

Comparison of Two Specific Allergen Screening Tests in Different Patient Groups

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UniCAP and multiple-antigen simultaneous test (MAST) are among the widely used specific allergen tests. The Phadiatop and Fx5 are the multi-allergen UniCAP screening tests for inhalant allergens and common food allergens, respectively. We compared the differences between Phadiatop-Fx5 and MAST as screening allergen tests to clarify the optimal tests for different groups of allergic patients. Serum samples of 224 consecutive allergic patients were tested with Phadiatop, Fx5 and MAST. Results of these allergen tests were compared and analyzed in subgroups categorized by age, serum IgE levels and the clinical departments where the patients were treated. We found that among the 224 patients, 155 patients (69.2%) tested positive with Phadiatop while 137 (61.1%) tested positive with MAST for inhalant allergens. Twenty patients were Phadiatop(+)/MAST(-), while only 2 were Phadiatop(-)/MAST(+). There were 57 patients (25.4%) who tested positive with Fx5, while 32 (14.3%) tested positive with MAST for food allergens. Thirty-eight patients were Fx5(+)/MAST(-), while 13 were Fx5(-)/MAST(+). The disagreement between these two tests was more apparent in food allergen tests than in inhalant allergen tests. Most cases of disagreement occurred in younger age groups and in the patient group with IgE > 500 IU/ml. These results suggested that UniCAP allergen screening tests might be more effective in certain patient groups as screening tests. (Acta Paediatr Tw 2006; 47:116-22)

Key words: *phadiatop, UniCAP, MAST, specific allergen test*

INTRODUCTION

The prevalence of allergy has been increasing in recent years in industrialized countries including Taiwan.¹⁻⁵ The identification of specific allergens for patients, especially in cases without clinically apparent symptom-provoking substances, is thus an important issue in clinical practice. There are several diagnostic tests available for the detection of specific IgE antibodies to relevant allergens in the serum of patients with allergy. Among the specific allergen tests, UniCAP and Multiple Antigen Simultaneous Test (MAST) are currently the most widely used.

MAST simultaneously detects specific IgE antibodies against 20 common inhalant allergens and 16 common food allergens⁶ using a semi-quantitative method, whereas UniCAP quantitatively measures IgE against

single allergen or allergen mixture in each test. Phadiatop and Fx5 are two of the multi-allergen UniCAP screening tests. Phadiatop contains a mixture of 23 inhalant allergens, while Fx5 contains a mixture of 6 common food allergens.⁷⁻¹⁰ As neither MAST nor UniCAP quantitatively measure IgE against individual antigens, further quantitative measurement for IgE against specific allergens should be performed in patients noted to have positive results in these screening tests. The selection of these screening tests thus determines whether the patients' allergens can be precisely identified in an economical manner.

Although both UniCAP and MAST are widely used by allergists in different clinical disciplines, few studies have been published comparing these tests as screening tools in different clinical settings.¹¹ Here we analyzed the differences between Phadiatop-Fx5 and MAST in order

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to clarify the differences of these tests in different groups of allergic patients.

MATERIALS AND METHODS

Patients

Serum samples were collected from 224 patients who were diagnosed by physicians to have allergy by history and physical examination at National Cheng-Kung University Hospital. The common diagnoses were allergic rhinitis, asthma and atopic dermatitis. Most patients from the department of dermatology were diagnosed with atopic dermatitis. Most ENT patients were diagnosed with allergic rhinitis. Patients from pediatric, internal medicine and family medicine mainly bore the symptoms of asthma, allergic rhinitis or both (Fig. 1). Informed consents were obtained from patients or their guardians as approved by the hospital's Human Research committee. The mean age of the subjects was 39 years, with a range from 1 to 77 years. Among these patients, 213 also had their total IgE level measured. These patients thus were grouped by age (< 6 years, 6-12 years, >12 years), serum total IgE level (< 150 IU/ml, 150-500 IU/ml, > 500 IU/ml) and the clinical department (pediatrics, internal medicine/family medicine/ otorhinolaryngology [ENT], dermatology) where they were treated (Table 1). Among these patients, thirty-two randomly selected patients (age range: 2-40 years) also had an allergen skin test to be compared with UniCAP and MAST.

Laboratory measurements of total and specific IgE

Total IgE in serum was measured with a nephelometric method (Dade Behring, Liederbach, Germany). Serologic testing for specific IgE was performed using both UniCAP Phadiatop-Fx5 (Pharmacia Diagnostics, Uppsala, Sweden) and Multiple- Antigen Simultaneous Test (MAST) (Hitachi Chemical Diagnostics, Mountain View, California, USA). UniCAP and MAST are both based on the principle of immunoabsorption of IgE and chemiluminescence detection. A 10- ml venous blood sample was taken to clot at room temperature for 2 hr. Then the serum for MAST and CAP was obtained by centrifugation at 2000 rpm for 15 minutes at room

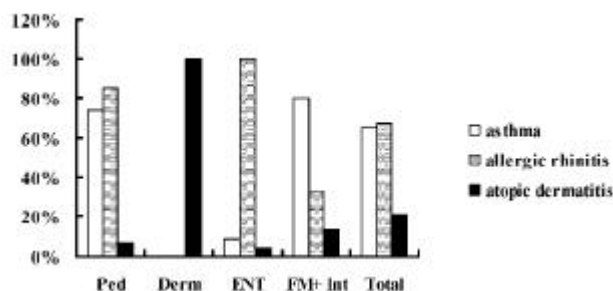


Fig. 1. Different distribution of clinical diagnosis in patients from different clinical departments. Allergic patients with the diagnosis of asthma (blank), allergic rhinitis (hatched) and atopic dermatitis (filled) are shown as percentage of patients from the particular department. A patient may have a single diagnosis or multiple diagnoses.

temperature and stored at -20°C until measurement. Then patient sera were first incubated with solid-phase absorbent coated with specific antigen. After washing, a labeled anti-human IgE was incubated with the solid-phase antigens. The amount of labeled anti-IgE was then measured. The MAST allergens include citrus, corn, wheat, vegetable mixture, crab, shellfish, shrimp, codfish, pork, beef, milk, yeast (brewers), soybean, peanut, egg (yolk and white), pine mixtures, willow, eucalyptus, mulberry mixture, grass mixture, Bermuda grass, ragweed mixture, pigweed mixture, molds (*Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Candida*), feather mixture, cat, dog, cockroach mixture, house dust and mites (*Dermatophagoides pteronyssinus* (*Der. p.*) and *farinae*). The Phadiatop comprises a mixture of 23 different inhaled allergens including mite (*Der. p.*), cat dander, dog dander, horse dander, molds (*Alternaria alternata*, *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*), *Anthoxanthum odoratum* (sweet vernal grass), *Lolium perenne* (rye grass), *Phleum pratense* (Timothy), *Secale cereale* (cultivated rye), *Holcus lanatus* (velvet grass), *Artemisia vulgaris* (mugwort), *Plantago lanceolata* (ribwort), *Chenopodium album* (lambs' quarters), *Solidago virgaurea* (golden rod), *Urtica dioica* (nettle),

Table 1. Clinical Characteristics of Allergic Patients

Age	< 6 yr	6-12 yr	> 12 yr	Total
Number	49	87	88	224
Department	Pediatrics	Int/FM/ENT	Dermatology	
Number	123	65	36	224
Total IgE	< 150	150-500	> 500	
Number	64	53	96	213

Alnus incana (grey alder), *Betula verrucosa* (common silver birch), *Coryhis avellana* (hazel), *Quercus alba* (oak) and *Salix caprea* (willow). The Fx5 contains a mixture of multiple food allergens including cow milk protein, egg white, wheat, codfish, peanut and soybean. UniCAP detected IgE antibodies in the range 0.35-100 kU/L. The cut-off point for positive results of Phadiatop-Fx5 was 0.35 kU/L in this study.¹²⁻¹⁴ Positive results of MAST were defined as ≥ 2 for at least one inhalant or food allergen.^{6,11}

Skin allergen test

Skin prick test with commercial extracts of 8 common inhalant and food allergens, including *Der. p.*, cockroach mix, dog dander, *Alternaria*, Bermuda grass, shrimp, egg white and cow milk protein (Center Laboratories, Port Washington, NY, USA), was performed. The histamine base (1mg/mL; Center Laboratories) was used as positive control and 50% glycerol saline as negative control. Skin prick tests were performed on the volar surface of forearms by means of a disposable prick tester (Stallerpointe, Stallergene, Joinville-le-Point, France). Wheal size >3 mm and itchy erythema more than 2 cm in diameter at the prick sites were judged as positive.¹⁵ The results were scored by one of the authors (YHL) blinded to the clinical impression and serological results. All the patients discontinued antihistamine for at least one week before the skin test.

Data analysis

The patients were classified into four groups (CAP+/MAST-, CAP-/MAST+, CAP+/MAST+, CAP-/MAST-) depending upon the agreement or disagreement between the 2 tests in each patient. The distribution of these classifications was compared among the subgroups of age, total IgE level and original clinical department. If a P-value ≤ 0.05 was revealed in Fisher's exact test, the subgroups were considered significant contributors to the differences between the two tests. Kappa statistic was performed in each subgroup to evaluate the agreement between the two specific allergen tests. The results of the UniCAP and MAST were also compared with allergen skin test used as a reference test.¹⁶ Kappa statistic was used to evaluate the agreement between the serological and skin tests.

RESULTS

Variable agreement of UniCAP and MAST screening tests in different patient groups

Among the 224 patients, 146 (65.2%) tested positive for UniCAP (either Phadiatop or Fx5, or both), while 107 (47.8%) tested positive with MAST (Fig. 2). The

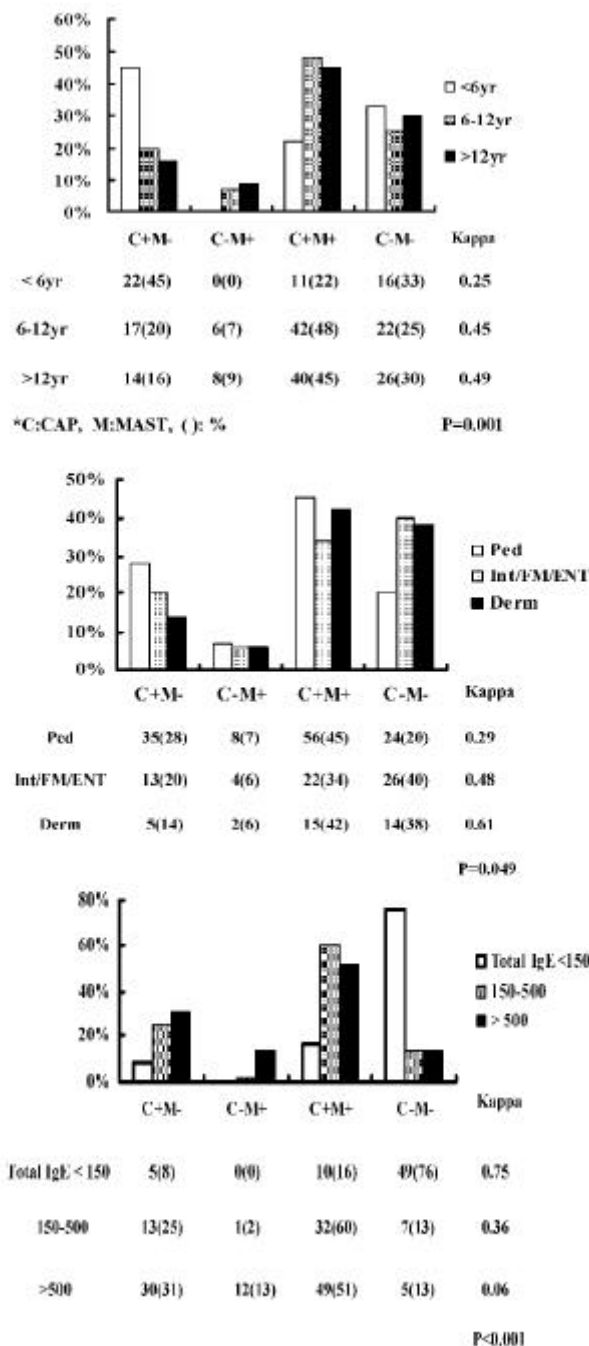


Fig. 2. Distribution of positive and negative results for MAST and UniCAP screening tests (all allergens) shown in different age groups (upper panel), different clinical departments (middle panel) and different IgE levels (lower panel). Histograms show the percentages of distribution of each group of patients as listed in the parenthesis. Kappa values for agreement between UniCAP (C) and MAST (M) are shown for each group. P values for the distribution of different groups are shown for each analysis.

distribution of agreement and disagreement of the results of UniCAP and MAST were different in patients of different age groups ($P=0.001$) and in patients with different levels of total IgE ($P<0.001$). The youngest patient group had a lower level of agreement between the two antigen-specific tests than those older patients (Kappa values for <6 yr, 6-12 yr, and >12 yr were 0.25, 0.45 and 0.49, respectively). The patients with higher total IgE level had a lower level of agreement between these two tests than those with lower IgE levels (Kappa values for total IgE level [IU/ml] >500, 150-500, <150 were 0.06, 0.36 and 0.75, respectively). The clinical departments of the patients also slightly contributed to the disagreement of these results ($P=0.049$).

High level of agreement in UniCAP and MAST screening test for inhalant allergens

In order to unravel the factors contributing to the variable agreement between UniCAP and MAST, we analyzed the results of inhalant and food allergens separately. Among the 224 patients, 155 (69.2%) tested positive with Phadiatop (UniCAP screening for inhalant allergens),¹⁷⁻¹⁹ while 137 (61.1%) tested positive for inhalant allergens with MAST (Fig. 3). The agreement of the two tests was generally high in patients belonging to different age groups and different clinical departments (Fig. 3, first and second panels) when compared with the overall results shown in Fig. 2. There was no significant difference between Phadiatop and MAST in different age groups ($P=0.062$). The clinical departments where the patients were treated did not affect the level of agreement of these results ($P=0.317$). However, there was an disagreement between Phadiatop and MAST in patients with different levels of total IgE ($P<0.001$). The patients with lower IgE level had a higher level of agreement between these two antigen-specific tests (Kappa values of total IgE <150, 150-500, >500 were 0.83, 0.46 and 0.53 respectively).

Lower agreement in UniCAP and MAST screening test for food allergens

Results of UniCAP screening test (Fv5) and MAST for food allergens were also compared. Among the 224 patients, 57 (25.4%) tested positive with Fv5, while 32 (14.3%) tested positive for common food allergens with MAST (Fig. 4). The agreement between the two screening tests was low when compared with the overall results and the results of inhalant allergen tests; hence the kappa values were lower. Moreover, the distribution of agreement and disagreement between the results of Fv5 and MAST was different in patients of different age groups ($P<0.001$) and in patients with different levels of total IgE ($p<0.001$). Low levels of agreement in these two antigen-specific tests were found in all age groups (Kappa

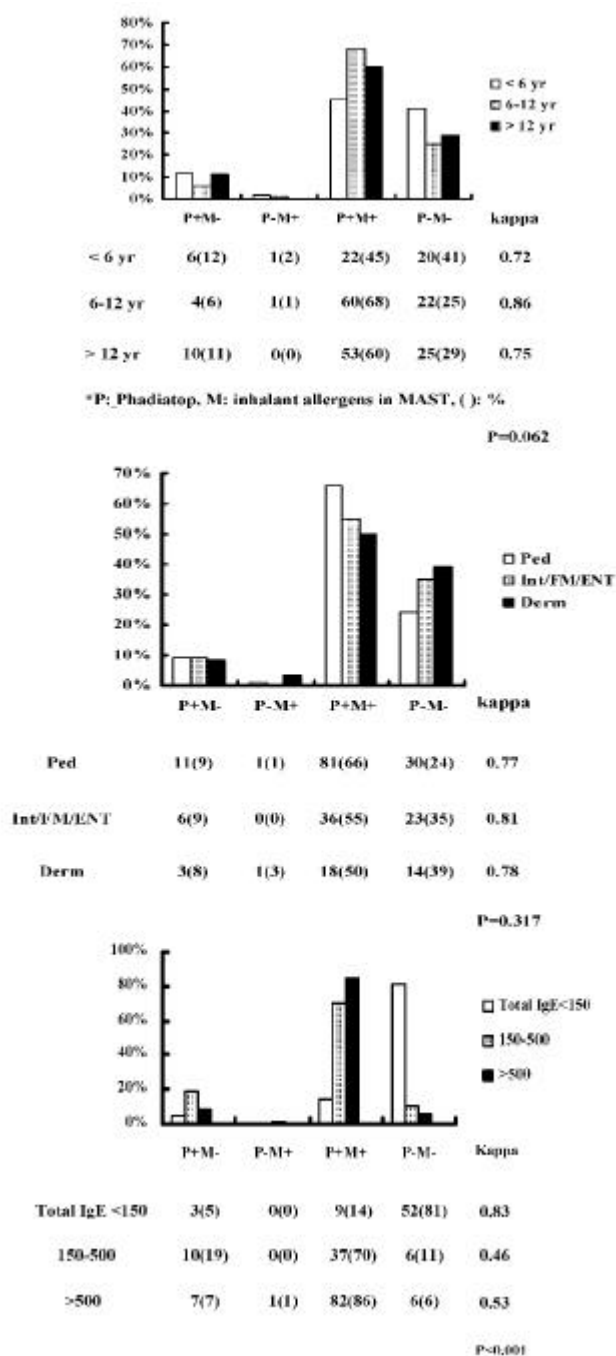


Fig. 3. Distribution of positive and negative results for MAST and UniCAP screening tests (inhalant allergens) are shown in different age groups (upper panel), different clinical departments (middle panel) and different IgE levels (lower panel). Histograms show the percentages of distribution of each group of patients as listed in the parenthesis. Kappa values for agreement and between UniCAP (C) and MAST (M) are shown for each group. P values for the distribution of different groups are shown for each analysis.

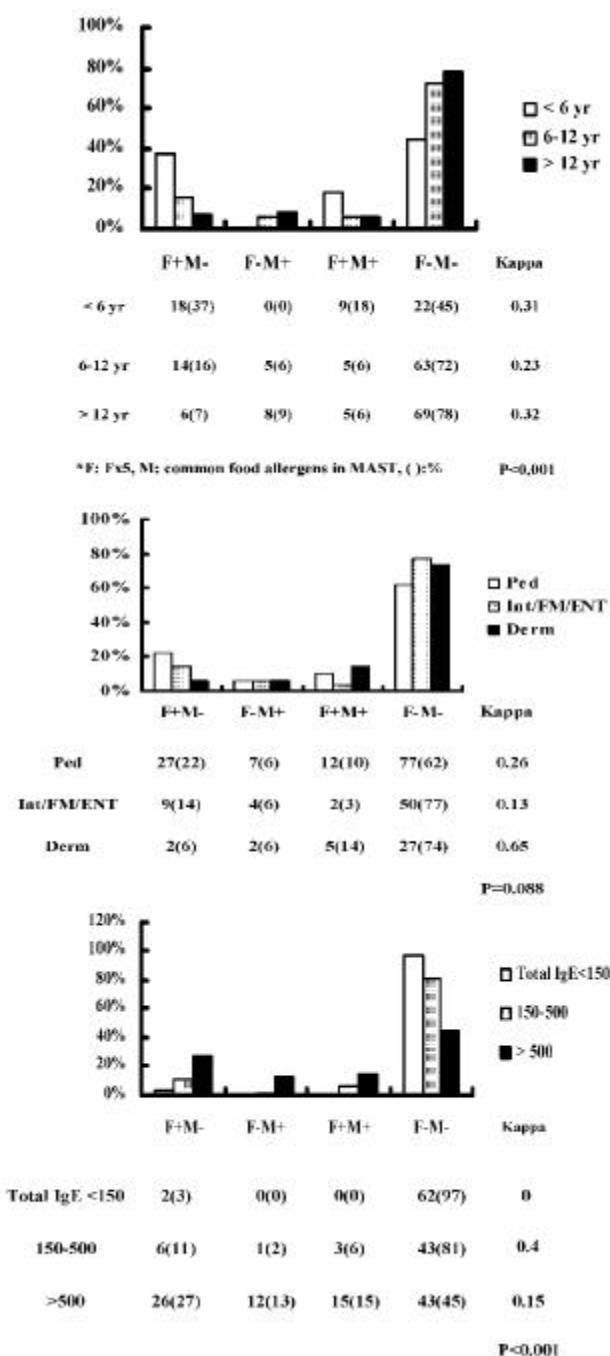


Fig. 4. Distribution of positive and negative results for MAST and UniCAP screening tests (food allergens) are shown in different age groups (upper panel), different clinical departments (middle panel) and different IgE levels (lower panel). Histograms show the percentages of distribution of each group of patients as listed in the parenthesis. Kappa values for agreement and between UniCAP (C) and MAST (M) are shown for each group. P values for the distribution of different groups are shown for each analysis.

value 0.31, 0.23 and 0.32) and patients with different IgE levels (Kappa value 0.00, 0.40 and 0.15). The clinical departments slightly affected the disagreement of the tests but did not reach statistical significance ($P=0.088$).

Low agreement of skin allergen test with MAST for food allergens

To address the discrepancy between MAST and UniCAP screening tests, we randomly selected patients from the serologically examined patients and performed allergen skin test. In the case of inhalation allergens, the skin test results of 32 patients showed that Phadiatop was slightly more sensitive than MAST in detecting patients with skin response to inhalant allergens (92 % vs. 85%; Table 2, left panel). The agreement between each allergen screening test and skin tests was high (the kappa values 0.52 for MAST and 0.67 for Phadiatop).

In the case of food allergens, the skin test also showed high agreement with Fx5, with a kappa value of 0.59 (Table 2, right panel). Similarly to the discrepancy between MAST and UniCAP screening test, a low level of agreement between MAST food allergen test and skin test was evident, with a low kappa value of 0.18.

DISCUSSION

Prevention of allergen exposure is the cornerstone of treatment for allergic diseases. The first step towards this goal is the detection of specific allergens for each patient.²⁰ Previous studies showed that specific allergens predispose patients to prolonged wheezing and severe asthma.^{21,22} Pinpointing the allergens causing airway inflammation in allergy thus empowers both the patient and the physician to effectively control and prevent the disease. To achieve this goal, UniCAP and MAST are both widely used in screening for allergen-specific IgE in patients' serum. MAST is used for detection of allergen-specific IgE antibodies using an enzyme-linked anti-human IgE and chemiluminescent assay which permits simultaneous detection of 36 different allergens.⁶ Phadiatop and Fx5 are two sensitive multi-allergen UniCAP screening tests that also detect inhalant allergens and common food allergens, respectively based on immunoabsorption.^{7,23}

In previous reports, UniCAP and MAST were both found to be useful for the detection of allergens, and the correlation between the results of CAP and MAST was generally good.¹¹ The Phadiatop test has a sensitivity ranging from 67% to 92% and high specificity 88%- 100%,¹⁷⁻¹⁹ while the sensitivity of MAST for inhalant allergens ranges from 56%-97%, with specificity 82%-98%.^{7,16,24} Kam et al, compared specificity and sensitivity of MAST and CAP for detecting specific IgE for individual

Table 2. Distribution of Positive and Negative Results for MAST, CAP and Skin Tests (Inhalant and Food Allergens)

Inhalant allergens				Food allergens			
M*+S [†] -	1	P*+S-	1	M+S-	6	F [§] +S-	5
M-S+	4	P-S+	2	M-S+	5	F-S+	1
M+S+	23	P+S+	25	M+S+	4	F+S+	8
M-S-	4	P-S-	4	M-S-	17	F-S-	18
Kappa	0.52		0.67	Kappa	0.18		0.59

*M: MAST, [†]S: skin test, *P: Phadiatop, [§]F: Fx5

inhalation allergens and found that CAP was the preferred method for this purpose.²⁵ The sensitivities of Fx5 and MAST for food, however, has been reported to be lower than those of inhalant allergen tests.^{7,26}

Even though Phadiatop-Fx5 and MAST employ similar principles for specific-IgE detection, there are differences in antigen coating and signal detection, and differences in the antigen mixture preparation may contribute to the differences in the final results. Both these methods include the most common allergens in Taiwan (i.e. mite (*Der. p.*), cockroach, *Alternaria*, Bermuda grass,²⁷ egg and cow milk), and thus are comparable in their scope of antigen detection as screening tests. Since Phadiatop-Fx5 has empirical values for positive results, adjustment of the cut-off value may lead to different sensitivity and specificity. However, disagreement did exist when the results from these tests were compared. In this study, the overall positive rates with Phadiatop-Fx5 and MAST were 65.2% and 47.8%, respectively. Phadiatop-Fx5 appeared to be more sensitive in comparison. Given the difference in the sensitivity between these tests, it becomes important to identify the factors that may affect the agreement between them. We included consecutive patients for whom specific allergen tests were performed in a university hospital during a one-month period in this study. Investigations on serum samples from this group of patients allowed us to analyze the clinical factors that may contribute to the differences in UniCAP screening and MAST results without the potential bias derived from selective recruitment of patients from a single clinic or a single clinical department. As the hospital where this study was performed is the referral center of the entire metropolitan area, we believe the results presented in this study reflect the trend in the general Taiwanese populace.

Our results showed that the overall disagreement was more apparent in patients with younger age and in patients with higher total IgE levels (Fig. 2). When inhalant allergens and common food allergens were separated for study, we found that even though the sensitivity of both Phadiatop (for inhalant allergens) and

Fx5 (for food allergens) were higher than that of MAST, the discrepancies among Phadiatop and inhalant allergens in MAST were less prominent, in comparison to Fx5 and common food allergens in MAST. Given the trend that younger children are more sensitive to food allergens, this result underscores the recent stress on the importance of using more sensitive tests in detecting allergens in younger pediatric patients.²⁸ The slight difference noted in patients from different departments also reflects this trend (Fig. 2, middle panel). The finding of the higher disagreement between UniCAP and MAST among higher IgE group patients may suggest that different thresholds of detection in these two tests leads to difference in detection of lower-level specific IgE in patients with high total IgE concentration. But the antigens difference between both tests exists and cross reaction remains a possible explanation.

It is worth noting that although most disagreement between these tests occurred as UniCAP (+) MAST (-) (53 cases), CAP (-) MAST (+) results still occurred in 14 cases. Although only two patients tested positive for MAST inhalant allergens and negative for Phadiatop, thirteen patients tested positive for MAST food allergens and negative for Fx5 test. The disagreement between Fx5 and common food allergens in MAST thus appears more prominent than that between Phadiatop and inhalant allergens in MAST. We suspect that this difference is due to the fact that the allergen mixture used in Fx5 test contains no shrimp antigens, which are among the important food allergens in Taiwan. Addition of shrimp antigens to the Fx5 antigen mixture thus may improve the sensitivity of Fx5 UniCAP screening test in Taiwanese patients.

Our findings that age and total IgE level affect the agreement between UniCAP and MAST tests are useful for the choice of appropriate specific antigen tests by physicians. For patients belonging to groups of lower agreement, the test with more quantitative results and generally higher sensitivity, a UniCAP screening test, might be a better choice. Moreover, as UniCAP costs only one third the price of MAST, it should be the test of choice in these groups of patients from the economical

point of view. However, for patients with positive results in either screening test, further quantitative specific allergen test should be required to follow up the change of IgE levels against specific allergens. Information from this investigation thus will be important for physicians to choose allergen-specific tests in a cost-effective manner.

In summary, although there was substantial agreement between results of the MAST and UniCAP screening tests (Phadiatop-Fx5) in allergic patients, significant differences existed in patient groups with younger age and higher total IgE level. The disagreement between these two tests was more apparent with food allergens. To get the best cost-effectiveness, we recommend that physicians take age, IgE level, and difference in food and inhalant allergens into consideration when choosing specific allergen screening tests.

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