

# The Usefulness of the Multiple Allergen Simultaneous Test-Chemiluminescent as Compared to the Phadia Immunocap IgE Test Panel System in Children and Adolescents

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**Abstract:** Several patents of methods for diagnostic work-up of IgE-mediated disease have recently been acclaimed. The aim of the present study was to assess the diagnostic usefulness of the Multiple allergen simultaneous test-chemiluminescent assay<sup>®</sup> (MAST CLA) as compared to the Phadia Immunocap<sup>®</sup> (Phadia CAP) allergen-specific IgE test panel system. The design was a prospective, observational study in which MAST CLA and Phadia CAP test panels were assessed in 105 children aged 1 to 17 years (46 girls and 59 boys) with a suspected diagnosis of rhinoconjunctivitis, asthma, eczema or food allergy. The MAST CLA screening panel included 23 and 13, the Phadia CAP 10 inhalant and 6 food allergens. The MAST CLA screening panel found 353 sensitizations which were not detected by Phadia CAP, 93 (26.3%) of which were class 3 or 4 results. The sensitizations gave clinically useful information in 7 patients (6.6%). As compared to the Phadia CAP the MAST CLA screening test panel adds little to the diagnostic work-up of IgE mediated disease in children and adolescents.

**Keywords:** Allergy, allergic rhinitis, asthma, eczema, IgE, IgE tests, multiple allergen simultaneous test-chemiluminescent assay, Phadia CAP test.

## INTRODUCTION

The diagnosis of allergic disease is based on a thorough clinical history, relevant examination and a measure of immunoglobulin E (IgE) sensitization. Recently, a method of providing a clinical decision support was patented [1]. It comprised a computer-implemented method, a computer program product, and a patient information carrier device containing a diagnosis result. In secondary and tertiary settings the skin prick test is often the preferred test of IgE-mediated allergy, and a novel needle-integrated skin diagnostic tester has been patented [2-4]. In primary care settings, however, *in vitro* allergen-specific IgE screening test panels are widely preferred, and during the past decade a marked increase in the number of screening tests has been recorded [1]. Several test panel systems are commercially available and there are, probably, more to come. A highly sensitive immunoassay using an immunometric sandwich procedure utilizing biotin-streptavidin signal amplification techniques and R-phycoerytherin fluorescent labels was acclaimed just recently [5]. The number and specificity of the allergens in the panels may vary considerably depending on the methods applied and the part of the world in which they are used. The Phadia Immunocap assay<sup>®</sup> (Phadia CAP) is preferred in some countries, the Multiple allergen simultaneous test-chemiluminescent assay<sup>®</sup> (MAST CLA) in others, and in some countries both are used [4-6]. The Phadia CAP and the MAST CLA assays have been evaluated with respect

to sensitivity and specificity finding comparable performances in detecting specific IgE sensitizations [7]. However, they have not been compared as clinical practice tools in the diagnosis of IgE mediated allergy. The aim of the present study was to assess the clinical usefulness of the MAST CLA as compared to the Phadia CAP allergen-specific IgE test screening panel system.

## MATERIAL AND METHODS

The design was a prospective, observational study. During a three year period skin prick tests, Phadia CAP inhalation and food screening test panels were performed in children and adolescents admitted to our secondary referral centre with a suspected diagnosis of food allergy, asthma, rhinitis or eczema. If IgE sensitization by an allergen which was not included in the Phadia CAP panels but included in the MAST CLA assay was suspected, in addition, a MAST CLA screening panel test was performed, Table 1. All patients were assessed by an experienced paediatric allergologist (ODW). Diagnosis was based on a combination of the allergen specific case history, physical examination and the results of skin prick testing and the Phadia CAP and MAST CLA screening panels. All data were entered into an electronic data base.

The clinics reference laboratory performed the Phadia CAP test according to the manufacturer instructions (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden) [8] and sera were sent to the manufacturer for analysis of Mast CLA (Unilabs, Copenhagen, Denmark) [9]. Both immunoassays operate on fully automated analyzers utilizing stored calibration curves that are capable of producing quantitative results.

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**Table 1. Distribution of IgE Sensitizations Detected by the MAST CLA (N = 688) and the Phadia CAP (N = 335) Screening Test Panels.**

| MAST-CLA          | Phadia CAP       |
|-------------------|------------------|
| Alder (21)        | Birch (26)       |
| Birch (25)        |                  |
| Hazel (28)        |                  |
| Meadow grass (34) |                  |
| Cocksfoot (35)    |                  |
| Rye grass (34)    | Timothy (39)     |
| Timothy (38)      |                  |
| Mugwort (19)      |                  |
| Dandelion (16)    |                  |
| Goldenrod (19)    |                  |
| D. Farinae (24)   | D. Farinae (1)   |
| D. Pteronys. (27) | D. Pteronys.(33) |
| Alternaria (15)   | Alternaria (0)   |
| Aspergillus (17)  | Cladosporium (5) |
| Cladosporium (11) |                  |
| Penicillium (7)   |                  |
| Mucor (7)         |                  |
| Cat (23)          |                  |
| Cow (9)           | Cat (24)         |
| Dog (28)          | Dog (34)         |
| Horse (16)        | Horse (22)       |
| Guinea pig (10)   | Milk (29)        |
| Rabbit (13)       |                  |
| Chicken (6)       |                  |
| Beef (5)          |                  |
| Pork (8)          |                  |
| Milk (27)         | Cod (6)          |
| Cod (7)           | Peanut (27)      |
| Shell fish (11)   |                  |
| Peanut (24)       |                  |
| Soy (24)          |                  |
| Pea (19)          |                  |
| Egg (26)          | Egg (22)         |
| Oat (20)          | Wheat (24)       |
| Wheat (18)        |                  |
| Rye (17)          |                  |

The Phadia CAP test uses a fluorescence tracer as report system, the Mast CLA test a chemiluminescent system. The methodology of both tests has been described in detail previously [8, 9]. The applied MAST CLA panel simultaneously measured 36 different (23 inhalant and 13 food) specific IgE antibodies, whereas the Phadia CAP tests measured 10 in an inhalation and 6 in a food IgE panel Table 1. The number of

allergens and the specific allergens in the two panels were such as were used in clinical practice at the time when the study was completed. The applied allergens had been defined by an ad-hoc group of pediatricians, allergists and clinical chemists under the auspices of Central Denmark Region (Phadia CAP) or by the manufacturers themselves (MAST CLA). None of the institutions or manufacturers were involved in the present study. The results of the Mast CLA analyses were given in a five-class system (class score 0-4) based on luminiscens units (LU) provided by the manufacturer:  $\leq 26$  LU, class 0; 27-65 LU, class 1; 66-142, class 2; 143-242, class 3;  $> 242$ , class 4. The results of the Phadia CAP tests were given as exact values in kU/L and scored in a five-class system (class score 0-4) modified from the seven-class system provided by the manufacturer:  $<0.35$  kU/L, class 0, 0.35-0.70 kU/L, class 1, 0.71-3.5 kU/L, class 2; 3.6-7.5 kU/L, class 3;  $>7.6$  kU/L, class 4. In both assays class  $>0$  was considered positive sensitization [6]. Clinical useful results of the MAST CLA test were defined as class 3 and 4 positive allergens which were not found with the Phadia CAP test and which caused revision of pharmacotherapy and/or elimination-provocation strategies to be undertaken.

## RESULTS

A total of 105 patients (46 girls and 59 boys) 3 to 17 years of age were studied. Forty (38.1%) were diagnosed with rhinoconjunctivitis, 54 (51.9%) with asthma, 10 (10.5%) with food allergy and 40 (38.1%) with eczema. Sensitizations were detected by at least one of the test panel systems in 68 patients (64.8%), whereas 37 (35.2%) did not have any sensitizations in any of the assays.

The Phadia CAP assay identified 335, the MAST CLA 688 positive specific IgE test results, Table 1. Table 2 gives the class distribution in the sensitizations which were included in both test panels (birch, timothy, mugwort, dermatophagoides farinae, dermatophagoides pteronyssinus, cladosporium, alternaria, cat, dog, horse, milk, cod, peanut, soy, egg and wheat). The MAST CLA screening panel identified 259 (77.3%) of the positive Phadia CAP results. Forty three were class 1, 43 were class 2, 19 were class 3 and 154 were class 4 results. Of the 75 sensitizations measured by the Phadia CAP which were not detected by the MAST CLA 73 were class 1, and 1 was class 2 sensitizations. Of the 353 sensitizations detected by MAST CLA which were not detected by Phadia CAP 93 (26.3%) were class 3 or 4 results.

In 21 children, (20%) class 3 or 4 positive reactions to alder and hazel pollen were found in the MAST CLA screening panel. All of them except one had timothy and birch pollen positive results in both tests. Clinical significance was found in 1 child, who was initiated on a nasal corticosteroid prior to the alder and hazel season. In 10 children (10.5%) class 3 or 4 positive reactions to dandelion were found; all 10 children were also positive to mugwort. No clinical significance of these results was detected. As seen in Table 1 the MAST CLA test detected 24 sensitizations to dermatophagoides farinae and 15 sensitizations to alternaria of which only one sensitization to dermatophagoides farinae was detected by the Phadia CAP test. All sensitizations to dermatophagoides farinae and all but one to alternaria were class 1 or 2 reactions. In 5 children, (4.8%) class 3 or 4 positive alter-

**Table 2. Distribution of Individual MAST CLA and Phadia CAP Class Score Values.**

| MAST CLA score | Phadia CAP score |    |    |    |    |
|----------------|------------------|----|----|----|----|
|                | 0                | 1  | 2  | 3  | 4  |
| 0              | 1161             | 73 | 2  | 0  | 0  |
| 1              | 39               | 45 | 0  | 0  | 0  |
| 2              | 25               | 42 | 1  | 0  | 0  |
| 3              | 5                | 18 | 0  | 0  | 1  |
| 4              | 19               | 85 | 33 | 17 | 19 |

Based on allergens included in both test panels (birch, timothy, mugwort, dermatophagoides farinae, dermatophagoides pteronyssinus, cladosporium, alternaria cat, dog, horse, milk, cod, peanut, soy, egg and wheat), (N = 1585).

aria, aspergillus, cladosporium, penicillium and mucor reactions were found. In one of them the finding of a class 4 positive alternaria reaction had clinical significance verified by elimination-provocation-elimination challenge and relevant avoidance procedures were established. Finally, in 8 children (7.6%) class 3 or 4 positive reactions were found to oat, in 8 children (7.6%) to rye. In 2 with oat and in 3 children with rye positive data, the results were clinically significant as verified by elimination-provocation-elimination test and a relevant diet was initiated. Prior to that all 5 of them had a clinical diagnosis of wheat allergy and the other oat and rye positive children all had sensitizations to wheat. So, in a total of 7 patients (6.6%) clinically useful results were obtained by MAST CLA test panel screening. None of the children were introduced to specific immunotherapy.

## DISCUSSION

In the interests of economy, time and human resources in primary centers *in vitro* allergen-specific IgE screening test panels are often preferred to skin prick testing in the diagnostic work-up of children. In secondary and tertiary centers among other reasons specific IgE in blood may often be used as an adjunct to skin prick testing because it is feasible, considered to be less affected by intake of anti-histamines, and because numerical results may be considered to be of importance for consecutive monitoring [10]. Such factors in combination with an increase in type-1 allergic diseases and a correspondent need for investigations of sensitization probably explain why allergen-specific IgE assays are extensively used [11]. As in most European countries in Denmark the immunoassay IgE market is dominated by the Phadia CAP system and to a lesser extent by the MAST CLA and others such as the Immulite® and ADVIA® test systems [11-13]. This may be the other way around in other parts of the world [6]. Performances of the immunoassays have been compared with each other and with the skin prick test [2, 7, 14]. Sensitivity and specificity have generally been in the range of approximately 40 to 90% and 40 to 80%, respectively, depending on specific allergens and diseases. Generally, a skin prick test performs better than a specific IgE immunoassay in most allergens and most diseases as reported in a com-

parative study of Phadia CAP and MAST CLA in adults [7]. Whether that applies to new patents remains to be evaluated [5].

Though assay performances of the Phadia CAP and MAST CLA have not been performed in children this was beyond the scope of the present study. In this study, we wanted to assess whether screening for 36 allergens by the MAST CLA test would improve clinical decisions as compared to screening for 18 allergens by the Phadia CAP test. Therefore, the definition of the outcome measure as class 3 and 4 MAST CLA positive allergens which were not found with the Phadia CAP test and which caused revision of pharmacotherapy CAP or elimination-provocation strategies to be undertaken was appropriate. Of the 7 children in whom additional clinically important sensitizations were measured by MAST CLA the 5 with oat or rye sensitization all had wheat sensitizations in the Phadia CAP test. Since oat and rye are often associated with wheat allergy they probably would have been detected by a specific Phadia CAP IgE analysis for oat and rye. Similarly, clinically insignificant alder and hazel sensitizations are closely associated to timothy and birch allergy, and in only 1 child were sensitizations to alder and hazel clinically important. There are no data available for the conspicuous finding of the MAST CLA detected 24 sensitizations to dermatophagoides farina and the 15 sensitizations to alternaria of which only one sensitization to dermatophagoides farinae was detected by Phadia CAP. A detailed analysis of the variations in sensitizations in the individual children did not reveal any other marked differences. We also looked for possible period and treatment effects, however, the time between blood sampling did not vary in comparison with the rest of the investigated population and neither did intake of anti-histamines or corticosteroids. The observation may warrant further attention in future studies.

So, little additional clinically important information was gained by using the MAST CLA 36 allergen screening panel. Probably, revision of the clinical history in combination with few specific IgE measurements would have provided similar information. One further argument against the MAST CLA screening test would be that the costs of MAST CLA and

Phadia CAP of approximately 180 EU and 50 EU per test, respectively, certainly are in favor of the Phadia CAP screening test. However, a screening test may not be warranted in all cases of suspected IgE mediated disease. Finally, it remains controversial in which clinical settings and to which extent *in vitro* IgE panel screening tests may improve diagnostic performances as compared to one or few specific IgE measurements based on an accurate clinical history.

## CURRENT & FUTURE DEVELOPMENTS

Rigorous assessments of the conventional use of specific IgE screening tests in individuals suspected of type-1 allergy with respect to clinical usefulness, costs or human resources have not been reported. Such assessments need to be performed when new patents are introduced in the diagnostic work-up of children and adolescents with suspected IgE mediated conditions [1, 3, 5].

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Authors' contributions: The study was planned by Ole D. Wolthers, and all patient related evaluations were performed by him. Ole D. Wolthers also took part in data collection, processing and analysis, and he wrote the paper. Data were collected and processed by Morten Staberg, and he took part in discussions of the results and approved of the paper.

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