ORIGINAL ARTICLE

Performance of Different in Vitro Techniques in the Molecular Diagnosis of Peanut Allergy

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Abstract

Introduction: Peanut allergy is an increasingly serious disorder with a heterogeneous pattern of sensitization across different countries. In vitro diagnostic techniques may help in establishing these patterns.
Objectives: To analyze the usefulness of determining specific immunoglobulin E (sIgE) with the ImmunoCAP fluorescence enzyme immunoassay (FEIA), the ImmunoCAP ISAC CRD103 microarray (ISAC), and the basophil activation test (BAT) in the molecular diagnosis of peanut allergy.
Methods: In 26 peanut-allergic patients, sIgE antibodies against allergic components were measured with FEIA, ISAC, and BAT.
Results: The major peanut component in our population was Ara h 9. The detection of sIgE to Ara h 9 using FEIA and BAT with this allergen yielded a sensitivity of 92% and 88% and a specificity of 95% and 100%, respectively. Overall diagnosis of peanut allergy by ISAC showed a sensitivity of 11% but a specificity of 95% since Ara h 9 was not present in the microarray version used. There was diagnostic agreement between the 3 techniques for the peanut allergens studied.
Conclusions: The determination of sIgE to Ara h 9 using FEIA and BAT offers high sensitivity and specificity in the diagnosis of peanut allergy in the Spanish population. The CRD103 version of ISAC is not of value in our region as it does not include the most common allergen, Ara h 9.

Key words: Peanut allergy. Molecular diagnosis. Basophil activation test. Ara h 9. Microarray.

Resumen

Introducción: La alergia a cacahuete es una patología importante cuya prevalencia va en aumento y que presenta diferentes patrones de sensibilización en los distintos países. Las técnicas in vitro pueden ser herramientas útiles para establecer dichos patrones.
Objetivos: Analizar la utilidad de la determinación de IgE específica mediante ImmunoCAP y micromatriz, así como la del test de activación de basófilos (TAB) en el diagnóstico molecular de dicha patología.
Métodos: Se determinó la IgE específica mediante ImmunoCAP y micromatriz ISAC CRD103, y se realizó TAB frente a diferentes componentes alergénicos del cacahuete en 26 pacientes alérgicos a cacahuete.
Resultados: El alérgeno mayoritario en nuestra población fue Ara h 9. La detección de la IgE específica mediante ImmunoCAP y el TAB frente Ara h 9 mostraron una sensibilidad (Se) de 92% y 88%, y una especificidad (Sp) de 95% y 100% respectivamente. El diagnostico global de la IgE frente a los alérgenos del cacahuete mediante la micromatriz presentó una sensibilidad de 11% y una especificidad de 95%, debido a la ausencia de Ara h 9 en la versión empleada. Las tres técnicas con los diferentes componentes alergénicos del cacahuete estudiados mostraron acuerdo diagnóstico .
Conclusiones: El TAB y la determinación de IgE específica mediante ImmunoCAP frente a Ara h 9 ofrecen una alta sensibilidad y especificidad en la alergia a cacahuete en la población española. La versión ISAC CRD103 de la micromatriz no es de utilidad en nuestra región debido a la ausencia de Ara h 9 en su panel de alérgenos.

Palabras clave: Alergia a cacahuete. Diagnóstico molecular. Test de activación de basófilos. Ara h 9. Micromatriz.

Introduction

Peanuts, as a cheap source of nutrients, are consumed throughout the world and are also used in many food and cosmetic products.

The estimated prevalence of peanut allergy in the US population is 1% [1] and incidence is increasing. Peanuts, together with other nuts, account for about 90% of all cases of anaphylaxis due to foods in the United States [2], with some series reporting that peanuts are involved in 55% of fatal allergic reactions [3].

While classically, Ara h 1 (vicilin), Ara h 2 (conglutinin), and Ara h 3 (glycinin) are considered to be the major peanut allergens [4-6], patterns of sensitization vary from one country to next. According to studies conducted in English-speaking countries, these 3 allergens are the allergenic components most frequently recognized by immunoglobulin (Ig) E in peanut-allergic patients. However, in Mediterranean countries, Ara h 9 is the main allergen. Studies published in Italy and Spain report that 90% of peanut-allergic patients have specific IgE (sIgE) against this protein [7-9].

In view of the above, it is crucial to determine the sensitization profile of peanut-allergic patients to ensure appropriate therapeutic management. To this end, it is essential to have available accurate diagnostic techniques capable of providing a reliable molecular diagnosis. While numerous in vitro techniques are available, their diagnostic accuracy requires further analysis.

The objective of this study was to determine the diagnostic accuracy of sIgE determination using the ImmunoCAP ISAC CRD103 microarray (ISAC) (ImmunoDiagnostics Thermo Fisher Scientific) and the basophil activation test (BAT) in the molecular diagnosis of peanut allergy in comparison with the determination of sIgE using the ImmunoCAP fluorescence enzyme immunoassay (FEIA) (ImmunoDiagnostics Thermo Fisher Scientific). We also analyzed the degree of agreement between the 3 techniques.

Materials and Methods

Patients

Twenty-six consecutive patients who had experienced type I hypersensitivity reactions to peanut on more than 1 occasion (excluding anaphylaxis) and who had a positive skin test to peanut extract and an sIgE to peanut extract of 2 kU_A/L or more, as defined in the recommendations of Perry et al [10], were enrolled for this study at the allergology departments of Hospital Carlos Haya in Málaga and Clínica Universidad de Navarra in Pamplona, Spain. The median age of the patients was 29 years (interquartile range [IQR] 23-35.5 years) and 30% were male. Peanut ingestion caused oral allergy syndrome (OAS) in 5 patients, nonanaphylactic systemic symptoms (e.g. urticaria, gastrointestinal symptoms) in 16, and anaphylaxis (defined according to the criteria of Simons [11]) in 5. All the participants were informed about the study by the medical team and signed an informed consent approved by the ethics committee.

Twenty-two of the 26 patients had symptoms of allergy to at least 1 other tree nut or seed, including walnut (n=19), hazelnut (n=13), almond (n=12), sunflower seed (n=10), pistachio (n=4), chestnut (n=6), and pine nut (n=1). Most of them (n=19) had allergic symptoms to at least 2 nuts or seeds apart from peanut. Only 5 patients had symptoms caused by legumes: one patient reported allergy to soybeans, another to peas, and 3 to green peas and lentils together with chickpeas in 2 cases, soybeans in 1 case, and beans in another case. Twenty-two of the 26 patients reported allergic symptoms after peach ingestion.

Eight atopic and 11 nonatopic controls were selected for the control group (19 patients in total). Their median age was 28 years (IQR, 26-33 years) and 16% were male. Two of the atopic patients were monosensitized to dust mites and 6 to pollen. One was sensitized to pollen from *Artemisia vulgaris* and peach and 5 to pollen from grasses; 1 of these 5 patients was also sensitized to *Parietaria judaica* and peach. None of the controls had plant-derived food allergy or a positive skin prick test to peanut extract. They all underwent the same in vivo and in vitro tests.

Skin Tests

Skin tests were performed with peanut extract (Bial-Aristegui), the most common aeroallergens in each location, and peach extract (30 mg/mL of Pru p 3) (ALK-Abelló), with reading of results after 20 minutes. Wheals with a 3-mm diameter were considered positive, as recommended by the European Academy of Allergy and Clinical Immunology guidelines [12]. All the skin tests were performed by the same experienced nurses.

Determination of sIgE With FEIA

We measured sIgE to peanut extract and the recombinant (r) allergens, rAra h 1, rAra h 2, rAra h 3, rAra h 8, and rAra h 9 with FEIA according to the manufacturer's instructions. All values above 0.35 kU_A/L were considered positive.

Determination of sIgE With ISAC

The ISAC CRD103 microarray was used to quantify serum sIgE to the following allergenic natural (n) and recombinant proteins: nAra h 1, nAra h 2, nAra h 3, and rAra h 8. sIgE detection was performed as recommended by the manufacturer and based on previously described protocols by Deinhofer et al [13] and Harwanegg and Hiller [14]. Positive values were considered those equal to or greater than 0.3 ISU (ISAC standardized units) as recommended by the manufacturer.

Basophil Activation Test

The percentage of activated basophils was determined following stimulation with peanut extract and the peanut allergens rAra h 1, rAra h 2 (Indoor Lab) and rAra h 9 [7]. BAT was performed as previously described by our group [15]. Briefly, blood was collected in 6-mL ACD tubes and resuspended in 100-µL HEPES calcium buffer, containing interleukin 3 (10 ng/mL). In the cellular stimulation phase, 2 final concentrations (1 and 0.1 µg/mL) were assayed for the peanut extract and all the components analyzed. As a positive control, a monoclonal

anti-IgE receptor antibody (Bühlmann Laboratories) at a concentration of 1 μg/mL was used. In order to evaluate baseline values without stimulation, 50 μL of stimulation buffer was added to another tube and 50 μL of cell suspension was added to all tubes. Soon afterwards, the tubes were centrifuged at 1000×g for 5 minutes at 4°C. The basophils from the cell pellet were double-labeled by adding 20 μL of anti-CD63 picoerythrin-labeled antibody diluted at 1:80 and 20 μL of anti-IgE fluorescein isothiocyanate–labeled antibody. Flow cytometric analysis was performed at 488 nm on a FACSCanto flow cytometer (Becton Dickinson). The results were analyzed with a DIVA software program.

A positive BAT result was considered when at least 1 of the 2 concentrations used was greater than 15% with a stimulation index (test value/background value) of over 2 [16].

Statistical Analysis

Quantitative variables were described as means (SD) or medians (25th and 75th percentiles), depending on whether or not the distribution of the data was normal, while qualitative variables were described as frequencies (percentages). Diagnostic accuracy was evaluated by means of sensitivity and specificity. Sensitivity and specificity values were obtained from contingency tables. Diagnostic agreement between the techniques was analyzed using the McNemar test for paired samples. All data were analyzed using SPSS 15.0. *P* values of less than .05 were regarded as significant.

Results

Skin Tests

In the group of patients with OAS, 5 patients (100%) had positive skin tests results with peach extract whilst in the groups with nonanaphylactic symptoms and anaphylaxis positive results were obtained in 13 (81%) and 4 (80%) patients, respectively. Only 2 of the 19 controls had a positive skin test to peach extract.

In Vitro Tests

The positive results for peanut extract and each allergenic component for the 3 diagnostic techniques are shown in Table 1.

Commercial Extract

BAT with peanut extract showed a sensitivity of 92% and a specificity of 95%. Considering positive results for all the peanut components in the ISAC CRD103 microarray, this technique showed a sensitivity of 11% and a specificity of 95%.

Peanut Components

Patients allergic to peanut had a low frequency of sensitization to Ara h 1, Ara h 2, and Ara h 3 in the 3 techniques. Specifically, sensitization to Ara h 1 and Ara h 2 with BAT (Ara h 1 sensitivity, 19%; Ara h 2 sensitivity, 15%), FEIA (Ara h 1 sensitivity, 4%; Ara h 2 sensitivity, 8%), and ISAC (Ara h 1 sensitivity, 4%; Ara h 2 sensitivity, 8%) was very low in our

Table 1. Summary of Positive in Vitro Results in Patients and Controls

	ImmunoCAP Fluorescence Enzyme Immunoassay					Bassophil Activation Test				ImmunoCAP ISAC CRD103 Microarray SPT				
	Peanut	Ara h 1	Ara h 2	Ara h 3	Ara h 8	Ara h 9	Peanut	Ara h 1	Ara h 2	Ara h 9	Ara h 1	Ara h 2	Ara h 3	Ara h 8	Peanut
OAS	5/5	0/5	0/5	0/5	1/5	5/5	5/5	1/5	0/5	5/5	0/5	0/5	0/5	0/5	5/5
Systemic/no anaphylaxis	16/16	0/16	1/16	1/16	1/16	15/16	14/16	2/16	1/16	12/16	1/16	1/16	0/16	1/16	16/16
Anaphylaxis	5/5	1/5	1/5	1/5	0/5	4/5	5/5	2/5	3/5	5/5	0/5	1/5	0/5	1/5	5/5
Atopic controls	1/8	0/8	0/8	0/8	0/8	1/8	1/8	0/8	0/8	0/8	1/8	0/8	0/8	0/8	0/8
Nonatopic controls	0/11	0/11	0/11	0/11	0/11	0/11	0/11	0/11	0/11	0/11	1/11	0/11	0/11	0/11	0/11

Abbreviations: OAS: oral allergy syndrome; SPT, skin prick test.

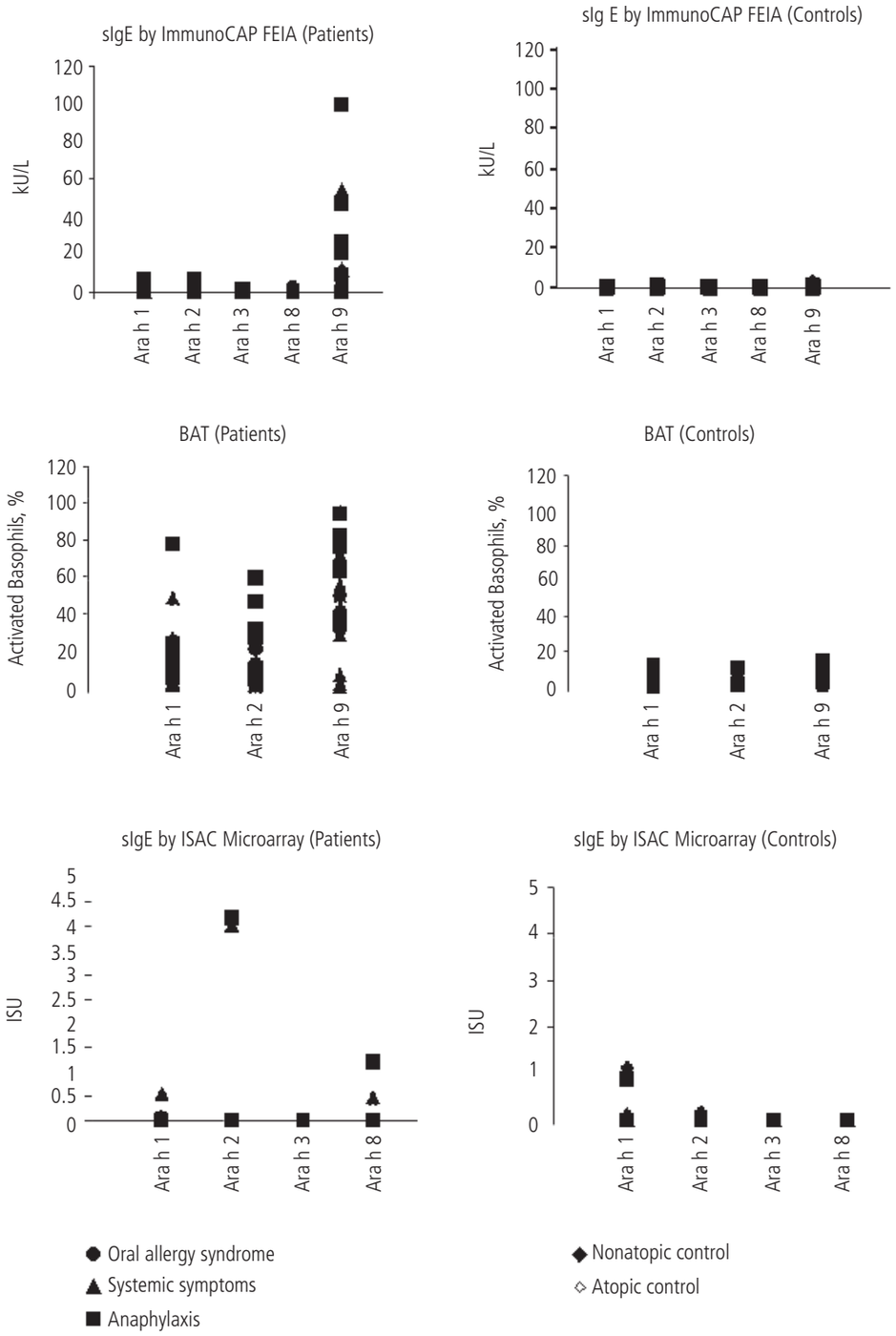

Figure. Specific immunoglobulin E (sIgE) levels in patients and controls determined using the ImmunoCAP fluorescence enzyme immunoassay (FEIA), the ImmunoCAP ISAC CRD103 microarray, and the basophil activation test (BAT). ISU indicates ISAC standardized units.

Table 2. Agreement Between the 3 in Vitro Techniques for the Different Allergens Studied.

		Peanut	Ara h 1		Ara h 2		Ara h 3		Ara h 8		Ara h 9	
		sIgE with FEIA	sIgE with FEIA	sIgE with ISAC CRD103 microarray	sIgE with FEIA	sIgE with ISAC CRD103 microarray	sIgE with FEIA	sIgE with ISAC CRD103 microarray	sIgE with FEIA	sIgE with ISAC CRD103 microarray	sIgE with FEIA	sIgE with ISAC CRD103 microarray
BAT	Agreement in patients	24/26	21/25	20/26	21/25	21/25					21/25	
	Agreement in controls	19/19	19/19	17/19	19/19	19/19					18/19	
	P value (McNemar)	.5	.125	.727	.625	.625					.375	
sIgE with FEIA	Agreement in patients		24/26		26/26		25/26		24/26			
	Agreement in controls		17/19		19/19		19/19		19/19			
CRD103 microarray	*P* value (McNemar)		.625		1		1		1			

Abbreviations: BAT, basophil activation test; FEIA, fluorescence enzyme immunoassay (ImmunoCAP); sIgE, specific immunoglobulin E.

country. Sensitivity for Ara h 3 and Ara h 8 was also low with FEIA (Ara h 3, 8%; Ara h 8, 8%) and ISAC (Ara h 3, 0%; Ara h 8, 8%) in our area. The highest sensitivity and specificity was observed for Ara h 9: 92% and 95%, respectively, for FEIA and 88% and 100%, respectively, for BAT. Ara h 9 was not included in the ISAC microarray panel studied.

The serum sIgE levels detected by the 3 techniques for the different components analyzed are shown in the Figure.

Agreement Between in Vitro Allergy Techniques

The agreement between the 3 techniques for the allergen components analyzed is shown in Table 2. The diagnostic performance of FEIA and BAT was similar for peanut extract (*P*=.5). Considering an overall positive result when any positive peanut component in ISAC was positive and an overall negative result when all of the components were negative, significant differences were found between ISAC and both BAT (*P*<.001) and FEIA for the diagnosis of peanut allergy using peanut extract (*P*<.001).

Discussion

In our study, the allergenic component that diagnosed peanut allergy with the greatest sensitivity and specificity was Ara h 9. Reactivity to Ara h 9 showed a good yield in the determination of sIgE with both FEIA and BAT. Indeed, the low sensitivity shown by ISAC in the diagnosis of peanut allergy, considering all the peanut allergenic components included, is due to the absence of the Ara h 9 component in the CRD103 microarray panel used. Furthermore, the low sensitivity of Ara h 1, Ara h 2, Ara h 3, and Ara h 8 in the different techniques is a result of the low frequency of sensitization to these allergens in our geographical area. The sensitization profile in our population is consistent with data reported by other authors in the Mediterranean region [7-9].

A strong association has been observed between sensitization to peanut and peach lipid transfer protein (LTP) [7,9,17], with high cross-reactivity observed between Pru p 3, the peach LTP, and Ara h 9 [7,9]. Indeed, it has even been suggested that Pru p 3 might be a primary sensitizer of Ara h 9 [9]. In a small sample including some patients in this study, we analyzed the timing of symptoms with peanut and peach, and found that almost two-thirds of the patients with symptoms to both foods developed symptoms first with peach and then with peanut. Nevertheless, further studies are needed to prove that the peach LTP is the main sensitizer of peanut allergy in the Mediterranean area.

The microarray analyzed in this study, the ISAC CRD103, offered low diagnostic value for peanut allergy given that the panel of allergens it contains

is not adapted to the sensitization patterns of peanut-allergic patients in the Mediterranean region. However, the new version of this microarray does contain Ara h 9 and it would therefore be interesting to reassess its diagnostic accuracy in the Spanish population.

BAT offers a similar diagnostic accuracy to FEIA for peanut extract and all the components studied. This finding attests to the usefulness of BAT in the molecular diagnosis of peanut allergy, as was shown for peach allergy in a previous study by our group [18].

In conclusion, the determination of specific IgE to peanut allergen components using FEIA and BAT is useful for the diagnosis of peanut allergy. Ara h 9 is the major allergen in our population and these techniques are reliable diagnostic tools for the study of this allergen given their high sensitivity and specificity. ISAC did not provide high diagnostic accuracy for peanut allergy in our geographical area as the CRD103 microarray used did not contain Ara h 9.

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