent positivity for 30 or more allergens via other tests; in such a situation, the original test results become meaningless.

The MAST-immunoblot assay, which was recently introduced and is being used with increasing frequency, is simpler, faster, and requires a smaller serum sample than MAST-CLA. In South Korea, of many MAST-immunoblot assays, the RIDA AllergyScreen (R-Biopharm AG, Darmstadt, Germany) is the most commonly used,⁴ although others, such as the AdvanSure Allergy Screen (LG Life Sciences, Seoul, South Korea) and the Polycheck Allergy System (Biocheck GmbH, Münster, Germany), are also used. Recently, another form of MAST-immunoblot, the EUROLINE (Euroimmun AG, Lübeck, Germany), was introduced. Unlike other immunoblots, the EUROLINE MAST-immunoblot test strip includes cross-reactive carbohydrate determinants (CCD), complex glycans present in glycoproteins from plants and some invertebrate organisms. The presence of IgE in CCDs may cause a false-positive reaction due to interference or cross-reactivity in allergen-specific IgE assays.⁶ Therefore, the CCD marker may provide useful information, especially with positive-specific IgE

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Abbreviations

MAST-CLA, multiple allergen simultaneous test-chemiluminescent assay; IgE, immunoglobulin E; SPT, skin prick test; MAST, multiple allergen simultaneous test; RAST, radioallergosorbent test; CCD, cross-reactive carbohydrate determinants

results that disagree with the clinical picture, and can aid health care professionals in interpreting overall test results showing concurrent positivity for multiple allergens.

The purposes of this study were to compare the EUROLINE MAST-immunoblot (known hereafter as MAST-immunoblot) with the MAST-CLA and to evaluate MAST-immunoblot as a potential alternative method for reducing concurrent positivity for multiple allergens.

Materials and Methods

Patient Specimens

Between October 2008 and March 2010, a total of 55 serum specimens were randomly selected from patients with positive reactions on MAST-CLA tests. The demographic characteristics of the patients are summarized in **Table 1**. The mean (SD) age was 20.1 years (16.5 years); the age range was 10 months to 70 years; 31 patients were male.

MAST-CLA Test

Among the 55 serum samples, 25 samples were tested using the Korean inhalant panel of the MAST-CLA; the remaining 30 samples were tested using the Korean food panel of the MAST-CLA. Allergens in the inhalant and food panels of MAST-CLA and the MAST-immunoblot are listed in **Table 2**. The CLA allergen-specific IgE assays of MAST-CLA were performed according to the manufacturer's instructions. The MAST pipette contained 38 discrete segments of cellulose thread, each coated with an allergen, including the positive procedural control, a negative blank control, total IgE, and 35 allergens. The MAST pipette was filled with 1.4 mL of patient serum and incubated at room temperature for 16 to 24 hours, after which it was washed with buffer to remove

Table 1 Patient Demographic Characteristics^a

Characteristic	No.
Sex, male to female ratio	31:24
Age, y ^b	
≤10	19
11- >21	13
21- >41	15
≥41	8
MAST-CLA panel	
Inhalant	25
Food	30
ImmunoCAP	47
Department	
Dermatology	20
ENT	22
Pediatrics	13
Diagnosis	
Acute urticaria	19
Allergic rhinitis	18
Allergic dermatitis	10
Asthma	4
Other	4

SD, standard deviation; MAST-CLA, multiple allergen simultaneous test-chemiluminescent assay; ENT, ear, nose, and throat.

^aN = 55.

^bMean (SD), 20.1 (16.5)

unbound serum components. Enzyme-labeled anti-IgE was then added and coupled with the bound allergen-specific IgE for 4 hours of incubation. After a second wash, the pipette was filled with a substrate that reacted with the labeled antibody to produce chemiluminescence. The amount of light emitted by each thread was measured by a luminometer; the results were interpreted as classes 0, 0/1, 1, 2, 3, or 4 based on the amount of light emitted, with classes 1 to 4 considered positive results.

Table 2 Allergens in the Korea 1 and Korea 2 Inhalant and Food Panels of the Multiple Allergen Simultaneous Test and the EUROLINE Multiple Allergen Simultaneous Test-immunoblot

Common Allergens in the MAST-CLA Inhalant and Food Panels

f1 Egg white	f2 Cow's milk	f14 Soybean	f23 Crab	f24 Shrimp	f95 Peach
d1 <i>D pteronyssinus</i>	d2 <i>D farinae</i>	h1 House dust	e1 Cat dander	e5 Dog dander	i6 Cockroach
g12 Cultivated rye grass	ts3 Birch-alder mix	t7 White oak	w1 Short ragweed	w6 Mugwort	
m2 <i>Cladosporium herbarum</i>	m3 <i>Aspergillus fumigatus</i>	m6 <i>Alternaria tenuis/alternata</i>			

Other Allergens in the MAST-CLA Inhalant Panel

g2 Bermuda grass	g3 Orchard grass	g6 Timothy grass	ts11 Planetree mix	ts14 Poplar mix	ts15 Ash mix
t4 Hazelnut	t12 Willow	T17 Japanese cedar	t19 Acacia		
w8 Dandelion	w11 Russian thistle	w12 Goldenrod	w14 Common pigweed	m1 <i>Penicillium notatum</i>	

Other Allergens in the MAST-CLA Food Panel

f3 Codfish	f4 Wheat	f6 Barley	f9 Rice	f13 Peanut	f26 Pork
f27 Beef	f40 Tuna	f41 Salmon	f45 Yeast	f47 Garlic	f48 Onion
f81 Cheddar cheese	f83 Chicken meat	fs15 Citrus mix			

Allergens in the MAST-immunoblot Only

w100 Sheep sorrel	t1 Elder	t16 White pine	ts8 Elm mix	g7 Common reed
f11 Buckwheat	f25 Tomato	f292 Corn	d70 <i>Acarus siro</i>	m5 <i>C albicans</i>

MAST, multiple allergen simultaneous test; CLA, chemiluminescent assay; D pteronyssinus, Dermatophagoides pteronyssinus; D farinae, Dermatophagoides farinae; C albicans, Candida albicans.

MAST-immunoblot Test

Multiple allergen simultaneous test-immunoblots were performed with Allergy Profile Inhalation Korea 1 and Korea 2 and Allergy Profile Food Korea 1 and Korea 2 kits (Euroimmun AG) using the EUROBlot Master processor (Euroimmun AG), according to the manufacturer's instructions. The test kit contained test strips coated with parallel lines of 22 different allergens, including the CCD marker, total IgE, and 20 allergens. Therefore, a total of 40 allergens were included in the inhalation and food profile panels. In this study, only 35 allergens shared between the 2 test platforms were included in the comparison analysis. In the first reaction step, the test strips were moistened with patient serum and incubated. If a specimen contained IgE for a specific allergen, the allergen-specific IgE would bind to that allergen. To detect the bound IgE, a second incubation was performed using an enzyme-labeled monoclonal anti-human IgE (ie, enzyme conjugate) to catalyze a color reaction. The test strips were scanned, and the EUROLineScan (Euroimmun AG) system was used to identify the bands, to measure their intensity, and to automatically sort the bands into classes from 0 to 6. Classes 1 to 6 were considered positive results, with classes 5 and 6 equal to MAST-CLA class 4.

ImmunoCAP Test

ImmunoCAP tests were performed using a Phadia 100 system (Phadia AB) according to the manufacturer's instructions. ImmunoCAP tests were used to resolve discrepancies between MAST-CLA and MAST-immunoblot results. A discrepancy was defined as a difference of 2 or more classes between the results, or a positive result by one method and negative result by the other. The results of ImmunoCAP were classified into 7 levels ranging from class 0 to class 6, with classes 1 to 6 considered to be positive results.

A total of 133 ImmunoCAP tests were performed on 47 of the 55 serum specimens. The number of specimens with concurrent positivity for more than 20 allergens was 12 of 47 (25.5%); 51 ImmunoCAP tests were performed on those 12 specimens. The 101 positive results for inhalant allergens in the ImmunoCAP were distributed as follows: *Dermatophagoides pteronyssinus* (n = 17), *D. farinae* (n = 15), house dust (n = 14), dog dander (n = 12), cockroach (n = 11), cat dander (n = 9), mugwort (n = 7), white oak (n = 6), *Alternaria tenuis/Alternate* (n = 4), birch/alder (n = 3), short ragweed (n = 2), and cultivated rye grass (n = 1). The 32 food allergens tested for were cow's milk (n = 7), shrimp (n = 7), soybeans (n = 6), egg white (n = 5), crab (n = 5), and peach (n = 2).

Statistical Analysis

Statistical analysis was performed using SPSS software, version 15.0 (SPSS Inc., Chicago, IL). The *t* test for continuous variables and the Pearson Chi square test for nominal variables were conducted to evaluate any differences in the results between the groups. Two-sided *P* < .05 was considered statistically significant.

Results

Comparison Between MAST-CLA and MAST-immunoblot

The positivity rates of 20 commonly existing allergens in the inhalant and food panels of MAST-CLA and MAST-immunoblot are summarized in **Table 3**. In MAST-CLA,

D. pteronyssinus showed the highest frequency at 92.7%, followed by *D. farinae* (89.1%), house dust (78.2%), dog dander (72.7%), cat dander (65.5%), white oak (63.6%), cockroach (56.4%), and *Aspergillus fumigatus* (52.7%). In cases of MAST-immunoblot, *D. farinae* (58.2%), house dust (56.4%), and *D. pteronyssinus* (50.9%) had frequencies greater than 50%; most of the remaining allergens showed between 10% and 20% frequency. Among the total 1100 tests for individual allergens (ie, 20 common allergens × 55 patients), the percentage of total positivity in MAST-CLA (53.4%) was significantly higher than in MAST-immunoblot (19.5%) (*P* < .01).

Comparison of MAST-CLA and MAST-immunoblot with ImmunoCAP

A total of 133 allergens showing a discrepancy in MAST-CLA and MAST-immunoblot were tested with the ImmunoCAP. The agreement rate of MAST-CLA with ImmunoCAP (75.9%) was greater than that of MAST-immunoblot with ImmunoCAP (62.4%), as shown in **Table 3**. In the cases of dog dander, white oak, cow's milk, crab, and shrimp, MAST-immunoblot showed particularly poor agreement with ImmunoCAP compared to MAST-CLA.

Concurrent Positivity Levels for Multiple Allergens

The concurrent positivity levels for multiple allergens in the MAST-CLA and MAST-immunoblot test are summarized in **Table 4**. The mean of positive allergens per patient in a single MAST-immunoblot test was 5.6. Also, most patients showed positivity for 1 to 3 (30.9%) or 4 to 6 (36.4%) allergens, with none showing concurrent positivity for more than 20 allergens. The mean positive allergen number per patient in a single MAST-CLA test, by contrast, was 15.9. Although 10 patients (18.2%) showed positivity for fewer than 7 allergens, 17 patients (30.9%) had concurrent positivity for 7 to 10 allergens, the largest number in the MAST-CLA test. Eight patients (14.5%) showed concurrent positivity for 31 or more allergens.

Among the 55 patients, 15 showed CCD positivity on MAST-immunoblot (**Table 4**). Of importance, 5 of 6 patients with concurrent positivity for 11 to 20 allergens in MAST-immunoblot showed CCD positivity; the MAST-CLA results of those 5 patients included 2 patients that were positive for 7 to 10 allergens, 2 patients that were positive for 21 to 30 allergens, and 1 patient that was positive for 31 to 40 allergens.

Discussion

Allergies are caused by several immune-system mechanisms. The identification of specific allergens that trigger these allergic mechanisms is important in the diagnosis and management of allergies. Measurement of allergen-specific IgE is a useful tool for identifying the sources of allergic reactions.^{7,8} Since its introduction, MAST-CLA has been widely used as a screening test for allergic diseases. However, it has been criticized as having no clear cutoff levels, variable sensitivity and specificity, and a lengthy testing time. Hence, the upgraded MAST-immunoblot assay has been introduced and is being used with increasing frequency.^{9,10}

Although the ImmunoCAP method is not considered the criterion standard for diagnosing an allergy, it has been the most extensively evaluated system among various in vitro allergy tests available for diagnostic use. The method has greater than 90% sensitivity, specificity, and positive

Table 3 Positive Results For 20 Common Allergens in the Korea 1 and Korea 2 Inhalant and Food Panels of the Multiple Allergen Simultaneous Test-Chemiluminescent Assay and the Multiple Allergen Simultaneous Test-Immunoblot^a

Allergen	Positive Results, No. (%) ^b		Agreements with ImmunoCAP, No. ^c		
	MAST-CLA	MAST-immunoblot	ImmunoCAP, No.	MAST-CLA	MAST-immunoblot
<i>D pteronyssinus</i>	51 (92.7)	28 (50.9)	17	13	12
<i>D farinae</i>	49 (89.1)	32 (58.2)	15	12	12
House dust	43 (78.2)	31 (56.4)	14	14	11
Dog dander	40 (72.7)	12 (21.8)	12	10	5
Cat dander	36 (65.5)	11 (20.0)	9	5	6
Cockroach	31 (56.4)	8 (14.5)	11	8	8
Cultivated rye grass	23 (41.8)	9 (16.4)	1	1	1
<i>C herbarum</i>	19 (34.5)	2 (3.6)	NT	NT	NT
<i>Aspergillus fumigatus</i>	29 (52.7)	6 (10.9)	NT	NT	NT
<i>A tenuis/Alternate</i>	22 (40.0)	9 (16.4)	4	1	3
Birch/alder	27 (49.1)	7 (12.7)	3	3	2
White oak	35 (63.6)	5 (9.1)	6	5	2
Short ragweed	27 (49.1)	7 (12.7)	2	2	2
Mugwort	24 (43.6)	10 (18.2)	7	4	4
Egg white	21 (38.2)	9 (16.4)	5	4	4
Cow's milk	27 (49.1)	7 (12.7)	7	5	3
Soybean	20 (36.4)	4 (7.3)	6	3	4
Crab	20 (36.4)	12 (21.8)	5	3	2
Shrimp	22 (40.0)	2 (3.6)	7	7	1
Peach	21 (38.2)	3 (5.5)	2	1	1
Total	587 (53.4%)	214 (19.5%)	133	101 (75.9%)	83 (62.4%)

MAST, multiple allergen simultaneous test; CLA, chemiluminescent assay; NT, not tested.

^an = 55 patients; P value determined by Pearson chi square testing of positive results between MAST-CLA and MAST-immunoblot.^bn = 1100 (calculated as 25 allergens × 55 patients); P < .01.^cn = 133; P < .02.**Table 4 Concurrent Positive Results for Multiple Allergens in Individual-Chemiluminescent Assay and Multiple Allergen Simultaneous Test-Immunoblot^a**

Positive Allergen Results, No.	Patients, No. (%)		Patients With Positive CCD Results, No.	
	MAST-CLA	MAST-Immunoblot	MAST-CLA	MAST-Immunoblot
0	0	4 (7.3)	NA	NA
1-3	5 (9.1)	17 (30.9)	1	3
4-6	5 (9.1)	20 (36.4)	1	5
7-10	17 (30.9)	8 (14.5)	5	2
11-20	9 (16.4)	6 (10.9)	2	5
21-30	11 (20.0)	0	3	NA
31-40	8 (14.5)	0	3	NA

CCD, cross-reactive carbohydrate determinant; MAST, multiple allergen simultaneous test; CLA, chemiluminescent assay; NA, not applicable.

^an = 55 patients. Percentages may not total 100 because of rounding.

predictive values. However, other systems have been less thoroughly evaluated and are considered less accurate.⁷ Therefore, the MAST-CLA and MAST-immunoblot were compared and their agreement evaluated using ImmunoCAP as a comparison method.

Until now, no standard cutoff classification for MAST-CLA had been established, to our knowledge; this study used class 1 as the cutoff classification for MAST-CLA positivity, in contrast to previous studies^{2,11} that used class 2 as the cutoff. Because class 1 status was believed to be meaningful in asymptomatic individuals^{5,12} and due to the lack of definitive evidence that positive results should be considered to indicate sensitivity to allergens for which cross-reaction or contamination should be avoided,^{2,13,14} we decided that class 1 of MAST-CLA should be considered positive; we then compared the results of MAST-CLA with those of MAST-immunoblot and ImmunoCAP.

Comparing MAST-CLA with MAST-immunoblot, MAST-CLA (53.4% positive) showed a 2.7-fold higher positivity than MAST-immunoblot (19.5% positive) for 20 common allergens included in the Korea 1 and Korea 2 inhalant and food panels. Compared to ImmunoCAP positive results, MAST-CLA showed a higher rate of agreement than MAST-immunoblot (75.9% vs 62.4%, respectively). Based on this comparison, MAST-CLA

appeared to be the superior assay. However, almost all 55 patients displayed concurrent positivity for multiple allergens in the MAST-CLA panel. The mean positive allergen number per patient in MAST-CLA was 15.9, a 3-fold higher increase compared with MAST-immunoblot (ie, 5.6). Greater than 50% of patients with MAST-CLA had concurrent positivity for more than 10 allergens; 8 of 55 patients showed concurrent positivity for 31 or more allergens in MAST-CLA. Using MAST-immunoblot, most patients (67%) showed positivity for less than 7 allergens; none of the patients was concurrently positive for 20 or more allergens by this method.

Considering the high positivity in MAST-CLA across all allergens, particularly the frequent concurrent positivity for multiple (and sometime all) allergens in the panel, the possibility of false-positive results observed in MAST-CLA could not be ignored, despite the fact that MAST-CLA showed higher agreement with ImmunoCAP

compared to MAST-immunoblot. Another explanation for concurrent positivity may be that cross-reactions between allergens result from similar protein-binding epitopes or cosensitization.^{13,15,16} However, the pattern of positive reactions was too broad to cover most of the inhalant and food allergens; also, the determination of cross-reactions or cosensitization was difficult. Similar to the high rate (37%) of false positives found by other researchers¹⁴ in the nonallergic patient group when class 1 was applied as the cutoff class in MAST-CLA, the results of MAST-CLA were considered to display numerous false positive results.

There was greater agreement between MAST-CLA and ImmunoCAP than MAST-immunoblot and ImmunoCAP. Approximately 38% of ImmunoCAP tests (51 of 133) were performed on 12 serum specimens that displayed concurrent positivity for greater than 20 allergens. The higher agreement rate of MAST-CLA compared with MAST-immunoblot may be due to a high false-positivity rate caused by cross-reactions or nonspecific reactions. In allergic diseases, screening and diagnosis include gathering the patient's medical and personal history. Therefore, allergy tests such as allergen-specific IgE should be specific rather than sensitive. Viewed from this perspective, MAST-CLA has limited value for the diagnosis of allergic diseases because retesting with another method is necessary when there is concurrent positivity for multiple allergens.

According to a recently published study,² the new automated MAST-CLA system AP720S (Hitachi Chemical Diagnostics) showed a strong concordance rate with manual MAST-CLA and decreased reactivity by class 0/1 to 1 for specimens below class 2. However, to our knowledge, no reports have been published regarding reductions in frequency of concurrent and false-positive results for most of the allergens in the panels through the use of the automated (vs manual) MAST-CLA system.

The MAST-immunoblot test strips used in this study included a CCD marker as an indicator of the clinical relevance of specific IgE results. Although the importance of specific IgE against CCDs is not yet fully understood, the determination of specific IgE against CCDs can be especially useful when a patient is concurrently positive for multiple allergens. Depending on the method, the numbers of patients recorded as having positive allergens in CCD was somewhat different; generally, CCD-positive patients reacted to more allergens in MAST-CLA than in MAST-immunoblot. For example, one CCD-positive patient showed reactivity toward 4 to 6 allergens in MAST-immunoblot but 31 to 40 allergens in MAST-CLA. These results indicate that the high, false positivity for overall allergens in MAST-CLA was caused by other factors, such as nonspecific reactions or cross-reactivity. Definitive reasons why nonspecific reactions occurred more frequently in MAST-CLA than in other methods are currently unknown. It is possible that the nonspecific reactions were caused by the differences in the allergens in the kit due to manufacturing methods, testing procedures such as incubation time and reagents, and in automated equipment or manual testing.

This study has several limitations. Subjects were patients who were MAST-CLA-positive for any allergen. This selection bias may have caused relatively higher positivity in MAST-CLA than in MAST-immunoblot. The reasons for high positivity in MAST-CLA were not known. Hypothetically, the high positivity in MAST-CLA could be caused by many false-

positive results occurring due to nonspecific reactions, cross-reactions, or cosensitizations.

Although agreement with MAST-immunoblot was higher for MAST-CLA than MAST-immunoblot, MAST-CLA cannot be conclusively considered superior to MAST-immunoblot for the diagnosis of allergic diseases because the high agreement rate with ImmunoCAP could have been caused by concurrent false-positive results for multiple allergens. Also, although MAST-immunoblot showed particularly lower agreements with ImmunoCAP for shrimp, cow's milk, and several other allergens, the frequency of concurrent positive results for multiple allergens, and the number of allergens showing concurrent positivity were lower in MAST-immunoblot than in MAST-CLA. Therefore, MAST-immunoblot may help health care professionals diagnose allergic diseases more quickly and help them avoid confusion arising from concurrent positivity for multiple allergens. LM



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