BRIEF COMMUNICATIONS
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Improved Diagnosis of the Polysensitized Allergic Rhinitis Patients Using Component Resolved Diagnosis Method

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ABSTRACT

Allergy diagnosis needs to be improved in polysensitized patients due to the existence of possible confounding factors in this type of patients. Component resolved diagnosis (CRD) is a new concept in the investigation of polysensitized patients. The aim of this study was to evaluate if the utilization of ImmunoCAP ISAC improve the diagnosis of the polysensitized allergic rhinitis patients.

Skin prick test (SPT) to 58 crude allergen extracts and CRD (ImmunoCAP ISAC) were carried out for 5 polysensitized allergic rhinitis patients.

Two patients had a shellfish allergy and avoidance of shellfish was the only way to prevent an allergic reaction. In contrast, although the remaining three patients had low risk for shellfish allergy, but they were the best candidates for immunotherapy using mite extracts.

CRD and particularly ImmunoCAP ISAC have proven to be a valuable diagnostic tool in polysensitized patients. ImmunoCAP ISAC helps refine the individual patient's sensitization profile and predict the potential risk of allergic reactions and improve the selection of patients for immunotherapy.

Keywords: Allergic rhinitis; Component resolved diagnosis; Skin prick testing

INTRODUCTION

Most allergic patients are sensitized to numerous allergen sources. In such cases it will be important for the clinician to know whether a patient is co-sensitized to several allergen sources and needs specific immunotherapy for each of them, or whether a patient is sensitized to several allergen sources due to sensitization or to cross-reactive components in each of the suspected allergen sources.[1] In Malaysia, the majority of polysensitization are caused by allergy to mites and shellfish.[2] Allergic cross-reactivity between these two groups was reported, in which tropomyosin

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seems to be involved.[3,4] Therefore, a correct diagnosis of these polysensitized patients is crucial for appropriate and potential life-saving management.[1] However, in our country, the diagnosis of polysensitized patients remains largely unchanged. SPT and/ or specific IgE to crude allergen extracts, together with a careful patient anamnesis, constitute the basis for diagnosis at present. Recent developments in allergy have introduced us with new diagnostic tool that is component resolved diagnosis (CRD) and particularly ImmunoCAP Immuno Solid-phase Allergen Chip (ISAC). Based on modern biochip technology, ImmunoCAP ISAC is a miniaturized immunoassay platform that allows for multiplex measurement of specific IgE antibodies to many allergen components using only 20 µl of serum or plasma.[1] Therefore, this study was conducted to evaluate if the utilization of ImmunoCAP ISAC improve the diagnosis of the polysensitized allergic rhinitis patients.

MATERIALS AND METHODS

This study involved adult patients (age>18 years) with polysensitized allergic rhinitis referred to ear, nose and throat (ENT) clinic of hospital Kuala Lumpur (HKL) from September to December 2013. The diagnosis of allergic rhinitis was made according to current guideline.[5] The polysensitization status was firstly established by SPT, which were carried out with 58 crude allergen extracts. Saline solution and histamine hydrochloride 1% (Alk Abello, Madrid, Spain) served as negative and positive controls, respectively. The local ethical committee approved the study and patients gave informed consent (research ID: JPP-IMR 13-022; ethical approval: NMRR-13-17594). Sera of all patients were analyzed for the presence of specific IgE to allergen components by the allergen microarray ImmunoCAP ISAC (VBC Genomics, Vienna, Austria/ Phadia, Uppsala, Sweden) according to manufacturer's guidelines. Different groups of 112 allergenic molecules are spotted on the allergen chip. Allergen-specific IgE-antibodies were detected by fluorescence-labeled anti-IgE antibodies. Fluorescence was measured with a laser scanner and results were evaluated using Phadia microarray image analysis (MIA) software. Results were reported in ISAC standardized units (ISU) giving indications of specific IgE antibody levels.

RESULTS AND DISCUSSION

A major problem using crude allergen extracts for testing, is that it is difficult and often impossible to precisely identify the disease-eliciting allergen, particularly in polysensitized patients.[6] The positive IgE results may be due to cross-reactive allergens instead of the specific allergens to a given biological source.[7] Many biological sources contain highly cross-reactive allergen components, presenting in broadly different botanical or zoological families, related or unrelated by taxanomy.[7] A sensitization towards such a cross-reactive allergen creates positive test results against numerous allergen extracts.

Therefore, CRD tests have the capability in resolving whether sensitization of patients towards a given allergen source was genuine or due to cross-reactive allergens causing cross-sensitization to several allergen sources.[1] As shown in table 1, it is clear that the presence of the cross reactive tropomyosin causes excess positive skin tests in patients no. 1 and 2 particularly for shellfish, mites, cockroach and the fish parasite, *Anisakis simplex*. Tropomyosins are well described as food allergens (crustaceans, mollusks and fish parasite *Anisakis simplex*) and also are found in arthropods identified as aero-allergens like mites and cockroaches.[7] The frequent cross-sensitization among different allergenic sources is due to the highly conserved tropomyosin sequences. In contrast to mites and cockroaches, tropomyosins are the major and clinically relevant allergens in shellfish.[8-10] Tropomyosins are digestion and heat resistant proteins and often associated with systemic and more severe reactions. Therefore, patients no. 1 and 2 have a shellfish allergy and avoidance of shellfish is the only way to prevent an allergic reaction.

The determination of the individual patient's sensitization profile by CRD also allowed the selection of the relevant patients for immunotherapy.[1] Patients no. 3, 4, and 5 are best suited for immunotherapy (IT) using mite extracts. Those patients were sensitized to group1 and/or group 2 of mites. In our previous study, the major mite allergens, group 1 (cysteine protease) and group 2 (NPC2 family) were a marker allergen for genuine sensitization to mite while tropomyosin was a marker for cross-reactivity between mites, crustaceans, mollusks and the fish parasite, *Anisakis simplex*.[11] IgE reactivity to group 1 and/or group 2 without reactivity to tropomyosin indicates sensitization only to the mite,

making them good candidates for IT.[11] The use of CRD is also helpful in predicting the severity of allergic reactions. Patients no. 3, 4 and 5 also showed IgE reactivity to arginine kinase which is prawn/shrimp specific allergen. Apart from prawn/shrimp, arginine kinase has also been identified as an allergen in other shellfish such as crab and lobster.[8,9] However, arginine kinase is thermo labile allergen; as a consequence allergic patients may potentially experience mild allergic reactions. Raw shellfish is more likely to produce allergic reactions due to arginine kinase than cooked shellfish. Therefore, these patients can safely eat cooked shellfish.

However, it must also be admitted that the current version of ImmunoCAP ISAC lack some allergen components as not all components in the various sources have been completely characterized and evaluated. It is apparently also noted that ImmunoCAP ISAC is unable to detect very low concentrations of IgE antibodies.[1]

Table 1. Patient's characteristics

Patient no.	Sex/age (y)	SPT		ISAC				
		Allergens	Wheal Diameter	Components			ISU	
1	F/25	White prawn	5	*A. simplex*	rAni s 3	Tropomyosin	0.6	Low
		Yellow prawn	6	Cockroach	nBla g 7	Tropomyosin	2.5	High
		Tiger prawn	5.5	*D. pteronyssinus*	rDer p 10	Tropomyosin	4.8	High
		King prawn	6	Shrimp	nPen m 1	Tropomyosin	2.4	High
		Sharp-rostum prawn	5					
		Giant Freshwater prawn	4					
		B. tropicalis	5					
		D. farinae	6					
		D. pteronyssinus	9					
		Cockroach	5					
		A. simplex	5					
		Barley	6.5					
		Soya	5					
2	M/30	White prawn	5	*A. simplex*	rAni s 3	Tropomyosin	6.2	High
		Yellow prawn	6	Cockroach	nBla g 7	Tropomyosin	7.6	High
		Tiger prawn	6.5	*D. pteronyssinus*	rDer p 10	Tropomyosin	29	Very high
		King prawn	5	Shrimp	nPen m 1	Tropomyosin	8.3	High
		Sharp-rostum prawn	6					
		Giant Freshwater prawn	5					
		B. tropicalis	7					
		D. farinae	6					
		D. pteronyssinus	10					
		Cockroach	6.5					
		Cockle	4.5					
		Squid	5					
		Clam	4					
		Blue crab	7.5					
		Wildflower honey	5					
		Black sesame	4					
		A. simplex	4.5					

Table 1. continued

ImmunoCAP ISAC for Polysensitization

Patient no.	Sex/age (y)	SPT	ISAC				
		Allergens	Wheal Diameter	Components			ISU
3	M/21	White prawn	7	Shrimp	nPen m 2	Arginine kinase	20	Very high
		Yellow prawn	5	*B. tropicalis*	rBlo t 5	Mite group 5	46	Very high
		Tiger prawn	6	*D. farinae*	nDer f 1	Cysteine protease	8	High
		King prawn	5	*D. pteronyssinus*	nDer p 1	Cysteine protease	12	High
		Sharp-rostum prawn	5					
		Giant Freshwater prawn	6					
		B. tropicalis	10					
		D. farinae	7					
		D. pteronyssinus	13					
4	M/18	White prawn	8.5	Shrimp	nPen m 2	Arginine kinase	3.5	High
		Yellow prawn	5.5	*D. farinae*	nDer f 1	Cysteine protease	2.4	High
		Tiger prawn	5	*D. pteronyssinus*	nDer p 1	Cysteine protease	1.4	High
		Sharp-rostum prawn	7.5					
		Giant Freshwater prawn	6.5					
		B. tropicalis	6					
		D. farinae	7.5					
		D. pteronyssinus	9					
5	F/27	White prawn	10	Shrimp	nPen m 2	Arginine kinase	1.9	High
		Yellow prawn	8.5	*D. farinae*	rDer f 2	NPC2 family	15	Very high
		Tiger prawn	10	*D. pteronyssinus*	rDer p 2	NPC2 family	18	Very high
		D. farinae	8					
		D. pteronyssinus	8					
		Scad	7.5					
		Mackerel	9					
		Tuna	9.5					
		Dhal	8					
		Green bean	7					
		Red bean	6.5					
		Blue crab	7.5					

In conclusion, CRD and particularly Immuno CAP ISAC is a precious diagnostic tool when scanty sensitization profiles are found in polysensitized patients. The results increase the possibility of identifying patients at risk of severe reactions and explain cross-reactivity. As a result, effective and optimized management can be started earlier, which in turn leads to improved patient health and quality of life.

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