Correlation between skin prick test and MAST-immunoblot results in patients with chronic rhinitis

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Summary

Background and Objectives: **The most reliable method for confirming the causative allergens of allergic rhinitis is the skin prick test, followed by the multiple allergen simultaneous test (MAST), which reportedly has acceptable sensitivity and specificity. This study was designed to confirm whether a novel MAST-immunoblot assay can reliably diagnose allergic rhinitis.**

Methods: **A retrospective chart review was conducted of chronic rhinitis patients who visited Yeouido St. Mary's Hospital between January 2010 and June 2011.**

Results: **In total, 193 subjects (111 male, 82 female) were included, with a mean age of 30.08 years (range 6–77). The skin prick test detected 132 subjects as having one or more positive responses to allergens, and MAST detected 105 subjects as having one or more positive response. The sensitivity, specificity, and efficiency of the MAST assay were 63.16%, 65.57%, and 63.92%, respectively. Sensitivity, specificity and efficacy for common allergens were not high enough for MAST to replace skin prick test in detecting causative allergens. When correlation was defined as a difference between the classes of MAST and SPT of less than 2, the correlation rates for Dermatophagoides farina and Dermatophagoides pteronyssinus were 65.80% and 59.07%, respectively.**

Conclusion: **The correlation between MAST and the skin prick test is not sufficiently strong to use MAST as a diagnostic test to confirm the causative allergen in allergic rhinitis. Further studies to confirm the reliability of MAST should be conducted.** ***(Asian Pac J Allergy Immunol 2012;31:20-5)***

Key words: *allergic rhinitis, multiple antigen simultaneous test, specific immunoglobulin E, skin prick test, immunotherapy*

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Introduction

Allergic rhinitis is one of the most common diseases encountered by rhinologists. The prevalence of allergic rhinitis among the general population can be as high as 20% in South Korea,[1] and statistical reports from the government indicate that the number of outpatients increases 10% annually, reaching 5,560,000 in 2010. A diagnosis of allergic rhinitis is confirmed by using an allergen confirmation test with either in vivo or in vitro methods. The former include the skin prick test (SPT) and the nasal provocation test. The latter includes several tools to measure serum total IgE, serum specific IgE (sIgE) and the eosinophil count, and a nasal smear test. Confirmation of causative allergens is important both for avoidance and specific immunotherapy (SIT). Various methods are used for diagnosis. The gold standard method is SPT, but this involves skin pricking and the results can be affected by recent medications.[2] Therefore, several serum-specific IgE measurement methods have been developed. These can be classified according to the measuring process: radioallergosorbent test (RAST), fluoroallergosorbent test (FAST), and multiple allergen simultaneous test (MAST).[3,4] MAST can be further divided according to the differences in the solid phase when the allergens are attached. For the last 20 years, the most commonly used MAST has been the MAST-chemiluminescence assay (CLA). Recently, the MAST–immunoblot assay was developed and entered the market.[5] MAST-CLA has increased in popularity because it has several advantages over RAST: it costs less and does not require radioactive

agents. However, it has disadvantages such as a long laboratory time (24–48 hours), higher positive rates, and lower sensitivity compared to SPT.[6] A recently developed MAST-immunoblot assay is much simpler and requires a shorter laboratory time (3 hours).[5] In clinical practice, it is important to know the reliability of serum sIgE test systems. Therefore, the primary purpose of this study was to compare sensitivity, specificity, and correlations between the new serum sIgE test system, AdvanSure AllergyScreen (AS; LG Life Science, Seoul, Korea), and SPT among South Korean patients with chronic rhinitis.

Methods

Subjects

Retrospective chart reviews were performed for patients who visited the rhinology clinic between January 2010 and June 2011 with nasal stuffiness, watery rhinorrhea, itching, and sneezing. This study was approved from the Internal Review Board of Yeouido St. Mary's Hospital. Subjects were included if the results of both SPT and AS were available; the final study population was 193 patients (111 male, 82 female) with an average age of 30.08 years (range 6–77).

SPT

SPTs were performed following established methods using standard allergen extracts (Table 1), negative controls (saline), and positive controls (histamine) provided by Bencard, Brentford, UK. After dropping positive or negative control allergen extracts, epicutaneous pricks were performed. After 20 minutes, using histamine as a positive contrast solution, we compared the average value of the longest diameter and the longest diameter in the perpendicular plane of the former longest diameter with the positive contrast solution. Average values below 25% were judged as negative; values from 25–50% were judged as 1+; values from 50–100% were judged as 2+; values from 100–200% were judged as 3+; values from 200–300% were judged as 4+; values from 300–400% were judged as 5+; and values above 400% were judged as 6+. Results that were over 2+ were considered to be positive responses.[5]

MAST-immunoblot assay

MAST was performed using Advansure Alloscreen (AS: LG Life Science, Seoul, Korea). AS results were obtained for serum-specific IgE for 41 allergens (Table 1). Total IgE can be classified into

Table 1. Allergens tested with SPT and AS; a total of 20 allergens were matched in two tests.

	SPT	AS
1	Milk	
2	Egg	
3	Peach	
4	*Dermatophagoides farinae*	
5	*Dermatophagoides pteronyssinus*	
6	Cat fur	
7	Dog hair	
8	Cockroach	
9	Alternaria	
10	Aspergillus	
11	Penicillium	
12	Ash	
13	Hazelnut	
14	Oak	
15	poplar	
16	rye	
17	Bermuda	
18	Ragweed	
19	Mugwort	
20	Dandelion	
21	B2 grass pollen	Soybean
22	B3 tree pollen	Crab
23	Alder	Shrimp
24	Ash	Acacia
25	Beech	Sallow willow
26	Birch	Japanese cedar
27	Willow	Syacamore
28	Fat hen	Orchard grass
29	Cocksfoot	Timothy grass
30	Nettle	Goldenrod
31	Plantain	Pigweed
32	Elder	Russian thistle
33	Chrysanthemum	Housedust
34	Kapok	Sweet vernal grass
35	Fusarium spp.	Reed
36	Rhizopus nigricans	Pine
37	Cladosporium	Oxeye daisy
38	Plaice	Japanese hop
39	Cheese	Mackerel
40	Chocolate	
41	Wheat grain	
42	Cod	
43	Lobster	
44	Mixed nuts	
45	Pork	
46	Peanut	
47	Feather mixed	
48	Cow dander	
49	Goat hair	
50	Horse hair	
51	Sheep wool	
52	Hay dust	
53	Histamine control	
54	Prick control	

AS; AllergyScreen, SPT; Skin prick test

positive and negative, using 100 IU/mL as a standard. Testing was performed according to the manufacturer's recommendations. Briefly, 250 uL of each patient's serum were pipetted into a reaction trough containing the allergens on a nitrocellulose membrane and incubated at room temperature for 45 minutes. After washing, an antihuman IgE antibody coupled with biotin was added and incubated at room temperature for 30 minutes. After washing to remove unbound antibodies, 250 ml of streptavidin conjugated to alkaline phosphatase were added and incubated at room temperature for 20 minutes. Non-bound conjugate was removed by washing. After adding the luminescent solution and incubating at room temperature for 20 minutes, test strips were completely dried and read using AdvanSure AlloScan. The software determined the class of (0.0–6.0) the specific IgE concentrations. In clinical practice, allergens with results greater than that of class 2 (sIgE ≥ 0.7 kU/L) were considered positive.[7]

Analyses of results

After counting patients who were true positive (TP; positive results in both tests), true negative (TN; negative results in both tests), false positive (FP; positive in AS but negative in SPT), and false negative (FN; vice versa), the sensitivity, specificity, and efficacy of AS were also calculated according to the following formula using the results of SPT as the standard; sensitivity = TP/(TP+FN), specificity = TN/(TN+FP), and efficacy = (TP+TN)/(TP+TN+FP+FN). The agreement rate was calculated as the ratio of the number of patients in whom the difference between classes of AS and SPT was less than 2 over the total number of patients. Statistical analysis was done using SPSS 18.0 (SPSS; Chicago, IL, USA). Correlations between the classes of AS and SPT were calculated using Pearson's analysis and were considered significant if the correlation coefficient was over 0.3.[8]

Results

In total, 132 subjects were positive for at least one allergen in SPT, and 105 tested positive for at least one allergen in AS. The allergens that were most frequently positive in SPT were *Dermatophagoides farinae* (*Df* ; 79.69%, n=106), *Dermatophagoides pteronyssinus* (*Dp*; 68.42%, n=91), and oak pollen (12.78%, n=17). Those in AS were *Df* (69.52%, n=73), *Dp* (59.05%, n=62), and housedust (50.48%, n=53).

The sensitivity of AS over SPT was 63.16%, specificity was 65.57%, and efficacy was 63.92% (Table 2). The sensitivity, specificity, and efficacy of common allergens are also shown in Table 2.

Table 2. The number of patients that were positive in each test; sensitivity, specificity, and efficacy were calculated based on these results.

Allergens	SPT (+) AS (+)	(+) (-)	(-) (+)	(-) (-)	Sensitivity	Specificity	Efficacy
Df	57	39	10	87	59.38	89.69	74.61
Dp	38	52	9	94	42.22	91.26	68.39
Cat	4	12	5	172	25.00	97.18	91.19
Dog	5	8	2	178	38.16	98.89	94.82
Cockroach	0	7	10	176	0.00	94.62	91.19
Alternaria	1	0	0	192	100.00	100.00	100.00
Aspergillus	0	1	2	190	0.00	98.96	98.45
Penicillium	0	2	0	191	0.00	100.00	98.96
Ash	0	7	0	186	0.00	100.00	96.37
Hazelnut	0	12	1	180	0.00	99.45	93.26
Oak	0	17	2	174	0.00	98.86	90.16
Poplar	0	2	1	190	0.00	99.48	98.45
Rye	0	6	3	184	0.00	98.40	95.34
Bermuda	0	3	10	180	0.00	94.74	93.26
Ragweed	1	7	3	182	1.25	98.38	94.82
Mugwort	1	11	6	175	8.33	96.69	91.19
Dandelion	0	3	1	189	0.00	99.47	97.93
Milk	0	1	9	183	0.00	95.31	94.82
Egg	0	1	5	187	0.00	99.45	96.89
Peach	0	0	3	190	0.00	100.00	98.45
Total	84	49	20	40	63.16	65.57	63.92

AS; AllergyScreen, SPT; Skin prick test, Dp; *Dermatophagoides pteronyssinus*, Df; *Dermatophagoides farinae*

The agreement rate across all allergens was 91.89%, and those across *Df* and *Dp* were 65.80% and 59.07%, respectively (Tables 3, 4, 5). The agreement rate across all allergens was as high as 91.89%, as the true negative rate was high. With true negative counts excluded, the overall agreement rate was 38.79%. Agreement rates for allergens other than *Df* and *Dp* outranked *Df* and *Dp* because of the high true negative counts.

Pearson's rank correlation analysis revealed statistically significant positive correlations (p <0.01) between allergens such as *Df*, *Dp*, cat hair, and dog hair, although the correlation coefficients were all less than 0.3, with the exception of *Df*. Correlation coefficients for other allergens were not statistically significant (Table 6).

Table 4. Rank correlation of AS system serum sIgE test class with SPT grade in *Df*.

SPT grade	AS test class 0	1	2	3	4	5	6	Sum
-	86	1	8	1	1	0	0	97
1+	0	0	0	0	0	0	0	0
2+	14	0	3	0	0	1	1	19
3+	13	2	7	7	7	2	5	45
4+	5	2	0	4	2	1	3	17
5+	2	1	2	0	1	1	1	8
6+	0	0	0	0	3	2	2	7
Sum	120	6	20	14	14	7	12	193

AS; AllergyScreen, sIgE; specific Immunoglobuline E, SPT; Skin prick test, *Df*; *Dermatophagoides farinae*

Discussion

Although the prevalence of allergic rhinitis is high, it is difficult to differentiate it from other causes of rhinitis. Identification of the causative allergens, or serum sIgEs, is important in treating allergic patients because the fundamental treatment of causative allergens and allergy is generally SIT. The allergens that cause the allergy are the most important factors in selecting SIT as the main treatment method. The standard method for allergy diagnosis is the skin prick test (SPT), which has high sensitivity and good reproducibility. However, it requires multiple skin pricks and the results can be influenced by recent medications. Various methods to measure serum-specific IgE(sIgE) have been developed to overcome these limitations, and upon development were confirmed to have good reliability and correlation with SPT.[1,3,5-7,9-14] With the advent of new serum sIgE test kits, it is important to verify the agreement of new products with preexisting products.

RAST is a method for testing sIgE that was widely used for 20 years before MAST was developed in 1984. The sensitivity and specificity of RAST are as high as 88% and 83%, respectively,[9] and agreement with SPT ranges from 67–84.6%.[11,13] However, RAST requires a long time to perform, can test only one allergen at a time, uses radioisotopes, and requires use of an expensive device. Because of these disadvantages, MAST has recently replaced RAST.

The principle of FAST is similar to RAST but involves an antigen-antibody reaction that uses a fluorescent enzymatic detection system instead of the radioisotope used in RAST. The sensitivity and specificity of FAST were recently reported to be as high as 75.5% and 93.3%, respectively,[15] and agreement with SPT ranges from 63–70.3%.[11,13]

Table 3. Rank correlation of AS system serum sIgE test class with SPT grade in 20 allergens tested by both AS and SPT.

SPT grade	AS test class 0	1	2	3	4	5	6	Sum
-	3412	58	48	18	6	5	3	3550
1+	10	0	1	0	0	1	0	12
2+	79	0	8	1	2	1	2	93
3+	58	11	11	12	9	2	6	109
4+	17	4	6	8	6	3	3	47
5+	11	2	4	4	1	1	2	25
6+	9	0	3	3	5	2	2	24
Sum	3596	75	81	46	29	15	18	3860

AS; AllergyScreen, sIgE; specific Immunoglobuline E, SPT; Skin prick test

Table 5. Rank correlation of AS system serum sIgE test class with SPT grade in *Dp*.

SPT grade	AS test class 0	1	2	3	4	5	6	Sum
-	90	4	4	2	1	1	1	103
1+	0	0	0	0	0	0	0	0
2+	15	0	2	1	1	0	0	19
3+	14	8	3	2	1	0	1	29
4+	5	2	4	4	4	2	0	21
5+	5	1	2	3	0	0	1	12
6+	2	0	2	3	2	0	0	9
Sum	131	15	17	15	9	3	3	193

AS; AllergyScreen, sIgE; specific Immunoglobuline E, SPT; Skin prick test, *Dp*; *Dermatophagoides pteronyssinus*

Table 6. Correlation coefficients calculated using Pearson's correlation test (n=194).

Allergen	Correlation coefficient	*p* value
Df	0.363**	0.000
Dp	0.268**	0.004
Cat	0.292**	0.001
Dog	0.268**	0.000
Cockroach	0.007	0.927
Milk	0.019	0.791
Crab	0.062	0.394
Oak	0.033	0.644
Hazelnut	0.023	0.746
Mugwort	0.078	0.278
Ragweed	0.135	0.060
Bermuda	0.024	0.735
Rye	0.026	0.718

**: statistical relevance

Dp; *Dermatophagoides pteronyssinus*, *Df*; *Dermatophagoides farinae*

Nevertheless, MAST continues to be more widely used because it involves a much simpler procedure than FAST.

MAST can be subdivided based on the methods used during the solid phase when the allergens are attached. The first method developed was MAST-RIA: this method had advantages over RAST, as it required neither radioactive elements nor an expensive device and could test multiple allergens simultaneously. Nevertheless, MAST-RIA was withdrawn from the market after MAST-CLA was developed one year later; MAST-CLA required a shorter testing time and used easy-to-keep reagents.[16] MAST-CLA is a popular test method with sensitivity and specificity as high as 85% and 82%, respectively,[9] and its agreement rate with SPT is as high as 71.5%.[10]

The more recently developed MAST-immunoblot method reduces testing time from 48 hours to less than 3 hours and involves a simplified testing procedure. Jiang et al.[12] reported the sensitivity and specificity of Allergyscreen, one of the MAST immunoblot assay methods other than AS that we used in the present study. Sensitivity and specificity for *Dp* were 65.9% and 94.9%, respectively, and those for the total number of patients were 78% and 86.2%. One report that measured AS against another MAST method, RIDA Allergyscreen, concluded that AS results are compatible with those of Allergyscreen.[7] However, AS, a commercially-available MAST-immunoblot method used in the present study, has agreement rates with SPT of 65.89% for *DF*, 59.07% for *Dp*, and lower for other allergens. Its sensitivity, specificity, and efficacy rates are 63.16%, 65.57%, and 63.92%, respectively, calculated using SPT as a standard. From these results, we conclude that each MAST method may show different sensitivities and specificities. In addition, although AS is a good method that shows results that are compatible with other MAST methods, AS results themselves cannot represent specific allergens for SIT of allergic rhinitis patients because their compatibility with SPT is not satisfactory.

Based on these values, AS can diagnose allergic rhinitis in chronic rhinitis patients. However, the method may be insufficient to replace SPT as a confirmation test for causative allergens when preparing SIT. As SPT is the gold standard method but not a definitive method for finding causative allergens, these results do not mean that AS results are unreliable. Further studies are needed to evaluate which method is the best to diagnose allergic rhinitis or to detect the causative allergen.

In conclusion, AS has limitations in identifying causative allergens for SIT, but it is a valuable test for screening allergic rhinitis in chronic rhinitis patients. Further studies involving larger study populations are needed to compare AS with allergic symptoms, SPT results, and other serum sIgE tests to evaluate its efficacy.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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