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Original Article

Allergic children with extremely high total IgE but no allergen identified in the initial screening panel

I-Hui Lin ^a, Ming-Chin Tsai ^a, Jun-Peng Chen ^b, Lin-Shien Fu ^{c,*}^a Department of Pediatrics, Taichung Veterans General Hospital, Taichung, Taiwan^b Biostatistics Task Force of Taichung Veterans General Hospital, Taichung, Taiwan^c Division of Immunology and Nephrology, Department of Pediatrics, Taichung Veterans General Hospital, Taichung, Taiwan

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KEYWORDS

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Abstract *Background:* High serum IgE level in atopic children usually implies a highly sensitized condition. However, there is a subgroup of atopic children for whom a specific allergen cannot be identified. In this study, we analyzed follow-up data from these children.

Methods: From March 2014 to July 2017, we recruited 14 atopic children with serum total IgE level higher than 500 Ku/L, but with no specific allergen identified by repeated MAST tests initially. Follow-up studies of specific IgE were conducted by the OPTIGEN MAST Allergy test and ImmunoCAP assays (Thermo Fisher Scientific/Phadia), while total IgE and specific IgG were measured by ImmunoCAP.

Results: The patients were aged from 2 to 17 y/o. The follow-up MAST tests showed significantly positive results in 10 patients. There were no significant differences in any of the clinical characteristics between the MAST-positive and MAST-negative groups. In the MAST-negative group, five allergen-specific IgE antibodies, including those for cockroach, Euroglyphus maynei, Blomia tropicalis, shrimp, and crab, were strongly predictive of negative ImmunoCAP results, according to ROC (Receiver operating characteristic curve) analysis of the AUC (Area under the Curve of ROC) (0.70–0.95), with significance set at $p < 0.05$.

Conclusion: In two thirds of atopic children with a high serum IgE whose specific allergen had yet to be identified, it was possible to identify the specific MAST allergen(s) after an average follow-up of 33.2 months. For patients who still had negative results in follow-up MAST, mite DP, DF, and DM may be suitable choices for further allergen identification by ImmunoCAP.

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* Corresponding author. Division of Immunology and Nephrology, Department of Pediatrics, Taichung Veterans General Hospital, 1650 Taiwan Boulevard Sect. 4, Taichung, 40705, Taiwan, +886 4 23592525-5909; +886 4 23741359

E-mail address: linshienfu@yahoo.com.tw (L.-S. Fu).

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Introduction

The prevalence of allergic disease has increased in industrialized countries^{1,2} including Taiwan.³ Elevated serum total IgE and allergen-specific IgE are features of atopy. Serum total IgE may reach over 10 times the normal level in atopic individuals.⁴ Concentrations of allergen-specific IgE are correlated with symptoms.⁵ High total IgE levels may indicate greater dysregulation of the immune system in atopic disease⁶ and increase the probability of sensitization.^{7,8} Children with allergic symptoms and a high level of serum total IgE imply high sensitization. There is an increased likelihood of identifying allergen-specific IgE in patients with elevated total IgE.^{9–11} Increased total IgE levels are associated with detection of inhalant allergens and respiratory allergic symptoms.^{12–14} Allergen-specific IgE can be identified by skin test or in vitro immunoassays for IgE-specific allergens. Identification of allergens affecting these patients may help them avoid allergic responses, facilitate treatment decision-making, and improve prediction of prognosis.

Further allergen-specific IgE examinations yielded strong positive results in most patients with high total serum IgE. The OPTIGEN® assay (Hitachi Chemical Diagnostics, Inc., Mountain View, CA) contains 36 allergens that are common in Taiwan and is the most popular tool for allergen-specific IgE identification. However, there was a subgroup of these patients without positive allergen-specific IgE in the MAST. Previous study showed a greater prevalence of positive results from extended inhalant allergen tests in patients with elevated total IgE compared with those with normal total IgE.¹³ The study recruited patients aged 10–79 years and compared differences in expanded allergen-specific IgE panel results between patients grouped by a total IgE cut-off level of 116 kU/L.

We postulate that in patients with specific IgE that cannot be detected by MAST, it may be possible to use other commercial examinations to detect either the same antigen contained in MAST or additional antigens not included in the initial screening panel.

The aim of this study was to determine whether (1) further allergen-specific IgE could be identified by other tests, and (2) allergen-specific IgG may compete with corresponding specific IgE and bind to available binding sites of the allergen leading to false negative results.

Materials and methods

Participants

During March 2014 and July 2017, there were 434 patients with clinical history of allergic diseases and total IgE >500 kU/L from the Division of Allergy, Immunology and Rheumatology, Department of Pediatric and Child Health Care, Taichung Veterans General Hospital. Among these patients, 399 patients had at least one positive result in MAST and 35 patients had negative result in MAST. 14 patients with negative result in MAST were recruited into this study. Approval for the study was granted by the Taichung Veterans General Hospital Institutional Review Board (approval number: CF17232A#1). Informed consent was

obtained from each patient and his or her parents/guardians. All participants received a questionnaire about allergic symptoms, family history, birth history, and other basic information. Serum samples were drawn for measurement of serum total IgE, allergen-specific IgE, and allergen-specific IgG from each patient. The inclusion criteria were as follows: (1) patients had clinical symptoms of allergic disease; (2) total serum IgE >500 IU/ml; (3) none of the results of the MAST examination > class 1 by repeated tests; (4) had no cancer nor other immunologic disease.

Retrospective comparison of MAST_{initial-} and MAST_{initial+}

The 14 participants in this study were termed as MAST_{initial-}. Four age-, sex- and total IgE-matched controls for each MAST_{initial-} case were randomly selected. Among 399 patients who had at least one positive result in MAST, 56 control subjects were randomly selected as MAST_{initial+}. The basal characteristics and allergic symptoms between two groups were compared.

Serum samples

About 6 ml venous blood was collected from every participant. A serum sample was obtained after centrifugation followed by storage at –79 °C until analysis.

Serum total IgE and allergen-specific IgG assays

Serum total IgE and allergen-specific IgG were detected by ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). There were 25 allergens for specific IgG comprising 8 inhalant allergens *Dermatophagoides pteronyssinus* (mite DP), *Dermatophagoides farina* (mite DF), *Dermatophagoides microceras* (mite DM), *Euroglyphus maynei* (mite EM), *Blomia tropicalis* (mite BT), cat dander, dog dander, cockroach mix, and 17 food allergens (egg white, shrimp, crab, cow's milk, wheat, rice, peanut, pecan nut, cashew nut, pistachio, walnut, kiwi, melon, mango, banana, peach, pineapple).

Allergen-specific IgE assays

The serum allergen-specific IgE were assayed by OPTIGEN® Allergen-Specific IgE Assay (Hitachi Chemical Diagnostics, Inc., Mountain View, CA) and ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). The results of ImmunoCAP were classified into 7 levels ranging from class 0 to class 6 (<0.35 kU/L, Class 0; 0.35–0.7 kU/L, Class 1; 0.7–3.5 kU/L, Class 2; 3.5–17.5 kU/L, Class 3; 17.5–50 kU/L, Class 4; 50–100 kU/L, Class 5; >100 kU/L, Class 6). The OPTIGEN® assay was used in the semi-quantitative determination of allergen-specific IgE concentrations. The results were interpreted as classes 0–4. MAST pette chambers contain 36 kinds of allergens including latex, avocado, pork, beef, milk, cheddar cheese, shrimp, crab, clam, codfish, tuna, peanut, soybean, wheat (food), brewer's yeast, egg yolk, egg white, chicken feathers, Bermuda grass, black willow,

eucalyptus, Japanese cedar, white mulberry, pigweed, ragweed mix I, Timothy grass, Alternaria, Aspergillus, Cladosporium, Penicillium, cat, dog, housedust, cockroach mix, mite DF, and mite DP. Twenty-four allergens including 8 inhalant allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Dermatophagoides microceras*, *Euroglyphus maynei*, *Blomia tropicalis*, cat dander, dog dander, cockroach mix) and 16 food allergens (egg white, shrimp, crab, wheat, rice, peanut, pecan nut, cashew nut, pistachio, walnut, kiwi, melon, mango, banana, peach, pineapple) were chosen for ImmunoCAP for comparison.

Definition of specific IgE positive in the two methods.

Values of class 2 or above were considered positive for both methods.

Statistical analyses

Statistical analyses were performed by IBM SPSS version 22.0 (International Business Machines Corp., New York, USA). Continuous variables were compared using the Mann–Whitney U-test. Group comparisons of categorical variables were performed using the Chi-square test. Receiver operating characteristic (ROC) curves analysis of the results of two tests and Kappa coefficient were applied using the MedCalc statistical software version 14.0. P-values <0.05 were considered indicative of statistical significance.

Results

Retrospective comparison of MAST_{initial-} and MAST_{initial+}

The basal characteristics and allergic symptoms between MAST_{initial-} and MAST_{initial+} were compared (Table 1). The MAST_{initial-} did not differ significantly from the MAST_{initial+} regarding age, sex, asthma, allergic rhinitis, atopic dermatitis, urticaria nor food allergy.

Table 1 Retrospective comparison of MAST_{initial-} and MAST_{initial+}

	MAST _{initial-} (n = 14)	MAST _{initial+} (n = 56)	p value
	n (%)	n (%)	
Age (now), median (range)	5.5 (2.0–7.3)	5.5 (3.0–7.0)	0.842
Total IgE	1135.0 (845.5–2539.8)	993.0 (749.5–1586.5)	0.340
Male	8 (57.1%)	32 (57.1%)	1.000
Asthma	7 (50.0%)	23 (41.1%)	0.763
AR	13 (92.9%)	40 (71.4%)	0.162
AD	9 (64.3%)	30 (53.6%)	0.674
Urticaria	2 (14.3%)	5 (8.9%)	0.621
Food allergy	1 (7.1%)	9 (16.1%)	0.674

Chi-square test. †Mann–Whitney U test. *p < 0.05, **p < 0.01.

Participants

The follow-up period ranged from 6 to 68 months with median 30.5 months (Interquartile range [IQR] 16.3–35.5). The follow-up MAST assay showed negative results in four patients who were thus classified as MAST-negative. The other 10 patients had positive results in the follow-up MAST assay and were classified as MAST-positive. Table 2 summarizes all of the patients' characteristics. The MAST-negative group did not differ significantly from the MAST-positive group with regard to age, sex, asthma, allergic rhinitis, atopic dermatitis, urticaria, food allergy, age at the first examination, period between two examinations, nor allergic histories in first-degree relatives. The first and second total IgE levels were 1686.71 ± 1248.66 (mean $\pm$ SD) and 1622.71 ± 1430.82 (p = 0.433). There was no statistically significant difference in first total IgE and second total IgE between MAST-positive and MAST-negative patients (Table 3).

Specific IgE in MAST and ImmunoCAP

In MAST-positive group, the most common positive allergens were mite DP (10/10) and mite DF (10/10). The results were similar in MAST Optigen and ImmunoCAP assays.

We chose the 10 most common allergens including 5 inhalant allergens and 5 food allergens for specific-IgE tests in both the MAST and ImmunoCAP assays. Agreement between the two assays for these 10 allergens showed a high Kappa coefficient and statistical significance except cockroach, shrimp, and egg white (Table 4). The specific IgE to 24 allergens in the ImmunoCAP results of the 4 patients in the MAST-negative group was positive in only 6 tests of 2 patients (class 3 in mite DP and class 2 in mite DF and mite

Table 2 Characteristics of the participants.

	MAST- (n = 4) n (%)	MAST+ (n = 10) n (%)	p value
Age (now), median (IQR)	4.0 (2.3–8.0)	8.5 (5.5–13.0)	0.102
Male	3 (75.0%)	5 (50.0%)	0.580
Asthma	2 (50.0%)	5 (50.0%)	1.000
AR	4 (100.0%)	9 (90.0%)	1.000
AD	4 (100.0%)	5 (50.0%)	0.221
Urticaria	1 (25.0%)	1 (10.0%)	0.505
Food allergy	0 (0.0%)	1 (10.0%)	1.000
Age at 1st test, median (IQR)	3.4 (1.4–5.8)	6.0 (3.5–9.5)	0.117
Period between the two examinations, (months), median (IQR)	19.0 (7.3–30.8)	32.5 (18.5–43.3)	0.076
Allergic histories in first-degree relatives	4 (100.0%)	8 (80.0%)	1.000

Chi-square test. †Mann–Whitney U test. *p < 0.05, **p < 0.01.

Table 3 Total IgE between MAST-negative and MAST-positive group.

		MAST- (n = 4)		MAST+ (n = 10)		p value
		n	Median (range)	n	Median (range)	
1st tlgE	4	820.5	(514.3–2209.8)	10	1340.5	0.258
2nd tlgE	4	549.5	(256.3–3038.0)	10	1424.5	0.203
					(793.5–2494.0)	

Mann–Whitney U test. * $p < 0.05$, ** $p < 0.01$.

Table 4 Agreement of specific IgE in two assays, MAST and ImmunoCAP.

	Kappa coefficient	p value
Cat dander	1.000	<0.001**
Dog dander	1.000	<0.001**
Cockroach	0.243	0.164
D. pteronyssinus	0.837	0.001**
D. farinae	0.650	0.015*
Shrimp	0.432	0.099
Crab	0.576	0.031*
Peanut	1.000	<0.001**
Egg, White	—	^a
Wheat	1.000	<0.001**

^a The kappa coefficient could not be calculated due to specific IgE to egg white in the follow-up MAST assay was negative in all fourteen participants in this study.

DM of patient 4; class 1 in mite DF, mite DM, and pistachio of patient 5).

When the cut-off value was >0 , five allergen-specific IgE in the ImmunoCAP assay had an excellent ability to predict negative results in the MAST-negative groups, including cockroach (AUC = 0.75, $p = 0.003$), mite EM (AUC = 0.95, $p < 0.001$), mite BT (AUC = 0.90, $p < 0.001$), shrimp (AUC = 0.70, $p = 0.014$), and crab (AUC = 0.75, $p = 0.003$) (Table 5 and Table 6).

Comparisons of specific IgG between the MAST-positive and MAST-negative groups

Comparisons of 25 allergen-specific IgG levels between the MAST-positive and MAST-negative groups were conducted

by Mann–Whitney U test. Specific IgG of mite DP and mite DF showed a higher level in the MAST-positive group than in the MAST-negative group ($p = 0.047$ and 0.048 respectively, Table 7). There were no significant differences in the specific IgG level of the other 23 allergens between two groups (Table 7).

Discussion

In this study, we investigated total IgE, specific IgE and specific IgG in patients with significant allergic symptoms and high total IgE but negative results in the original specific IgE screening panel, MAST Optigen. Our results revealed the following: (1) Ten out of fourteen participants had positive results in the follow-up MAST Optigen examination with an average interval of 33.2 months. Ten common allergens in the MAST Optigen and other additional allergens were chosen for specific IgE tests using the ImmunoCAP assay in this study. (2) Agreements between the MAST Optigen and ImmunoCAP were moderate to almost perfect with a high Kappa coefficient and statistical significance, except for cockroach, shrimp, and egg white. (3) In the MAST-negative group, five allergen-specific IgE including cockroach, mite EM, mite BT, shrimp, and crab had a high true negative rate in the ImmunoCAP assay, according to the ROC analysis. (4) Higher mite DP- and DF-specific IgG in the MAST-positive group compared to the MAST-negative group were noted. The specific IgG to the other 23 allergens showed no difference between the two groups.

A previous study investigated a birch atopy group: specific IgE to Bet V1 was first detected in patients before the age of 3 years and increased rapidly, reaching 100% at the age of 10 years.¹⁵ The highest levels for specific IgE to grass and house dust mite were observed in those who were 10–15 years of age.¹⁶ Dividing the adult population into four age groups, it was found that specific IgE to any allergen and to grass were highest in those who were 20–27.5 years of age and declined progressively in the other three age groups (27.5 to <35, 35 to <42.5, and over 42.5 years).¹⁷ All of the results are in agreement with the widely held notion that IgE sensitization tends to increase with age during childhood, reaches a peak in adolescence, and decreases with age in adults after the age of 20 years. Participants in this study were under 18 years old. Repeated specific IgE screening test eventually yielded a positive result in more than two thirds of our participants, which is compatible with previous reports.

Table 5 ROC-curve analysis of specific IgE (Inhalant Allergen).

Allergens	AUC	(95% CI)	p	Optimal cutoff	Sensitivity	Specificity
Cat danger	0.55	(0.27–0.81)	0.317	>0	10.00%	100.00%
Dog danger	0.60	(0.31–0.84)	0.134	>0	20.00%	100.00%
Cockroach	0.75	(0.46–0.94)	0.003	>0	50.00%	100.00%
D. pteronyssinus	0.98	(0.73–1.00)	<0.001	>3	90.00%	100.00%
D. farinae	0.93	(0.66–1.00)	<0.001	>2	90.00%	100.00%
D. microceras	0.93	(0.66–1.00)	<0.001	>2	90.00%	100.00%
E. maynei	0.95	(0.69–1.00)	<0.001	>0	90.00%	100.00%
B. tropicalis	0.90	(0.62–0.99)	<0.001	>0	80.00%	100.00%

Table 6 ROC-curve analysis of specific IgE (Food Allergen).

Allergens	AUC	(95% CI)	<i>p</i>	Optimal cutoff	Sensitivity	Specificity
Shrimp	0.70	(0.41–0.91)	0.014	>0	40.00%	100.00%
Crab	0.75	(0.46–0.94)	0.003	>0	50.00%	100.00%
Peanut	0.60	(0.31–0.84)	0.134	>0	20.00%	100.00%
Egg, White	0.60	(0.31–0.84)	0.134	>0	20.00%	100.00%
Wheat	0.60	(0.31–0.84)	0.134	>0	20.00%	100.00%
Rice	0.55	(0.27–0.81)	0.317	>0	10.00%	100.00%
Pecan nut	0.50	(0.23–0.77)	1.000	>0	0.00%	100.00%
Cashew nut	0.50	(0.23–0.77)	1.000	>0	0.00%	100.00%
Pistachio	0.51	(0.24–0.78)	0.927	≤1	90.00%	0.00%
Walnut	0.50	(0.23–0.77)	1.000	>0	0.00%	100.00%
Pineapple	0.65	(0.36–0.88)	0.050	>0	30.00%	100.00%
Kiwi	0.60	(0.31–0.84)	0.134	>0	20.00%	100.00%
Melon	0.60	(0.31–0.84)	0.134	>0	20.00%	100.00%
Mango	0.55	(0.27–0.81)	0.317	>0	10.00%	100.00%
Banana	0.65	(0.36–0.88)	0.050	>0	30.00%	100.00%
Peach	0.55	(0.27–0.81)	0.317	>0	10.00%	100.00%

Table 7 Comparisons of specific IgG to 25 allergens in the MAST-positive and MAST-negative groups.

	MAST- (n = 4)		MAST+ (n = 10)		<i>p</i> value
	Median	range	Median	range	
Cat dander	2.8	(1.32–6.06)	3.0	(2.24–3.43)	0.944
Dog dander	10.3	(7.48–17.45)	13.3	(10.07–15.25)	0.396
Cockroach	6.1	(3.47–10.07)	8.2	(6.07–9.89)	0.396
<i>D. pteronyssinus</i>	7.5	(4.16–13.21)	21.6	(11.87–22.28)	0.047*
<i>D. farinae</i>	9.1	(5.64–14.23)	19.6	(11.89–26.20)	0.048*
<i>D. microceras</i>	10.2	(6.57–12.90)	17.1	(12.86–23.23)	0.034
<i>E. maynei</i>	5.3	(3.27–8.21)	15.5	(6.14–20.33)	0.157
<i>B. tropicalis</i>	3.8	(2.47–5.64)	10.1	(4.43–12.55)	0.066
Shrimp	1.6	(1.00–3.96)	3.3	(2.52–4.74)	0.118
Crab	1.0	(1.00–3.06)	2.1	(1.00–3.43)	0.270
Peanut	4.5	(2.50–8.20)	4.0	(2.17–4.92)	0.671
Egg, White	23.2	(12.36–36.95)	27.2	(4.50–37.40)	0.888
Wheat	15.2	(8.35–29.95)	16.0	(7.22–19.73)	0.571
Rice	4.6	(1.49–7.57)	5.7	(4.24–7.91)	0.572
Pecan nut	2.2	(2.14–5.00)	2.6	(2.23–3.32)	0.479
Cashew nut	3.7	(1.57–5.37)	2.4	(1.00–4.03)	0.353
Pistachio	4.5	(2.33–6.20)	2.9	(1.00–4.22)	0.286
Walnut	3.4	(1.27–5.55)	1.8	(1.00–3.64)	0.418
Pineapple	7.9	(4.65–34.98)	11.8	(5.48–18.13)	1.000
Kiwi	8.2	(3.09–19.98)	6.8	(3.88–12.05)	0.888
Melon	8.2	(7.15–10.03)	5.4	(3.62–9.19)	0.157
Mango	6.3	(3.18–8.87)	4.0	(3.06–5.76)	0.396
Banana	40.7	(16.13–55.58)	19.8	(11.53–41.15)	0.396
Peach	10.1	(4.74–13.30)	6.9	(4.77–9.64)	0.480
Milk	84.1	(66.30–112.25)	71.5	(55.43–89.58)	0.322

Mann–Whitney U test. *statistically significant with *p*-value <0.05.

Comparisons of specific IgE to 10 common allergens in both the MAST and ImmunoCAP assays showed perfect agreement between the two assays for 7 of the allergens, but differences were found for cockroach, shrimp, and egg. Although MAST and ImmunoCAP utilize similar fundamental techniques for detecting specific IgE, there are differences between these two tests with respect to allergen coating,

signal detection, and antigen preparation that may contribute to differences in the results.¹⁸ Cockroach allergen in the ImmunoCAP assay uses German cockroach, while MAST uses mixed cockroach, American cockroach, and German cockroach. Allergenic cross-reactivity¹⁹ between German cockroach allergen, Bla g1, and American cockroach allergen, Per a1 has been demonstrated.²⁰

In Taiwan, most patients with bronchial asthma had positive skin tests with reaction to both American and German cockroach extracts (78/236). However, a few of these patients were allergic to only one of the two species of cockroaches (15/236 to only American cockroach and 8/236 to only German cockroach).²¹ The use of different allergen sources and the possibility that patients may be allergic to American cockroach but not to German cockroach may explain the low kappa value. Specific IgE levels to food allergens may be different according to which allergenic component induced the IgE antibody,²² the amount and frequency of consumption of the food,²³ and the age²⁴ of an individual patient. Different allergen preparation and examination methods, as previously mentioned, between the two types of tests may have contributed to the relative disagreement between the two tests for shrimp and egg. Specific IgE to egg white in the follow-up MAST assay was negative in all fourteen participants in this study. Therefore, the kappa coefficient could not be calculated. Specific IgE to egg white in ImmunoCAP revealed one class 2 and one class 1 in the fourteen participants. Interpretations of the results of specific IgE to food allergens should be supported by the clinical histories and ages of the patients. Cut-off level of specific IgE to egg of 7 kU_A/L and 2 kU_A/L for children over 2 years of age and children two years or younger, respectively, predicted clinical reactivity with greater than 95% certainty.²⁵ Therefore, the results of specific IgE to egg white in the two tests were compatible. Cross-reactivity due to tropomyosin²⁶ and hemocyanin^{27,28} among shrimp, cockroach, and house dust mite may also explain, at least in part, the discrepancy found in these results.

With elevation of total IgE, there was predicted to be more positive results in the specific IgE tests.¹¹ When results of multiallergen tests were negative, an individual test on the expanded panel may be positive to inhalant allergens.¹³ Two out of four participants in the MAST-negative group had positive results in the extended specific IgE tests of the ImmunoCAP assay. The positive results are specific IgE to mite DP, DF, DM, and pistachio. Specific IgE tests of the ImmunoCAP assay for the other 20 allergens were also negative in the MAST-negative group. In the MAST-negative group, further tests for IgE sensitization to five allergens including cockroach, mite EM, mite BT, shrimp, and crab in the ImmunoCAP assay were expected to be true negative according to the ROC analysis. Therefore, these five allergens should not be chosen from the extended allergen tests in the ImmunoCAP assay for patients in the MAST-negative group. The prevalence of 5 different house dust mites in studies conducted in northern, central, and southern Taiwan was highest for DP, followed by DF, DM, BT, and EM.^{29–31} Based on the mite prevalence in Taiwan and the results shown in this study, we suggest testing MAST-negative patients for perennial inhalant allergens such as mite DP, DF, and DM by ImmunoCAP assay.

B cells respond to an antigen by first producing IgM and then switching to production of either IgG or IgE. The amount of serum IgG was tens of thousands of times higher than the amount of serum IgE. We had initially hypothesized that in vitro specific IgE tests may have been disturbed by specific IgG to the same antigen. However, the

results in this study refuted our original hypothesis. Significantly higher levels of specific IgG to mite DP and DF were detected in the MAST-positive group than in the MAST-negative group. Serum levels of specific IgG to the other 23 allergens in the MAST-positive group did not significantly differ from those of the MAST-negative group. The pathogenicity of IgE derived from IgG1 memory B cell was proved in a recent report.³² The levels of specific IgG to peanuts,³³ airborne molecules,³⁴ Saffron pollen,³⁵ and Bet V 1¹⁵ (a major birch pollen allergen) were demonstrated to be correlated to the level of specific IgE levels for the corresponding allergen in previous studies. The results support this response pattern because a strong specific IgE response to mite DP and DF was found in almost all of the participants in the MAST-positive group.

Limitations

The small sample size was the major limitation in this study. Second, we did not perform provocation test or skin prick tests to compare the positive findings in specific IgE, because cross-reaction between crust-extract allergens had been reported.^{36,37} In addition, there was no available allergen-extracts for provocation test in Taiwan. Levels of allergen-specific IgE were affected by routes, frequency, and amount of allergen exposures. Therefore, a combination of clinical symptoms, disease history and treatment response is important to decipher the results of specific IgE.

Conclusion

IgE plays an essential part in the pathogenesis of allergic disease. The results of this preliminary studies demonstrated patients who had significant allergic symptoms and high total IgE but had no allergen findings in the initial screening test panel had a high probability of a positive result in repeated tests after an average of 33.2 months. For patients who still have negative results in the follow-up MAST assay, we recommend testing for mite DP, DF, and DM by ImmunoCAP assay. However, following identification of an allergen, the interpretation of test results and application of therapeutic strategies should be based on the individual patient's clinical manifestations, disease history, physical examinations, and environment. Further research with higher patient numbers and the use of a *component-specific IgE test* is required to clarify the cause of high total IgE in patients with allergic disease whose allergens can not be identified in the initial screening panel.

Declaration of Competing Interest

All authors declare no conflicts of interest in this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmii.2020.01.001>.