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Component-Resolved Diagnosis (CRD): Is It Worth It? Frequency and Differentiation in Rhinitis Patients with Mite Reactivity

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ABSTRACT

Component-resolved diagnosis (CRD) using microarray technology has recently been introduced with the aim to improve diagnosis of allergy. The aim of this study was to compare performance of this allergen microarray to those of an established extract-based skin prick testing (SPT).

45 patients with allergic rhinitis were studied (16 children and 29 adults). SPT to *Dermatophagoide s pteronyssinus*, *Dermatophagoide s farinae* and *Blomia tropicalis* extracts and allergen microarray ImmunoCAP ISAC were carried out for all patients.

Forty out of 45 patients demonstrated positive SPT to all mite extracts tested. These 40 patients were considered to be mite- allergic based on the positive SPT results. The remaining 5 patients with negative SPT to any mite extracts were classified as non-mite allergic. Comparatively, based on the microarray results, only 34 mite-allergic patients had detectable serum IgE to at least one of the mite allergen components tested whereas 6 patients with positive SPT to mite extracts showed no detectable IgE reactivity to any of the components tested. One non-mite allergic patient had a positive test- Blo t 5. Der p 10- positive patients also reacted to other cross-reactive tropomyosin from anisakis (Ani s 3) (25%), cockroach (Bla g 7) (50%) and shrimp (Pen m 1) (75%).

CRD is a reliable tool for the diagnosis of allergy to mites. Der p 10 might be a useful indicator to identify a subset of mite-allergic patient that have additional sensitization due to cross-reactivity and thus allows selection of patients for immunotherapy.

Keywords: Allergic rhinitis; Component resolved diagnostics (CRD); Cross-reactive tropomyosin; Immunotherapy; Mite allergy; Skin prick testing

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INTRODUCTION

Mites represent one of the most important allergen sources worldwide, with 20% of the respiratory allergic population having mite allergy and an extra 30% having asymptomatic sensitization.¹ The two perennial indoor house dust mite (HDM), *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, are major sources of allergens, involved in asthma, rhinitis, rhinoconjunctivitis and atopic dermatitis.²⁻⁴ The storage mites are also recognized as sources of allergens. The mite genera *Blomia*, *Lepidoglyphus* and *Glycyphagus* belong to the Glycyphagidae family which together with the Acaridae family (*Tyrophagus* and *Acarus* genera), are collectively called storage mites.⁵

Generally, mite allergy work-up is based on a clinical history supported by positive specific serum IgE (sIgE) and/or skin prick test (SPT) to mite allergen crude extracts.^{1,6} The symptoms of mite allergy may be mitigated by pharmacological treatment, but only allergen-specific immunotherapy (SIT) can prevent long term disease progression.^{7,8} SIT has been shown to be highly effective when properly implemented. The purpose of allergen-specific immunotherapy is to induce immunological changes or tolerance against the administered allergens and is thus indicated for patients with IgE antibodies to clinically relevant allergens.^{7,9}

Mite allergen crude extracts are currently used throughout the world for both diagnostic and specific immunotherapy procedures.⁷ Mite allergen crude extracts contain varying concentrations of allergens as well as non-allergenic components.^{10,11} The group 1 (cysteine protease) and 2 (NPC2 family) allergens are usually present in high concentrations in whole mite extracts.¹² The contents of other important allergens (e.g. groups 5, 7 and 10) in mite extracts have so far not been evaluated in depth but some allergens (e.g. group 7) seem to be lacking in natural extracts.¹¹⁻¹³ For diagnosis and immunotherapy, the use of allergen components seem to be a promising alternative to allergen crude extracts, since they represent well defined components, which can easily be characterized and quantified.^{11,14-16} In order to use allergen components for diagnostic or therapeutic purposes, the importance of the individual allergen molecules should be evaluated as the IgE binding frequency of individual allergens may show high variability at least in certain populations^{11,15,17} and also to determine the allergen components necessary for immunotherapy.¹¹

Component resolved diagnosis (CRD), which used

the microarray technology, has recently been introduced into the field of clinical allergology.¹⁸⁻²⁰ The technique uses multiple allergen components spotted onto a microarray plate, allowing multi-allergen analyses with minute amounts of serum (20 µl) in a single measurement and enable a comprehensive analysis of the patient's IgE binding pattern to a large number of individual allergens.²⁰ The ImmunoCAP ISAC microarray (VBC Genomics, Vienna, Austria/Phadia, Uppsala, Sweden) is relatively new in the market. Due to the importance of CRD, this study was conducted to compare the performance of this allergen microarray to those of an established extract-based SPT.

MATERIAL AND METHODS

Study Population

We studied 45 patients with mite allergic rhinitis (29 adults and 16 children; age: 7-67 years; median age; 24 years) referred to Ear, Nose and Throat (ENT) Clinic of Hospital Kuala Lumpur (HKL). The diagnosis of mite allergy was based on clinical history and positivity of the SPTs to *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Blomia tropicalis* crude extracts (Alk Abello, Madrid, Spain). The local ethical committee approved the study and patients gave informed consent.

Skin Prick Test (SPT)

SPT to *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Blomia tropicalis* crude extracts (Alk Abello, Madrid, Spain) were performed in all patients. One drop of each mite crude extracts was applied to the patient's forearm and 1 mm single-peak lancets were used for SPT. Saline solution and histamine hydrochloride 1% (Alk Abello, Madrid, Spain) served as negative and positive controls, respectively. All reactions eliciting a mean wheal diameter of 3 mm or greater than the negative control read 15 min after application were considered positive.

Blood Sampling

Venous blood samples of all patients were collected prior to SPTs and obtained serum were aliquoted and stored at -80°C until further analysis.

Allergen Microarray Analysis

We used the allergen microarray ImmunoCAP ISAC (VBC Genomics, Vienna, Austria/ Phadia,

Uppsala, Sweden). This ambient analyte assay consists of a microscopy glass slide modified with a Teflon™ mask in order to create four individual reaction sites. These were coated with amine-reactive polymers allowing covalent immobilization of allergenic molecules. In the present study, we focused on mite allergen components that had been spotted onto the microarray: cysteine protease (Der f 1, Der p 1), NPC2 family (Der f 2, Der p 2, Lep d 2), mite group 5 (Blo t 5) and cross-reactive tropomyosin components (Der p 10, Ani s 3, Bla g 7, Pen m 1).

Allergen microarray ImmunoCAP ISAC (VBC Genomics, Vienna, Austria/Phadia, Uppsala, Sweden) was performed according to the manufacturer's recommendations. Microarray slides containing allergen chips were washed for 10 min with washing solution, rinsed with deionised water and dried. Then, each microarray reaction site was incubated with 20 µl of undiluted patient serum for 2 hours at room temperature in a humid chamber in order to capture allergen-specific serum IgE antibodies by their corresponding allergen molecules. In a second step, the microarray slides were washed with washing solution for 10 min, rinsed with deionised water and dried. Hereafter, microarray bound IgE was marked with a secondary, fluorescence-tagged anti-human IgE antibody for 30 min at room temperature in a humid chamber. After a second washing procedure, the corresponding fluorescence signals were scanned using

a microarray scanner (CapitalBio LuxScan™ 10K-A) for data acquisition. The images of each spot of the scanned chips were analysed using the MIA software (Microarray Image Analyzer) semi-quantitatively, where by allergen component sIgE antibodies are measured in ISU/L (ISAC Standardized Units/liter).

Statistical Analysis

The differences of the frequency of allergen-specific IgE reactivity were assessed with Fisher's exact test. P-values less than 0.05 were considered statistically significant.

RESULTS

Comparison of Skin Prick Testing (SPT) with ImmunoCAP ISAC Microarray

All the patients included in our study underwent SPT to *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Blomia tropicalis* extracts. Forty out of 45 rhinitis patients showed positive SPT to all mite extracts tested (data not shown). These 40 rhinitis patients were considered to be mite allergic based on the positive SPT results.²¹ The remaining 5 patients, who showed no SPT reactivity to any mite extracts used, were considered to be non-mite allergic (data not shown).

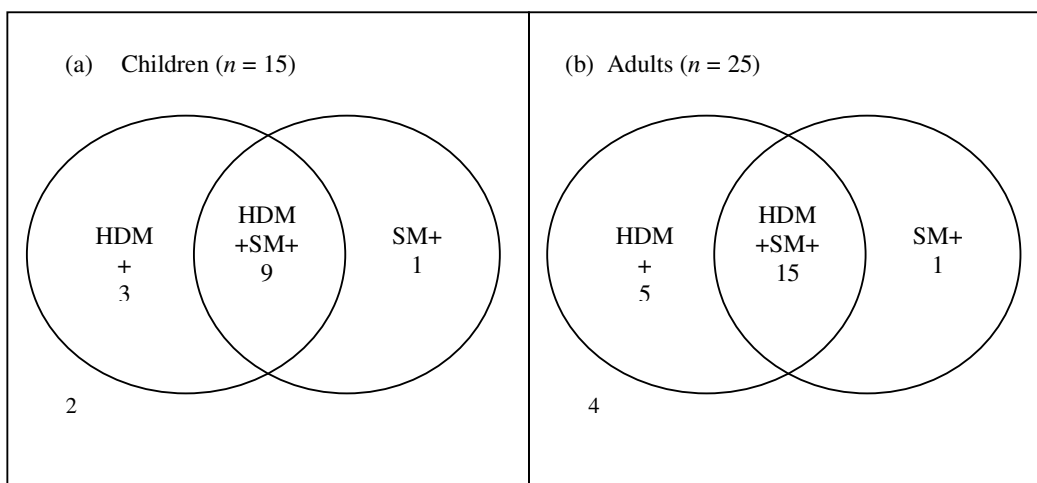


Figure 1. Sensitization profile of children (a) and adults (b) rhinitis patients with mite allergic to different mite allergen combinations based on microarray results.

HDM+: Patients with IgE sensitivity to at least one of the components Der p 1/ Der f 1/ Der p 2/ Der f 2/ Der p10

SM+: Patients with IgE sensitivity to at least one of the components Lep d 2/ Blo t 5

Component-Resolved Diagnosis in Rhinitis Patients with Mite Reactivity

Table 1. IgE reactivity to mite allergen components in rhinitis patients with mite-allergic (a) and non-mite allergic (b).

a)	Total		Children		Adults		P-value
	%	n= 40	%	n= 15	%	n= 25	
Der p 1	65	26	67	10	64	16	1
Der f 1	65	26	67	10	64	16	1
Der p 2	55	22	60	9	52	13	0.747
Der f 2	58	23	67	10	52	13	0.512
Lep d 2	50	20	53	8	48	12	0.1
Blo t 5	50	20	60	9	44	11	0.514
Der p10	30	12	33	5	28	7	0.999

b)	Total		Children		Adults		P-value
	%	n= 5	%	n= 1	%	n= 4	
Der p 1	0	0	0	0	0	0	1
Der f 1	0	0	0	0	0	0	1
Der p 2	0	0	0	0	0	0	1
Der f 2	0	0	0	0	0	0	1
Lep d 2	0	0	0	0	0	0	1
Blo t 5	20	1	0	0	25	1	1
Der p10	0	0	0	0	0	0	1

n, number of patients with IgE reactivity; %, percentage of patients with IgE reactivity

Comparatively, based on microarray results in Figure 1, only 34 out of 40 (85%) mite-allergic patients had detectable serum IgE to at least one of the mite allergen components tested. One patient classified as non-mite allergic based on a negative SPT, was tested positive to Blo t 5 on CRD (Table 1b).

IgE Reactivity Frequency to Mite Allergen Components

IgE reactivity frequency to mite allergen components was comparable between children and adults (Table 1).

The frequency of IgE reactivity of children to mite specific allergen components were 67% to Der p 1, Der f 1 and Der f 2, 60% to Der p 2, 53% to Lep d 2 and 60% to Blo t 5. The mite specific allergen components recognized in adults were 64% to Der p 1 and Der f 1, 52% to Der p 2 and Der f 2, 48% to Lep d 2 and 44% to Blo t 5. IgE reactivity to cross-reactive tropomyosin (Der p 10) was 33% in children and 28% in adults. There was no significant association between the frequency of IgE reactivity to mite allergen components and the two groups.

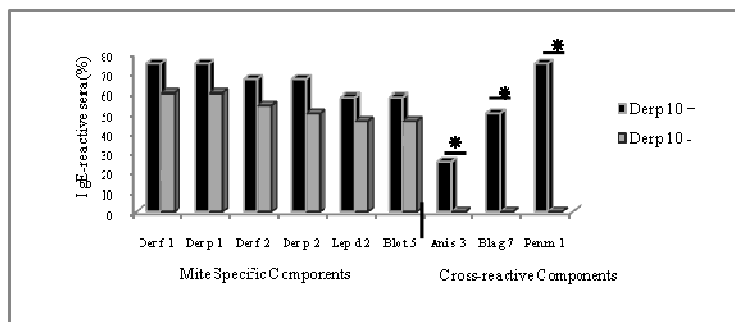


Figure 2. Frequencies (percent of reactive sera) of IgE recognition (y-axis) of mite allergens (x-axis) are shown for mite allergic patients with (black bars) or without (grey bars) IgE antibodies to Der p 10. Statistically significant differences between the two groups are indicated (* $P < 0.05$).

Der p 10, a Tropomyosin, is a Common Cause of Cross-Reactive Allergen between Mites and Other Invertebrates and Seafood

Comparing the frequencies of IgE reactivity in the cross-reactive tropomyosin Der p 10-positive patients ($n=12$; Children=5; Adults=7) and negative patients ($n=28$; Children=10; Adults=18), we observed striking differences regarding the reactivity profiles (Figure 2).

The majority of the Der p 10-negative patients were primarily sensitized to mite specific allergen components. By contrast, Der p 10-positive patients reacted to other cross-reactive tropomyosin from anisakis (Ani s 3), cockroach (Bla g 7) and shrimp (Pen m 1). The frequencies of IgE reactivities of Ani s 3 ($p=0.02$), Bla g 7 ($p=0.0002$) and Pen m 1 ($p<0.000$) were approximately twenty five to seventy five percent higher in the Der p 10-positive compared with the Der p 10-negative patients (Figure 2).

DISCUSSION

The development of microarrays with a large number of purified allergens has enabled the testing of a large number of allergen molecules simultaneously. However, this diagnostic tool requires validation as patients from different geographical regions have different sensitization profiles to mites. The microarray chip used had the following mite allergen components coated on the chip: Der p 1, Der p 2, Der f 1, Der f 2, Der p 10, Lep d 2 and Blo t 5. In our study, we found that the majority (85%) of the patients tested positive to mites on SPT also tested positive to at least one of the components of mite in the microarray panel. However, there were 6 patients who went undetected by these allergen components, indicating that other additional allergen components are needed (e.g. the inclusion of groups 6, 9, 11 and 12 allergens) for a complete-spectrum diagnosis of mite allergy.

There was also one patient who reacted to the individual allergen components used but not to the mite allergen crude extracts used in SPT (Table 1b). This could be due to an insufficient amount of allergenic components in the crude extract. Crude allergen extracts were prepared from natural allergen sources and it is possible that important allergens were present in small amounts or lacking and their biological potency subject to huge variability.^{11,12} Attempts have been made to control the quality of these crude extracts by using different standardization procedures. Despite

these efforts, their composition cannot be altered and products from different companies and even batches from the same company are not comparable. Therefore, it would be feasible and useful to use a panel of individual allergen components to replace the use of mite allergen crude extracts for more precise diagnosis of mite allergy.

There was an apparent disparity between frequencies of serum specific IgE to groups 1 and 2 in our study and other studies across the globe. This may be accounted for by the different geographical, age and clinical status-related in the study population.^{1,11,15,22,23} The frequency of group 1 IgE in our study (65%) was comparable to Singapore (63%)²³, Australia (74-77%)²⁴ and Brazil (77-83%).^{22,25} In contrast, reported frequency to group 1 IgE is higher in other European cohorts: 97% in a Strasbourg-based cohort,¹⁰ 91% in a Central Europe cohort¹⁵ and 85-100% in a collaborative French, Italian, Austrian and Swedish study.¹¹ The frequency of group 2 IgE in our study was 55% (Der p 2), 58% (Der f 2) and 50% (Lep d 2) which was in the low range as compared to the reported values in Europe, Brazil, Australia and Singapore: 63-96%,^{10,11,15} 79-81%,^{22,25} 62-74%²⁴ and 71%,²³ respectively. Der p 10 or tropomyosins are minor mite allergens and cross-reactivity is extremely frequent with tropomyosin from prawn, cockroach and anisakis.^{1,11,26,27} In our study, Der p 10 IgE sensitization was more prevalent in both children (33%) and adults (20%) than in previous European reports where it ranged from 6% to 18%.^{10,11,26} One possible explanation might be due to the higher intake of seafood that influences the tropomyosin IgE reactivity.

One of the strengths of CRD is that in some situations it may be able to discriminate between allergy and cross reactivity in patients, in whom the differentiation is difficult to establish from symptoms and signs.^{19,20} CRD allowed discriminating patients who were mainly sensitized to the mite specific allergens from others who exhibited reactivities also to highly cross-reactive tropomyosin. Der p 10, a tropomyosin is a cross-reactive allergen in invertebrates and seafood. We found that all Der p 10-positive patients also reacted to other cross-reactive tropomyosin from anisakis (Ani s 3), cockroach (Bla g 7) and shrimp (Pen m 1). This supports the theory that Der p 10 might be a useful indicator to identify a subset of mite-allergic patient that have additional sensitization due to cross-reactivity.

Component-Resolved Diagnosis (CRD) of Mites Reactivity

The use of CRD not only confirmed the discrimination of allergy and cross-reactivity in patients but also allowed the identification of those patients who are best suited for immunotherapy using mite extracts. Those patients who were sensitized to group 1 and/or group 2 should receive immunotherapy with mite extracts. This consideration was based mainly on the fact that, at present, mite extracts were only tested for the presence of group 1 and 2. There was no available information regarding the contents of allergens other than group 1 and 2 in mite extracts.¹⁰ Furthermore, it has been demonstrated that allergens other than group 1 and 2 are missing in most of the commercially available allergen extracts.⁶ It is very likely that patients from the Der p 10- positive group may not benefit so well from therapy with such extract.^{6,15} Accordingly, crude allergen extracts are heterogeneous mixtures of allergenic and non-allergenic components, which can theoretically induce new sensitizations to constituents to which patients were not originally sensitized. Therefore, the use of reagents with only the purified and well-characterized allergenic proteins that are relevant to the allergic diseases would probably improve the efficacy of immunotherapy.⁸

In conclusion, CRD provide us with a new tool to diagnose mite allergy and improve the selection of patients for immunotherapy. Nevertheless, our findings are derived from a relatively small group of subjects and replication of our findings with larger collection of sera samples is warranted.

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