

SHORT COMMUNICATION

Alternative to *In Vivo* Tests During the COVID-19 Pandemic: Multiple Allergen Simultaneous Tests as a Useful First Screening

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SUMMARY

Background: In allergy diagnosis we sometimes find some clinical or logistic limitations to be able to carry out *in vivo* tests, so the detection of serum allergic-specific IgE could be an alternative as a first screening step. Here, we compare the results from the routine diagnostic tools and multiple allergen simultaneous tests to detect inhalant allergen sensitization.

Methods: Thirty-two subjects with a positive ImmunoCAP Phadiatop screening were included, evaluating the accuracy of their diagnosis using (1) specific IgE determination by ImmunoCAP and (2) MAST EUROLINE Immunoblot.

Results: The MAST method showed a high agreement and correlation with the ImmunoCAP system for *Dermatophagoides pteronyssinus*, cat dander, orchard grass and *Alternaria alternata*. Of the subjects, 94% were sensitized to at least one of the allergens using MAST EUROLINE immunoblot, whereas 79% of individuals with a positive Phadiatop went undetected when we analyzed only the 4 allergens mentioned before.

Conclusions: The study showed the usefulness of MAST EUROLINE immunoblot for screening detection of specific IgE antibodies directed against a broad spectrum of inhalant allergens as a first screening tool. Furthermore, its performance is not affected by the possible *in vivo* test limitations and avoids the arbitrary selection of allergenic sources for evaluation, which may lead to incorrect patients' diagnosis and management.

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multiple allergen immunoblot, sensitization screening, specific IgE, Phadiatop

INTRODUCTION

For an allergy diagnosis, the presence of allergen-specific immunoglobulin E (IgE) is usually detected either by *in vivo* skin prick test (SPT) or by *in vitro* tests measuring specific IgE in sera [1]. *In vivo* tests have some limitations when the patient suffers from skin disorders such as dermatographism or atopic dermatitis [2], both cases less suitable to be tested by allergy skin testing. Additionally, due to the global COVID-19 pandemic, current contact restrictions have led to a lack of STPs as

a result of activity decrease at hospitals, lengthening waiting lists.

Therefore, detection of serum allergic-specific IgE could be useful as a screening first step when there are clinical or logistic limitations for doing STPs; furthermore, also when it is desired to avoid risky contacts.

In contrast to single allergen-specific IgE detection methods, multiple allergen simultaneous tests (MAST) can detect a higher number of allergen-specific IgE [3-6] and is a useful screening tool in these situations. Moreover, MAST may be an alternative to SPT or to the broad screening of serum using singleplex *in vitro* testing, common routine methods for sensitization screening in case of suspicion of allergy disease.

The aim of this study is to use a multiplex system to evaluate inhalant allergen sensitization in individuals with a suspicion of allergic disease referred from primary care and to compare the results with the current approach followed by that center.

MATERIALS AND METHODS

Subject selection

The study was carried out in the Immunology Department of Hospital Clonic de Barcelona during June 2020. Data from all individuals with a positive ImmunoCAP Phadiatop screening test in that period (ThermoFisher Scientific, Sweden; cutoff ≥ 0.35 kU_A/L) from a regional hospital and their primary care centers were retrospectively collected and analyzed. The study was approved by the Committee on ethics of the Hospital Clinic, Barcelona, Spain.

ImmunoCAP assay

Based on the indications of the requesting hospital, the current protocol of serum testing consists in first using the fluorescence enzyme immunoassay Phadiatop (ThermoFisher Scientific, USA) and in the case that Phadiatop values are > 2.5 kU_A/L, automatically the same serum is tested for sensitization to four allergens: *Dermatophagoides pteronyssinus* (d1), cat dander (e1), orchard grass (g3), and *Alternaria alternata* (m6) by ImmunoCAP (ThermoFisher Scientific, USA, cutoff 0.35 kU_A/L). These four allergens have been selected by the petitioner based on prevalence of sensitization in the geographical area of origin. Those individuals with Phadiatop levels < 2.5 kU_A/L were also analyzed for the mentioned extracts to further analyze and compare both groups.

MAST-immunoblot

Data from immunoblot using Euroline Mediterranean Inhalation 2 (Euroimmun AG, Lubeck, Germany) were also evaluated. Cutoff used for Euroline was ≥ 0.35 kU_A/L. The profile includes the detection of the specific IgE antibodies against the following 32 different allergens simultaneously: *D. pteronyssinus* (d1), *D. farinae* (d2), *Acarus siro* (d70), *Lepidoglyphus destructor*

(d71), *Blomia tropicalis* (d201), cat (e1), dog (e2), horse (e3), *Cladosporium herbarum* (m2), *Aspergillus fumigatus* (m3), *Alternaria alternata* (m6), German cockroach (i6), Bermuda grass (g2), Orchard grass (g3), ryegrass (g5), Timothy grass (g6), Meadow grass (g8), Cultivated rye (g12), Alder (t2), Birch (t3), Hazel (t4), Olive tree (t9), Plane tree (t11), Cypress (t23), Ragweed (w1), Mugwort (w6), English plantain (w9), Goosefoot (w10), Russian thistle (w11), Wall pellitory (*P. officinalis*) (w19), Wall pellitory (*P. Judaica*) (w21), Latex (u85), and CCD marker.

Statistical analysis

Quantitative variables were described by median, range, interquartile range (IQR), and qualitative variables with percentages. The agreement of sIgE test performed by MAST EUROLINE immunoblot and ImmunoCAP was calculated as follows: (total number of results - number of discrepancies) x 100/total number of results. To evaluate the agreement of detection results, Cohen's kappa analysis was performed. Kappa values were categorized as almost perfect (0.8 - 1.0), substantial (0.6 - 0.8), moderate (0.4 - 0.6), fair (0.2 - 0.4), and poor (below 0.2). All statistical analyses were performed using GraphPad Prism 8.2.1, GraphPad Software Inc., San Diego, CA, USA.

RESULTS

Sera of 32 subjects (16 women (50%); mean (range) age 28 (10 - 40.75) years) were analyzed. Performing the analysis of the four allergens by ImmunoCAP when Phadiatop values were between 0 - 1.5 kU_A/L, 1.5 - 2.5 kU_A/L, and ≥ 2.5 kU_A/L only found sensitization to at least one of the four allergens in 21% (5/24), 29% (7/24), and 35% (28/80), respectively. While, by MAST EUROLINE Immunoblot, an important number of new allergen sensitizations were detected with a median (interquartile range, IQR) of 6 (2 - 10.5) new sensitizations (Figure 1). Remarkably, the number of new sensitizations detected by Immunoblot increased as higher Phadiatop values were presented by subjects (Table 1). The highest sensitization values were detected in olive tree with a median (IQR) of 61.3 (14.6 - 79.5) kU_A/L, dog dander (26.2 (0.5 - 55.4) kU_A/L), meadow grass (12.4 (2.1 - 26) kU_A/L), Wall pellitory (*P. officinalis*) (9.5 (0.5 - 21.7) kU_A/L) and *A. alternata* (8 (0.4 - 22.4) kU_A/L). Neither olive tree, dog dander, meadow grass or wall pellitory were among the selected four to be tested by ImmunoCAP.

Focusing on the 11 individuals with Phadiatop results below 2.5 kU_A/L, only two (subject #3 and #21) were found negative by MAST. The rest showed a high sensitization to at least one allergen (subject #17, #18, #19, #20, #24, #25, #26, #28, #29). Regarding the 21 individuals with Phadiatop values ≥ 2.5 kU_A/L, 17 of them were sensitized to olive tree, being the allergen with the highest rate of positive results.

Table 1. Number of sensitizations detected by Immunoblot (median [IQR]) according to Phadiatop values.

Phadiatop (kU _A /L)	Immunoblot (no. new sensitizations)
0 – 1.5	3 [2 - 4.75]
1.5 - 2.5	4 [2 - 12.75]
> 2.5	9.5 [2 - 12.5]

Table 2. The agreement of specific IgE allergic tests performed by Immunoblot and ImmunoCAP system.

Allergen		ImmunoCAP		Kappa index	Agreement
d1-D. pteronyssinus		n *	p #	0.5	75%
Immunoblot	n	14	2		
	p	6	10		
e1-Cat					
Immunoblot	n	22	1	0.76	90%
	p	2	7		
g3-Orchard grass					
Immunoblot	n	15	3	0.75	87%
	p	1	13		
m6-Alternaria alternata					
Immunoblot	n	24	0	0.71	90%
	p	3	5		

* The number of patients with negative results, # - the number of patients with positive results. The total number of tested sera was 32 of the following allergens: *D. pteronyssinus* (d1), Cat dander (e1), orchard grass (g3) and *Alternaria alternata* (m6).

Moreover, the use of MAST EUROLINE Immunoblot pointed out some interesting results. Subject #6 and #24 showed sensitization exclusively to wall pellitory. In another individual (#7) it highlighted an elevated sensitization to dog dander that could not be detected in a first evaluation due to the presence of other several sensitizations (grasses, olive, and cat). Further, subject #30 showed latex sensitization that could alert about cross-reactivity between other allergenic sources not included in this MAST EUROLINE profile (i.e., latex-fruit syndrome). Finally, in samples #10 and #19 the inclusion of the CCD marker could give additional information on allergen-nonspecific cross-reactivity.

Additionally, a substantial agreement between MAST EUROLINE Immunoblot and ImmunoCAP was found for cat dander, orchard grass, and *Alternaria alternata* with a kappa index of 0.76, 0.75, and 0.71, respectively. One allergen (*D. pteronyssinus*) presented moderate agreement with a kappa index 0.5 (Table 2).

DISCUSSION

Inhalation allergies can be triggered by seasonal allergens (pollen from trees, grasses and weeds) or year-round indoor allergens (house dust mites, domestic animals, mold spores). The allergic symptoms intensify with every increased exposure to the allergen and could result in life-threatening reactions (anaphylactic shock) [7]. Consequently, an accurate strategy to detect allergen sensitization is needed for better diagnosis and management of patients.

The MAST method showed a high agreement and correlation with ImmunoCAP system for the four allergens tested. Using MAST EUROLINE immunoblot, we found that 94% (30/32) were sensitized to at least one of the allergens. Whereas when we analyzed only the 4 allergens mentioned before, about 79% of individuals with positive Phadiatop results go undetected. Therefore, a broad spectrum of allergens in the first screening is recommended.

In the present study we revealed that MAST EUROLINE Immunoblot, with a panel of whole extracts of allergenic sources representative of our geographical area, could be an option and successfully used for screening detection of specific IgE antibodies directed against a

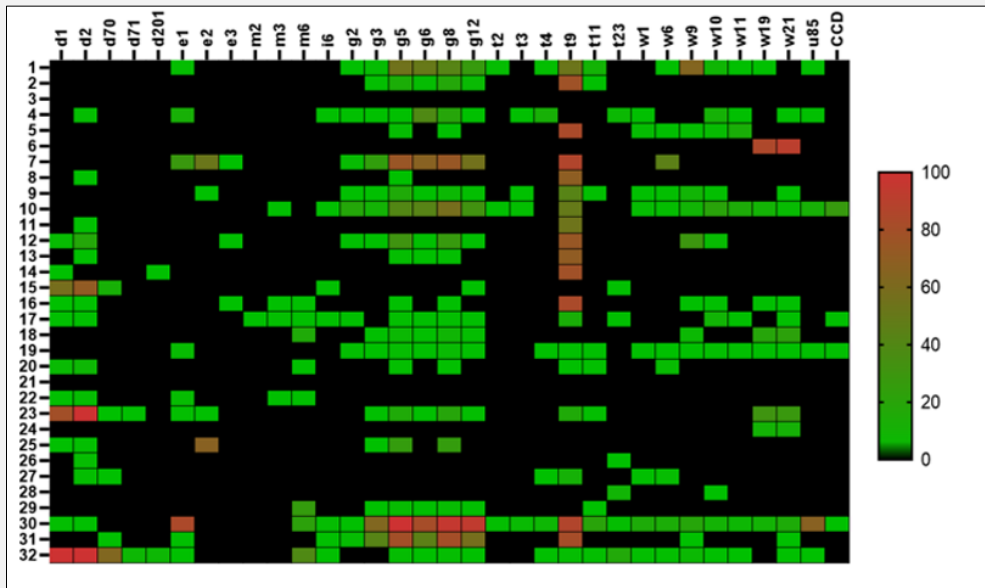


Figure 1. The heatmap represents the sensitization detected by MAST EUROLINE Immunoblot (kU_A/L).

The patients analyzed are represented in rows and allergens tested in columns (d1, *D. pteronyssinus*; d2, *D. farina*; d70, *A. siro*; d71, *L. destructor*; d201, *B. tropicalis*; e1, cat; e2, dog; e3, horse; m2, *C. herbarum*; m3, *A. fumigatus*; m6, *A. alternata*; i6, German cockroach; g2, Bermuda grass; g3, Orchard grass; g5, Ryegrass; g6, Timothy grass; g8, Meadow grass; g12, Cultivated rye; t2, Alder; t3, Birch; t4, Hazel; t9; Olive tree; t11, Plane tree; t23, Cypress; w1, Ragweed; w6, Mugwort; w9, English plantain; w10, Goosefoot; w11, Russian thistle; w19, *P. officinalis*; w21, *P. Judaica*; u85, Latex and CCD marker).

wide spectrum of inhalant allergens as a first screening tool in primary care upon a suspicion of a respiratory allergic disease prior to the referral to the allergist. Thus, the MAST method used for this preliminary study is useful in identifying allergen sensitization and has the additional benefit that its performance is not affected by the possible limitations when performing *in vivo* testing and also avoids the arbitrary selection of allergenic sources for evaluation based on prevalence or economic criteria, which may lead to incorrect diagnosis or patient management.

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Declaration of Interest:

Mariona Pascal has received lecture honoraria from EUROIMMUN. All other authors have declared they have no competing interests related to this work.

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