

Feasibility of the ALEX multiplex platform in the diagnosis of nut allergy from a Mediterranean population

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ABSTRACT

Background: ALEX multiplex platform has been recently commercialized but its clinical utility as quantitative technique respect to ImmunoCAP-singleplex as the reference method has not yet been confirmed on patients suffering from nut allergy and co-sensitization to different nuts.

Methods: 58 serum samples from patients with nut allergy from a Mediterranean population were assayed in parallel by ALEX-multiplex and ImmunoCAP-singleplex techniques. Patients were diagnosed based on clinical symptoms and positive skin prick tests (SPTs). The following whole extracts were compared between both techniques: walnut, hazelnut, peanut, almond, pistachio and sunflower seed; besides the recombinant Pru p 3. A qualitative and quantitative study was carried out.

Results: Both techniques had similar sensitivities respect to whole extracts from walnut, hazelnut and peanut as well as to Pru p 3 ($p > 0.05$). However for whole extracts from almond, pistachio and sunflower seed the sensitivity obtained by ALEX was much lower than ImmunoCAP (9.09 % vs 88.63 %; 14.81 vs 70.37 %; and 8.51 % vs 88.88 %; respectively). The concordance between both techniques showed only a substantial agreement for Pru p 3 ($k = 0.791$); moderate agreement for hazelnut and peanut ($k = 0.550$ and $k = 0.544$, respectively); fair agreement for walnut ($k = 0.386$) and poor agreement for almond, pistachio and sunflower seed ($k < 0.2$). Quantitative analysis showed that ImmunoCAP for walnut, peanut and sunflower seed had higher mean values than ALEX. Relationships were significant for all specific IgE levels except to for almond, pistachio and sunflower seed.

Conclusions: ALEX platform is a suitable technique to patients with nut allergy from the Mediterranean area except to for those suffering from allergy to almond, pistachio and sunflower seed.

1. Introduction

Nuts are an important allergen source in food allergy both in pediatric and adult populations [1,2]. Under the term of *nut allergy* are included those allergic reactions triggered by peanut, tree nuts [2] and even by seeds such as the sunflower seed although is not strictly a nut [3]. Among symptomatic reactions to nuts, anaphylaxis is very common representing around 70 %-80 % of fatal food-induced reactions [4]. The consumption of nuts is very extended in the world by their high nutritional content. Thus, the worldwide prevalence described to tree nut allergy is between 0.05 % and 4.9 % [5] and peanut allergy ranging from

0.5 % to 3 % [2]. Therefore, nut allergy is a relevant health issue and its diagnosis based in clinical symptoms as well as *in vivo* and *in vitro* sensitization tests must be performed properly [6].

Molecular allergy based on component-resolved diagnostics (CRD) has emerged in recent years as the third-level diagnostic in clinical algorithms [7]. Currently, the quantification of specific IgE (sIgE) levels to whole extracts and recombinant allergens by singleplex techniques represents the gold standard of *in vitro* diagnosis for the second- and third-level diagnostics, respectively [7]. The development of multiplex platforms based on multiple recombinant allergens in a single array has meant a qualitative improvement in allergy diagnosis [8]. Immuno

Abbreviations: ALEX, Allergy Explorer; CCD, cross-reactive carbohydrate determinant; CRD, component-resolved diagnostics; ISAC, Immuno Solid-Phase Allergen Chip; nsLTP, non-specific lipid-transfer protein; OAS, oral allergy syndrome; OFC, oral food challenge; PR10, pathogenesis related-10 protein; sIgE, specific IgE; sIgG, specific IgG; SPT, skin prick test.

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Solid-Phase Allergen Chip (ISAC) assay (Thermo Fisher) was the first to be developed in the year 2000 [9], in addition this platform is the most widely used to date [10]. Allergy Explorer (ALEX) assay (MacroArray Diagnostics) is a new multiplex system containing 157 whole extracts and 125 recombinant allergens attached to a solid-phase [11] in contrast to ISAC platform which is compound of only 112 allergen components [10]. Both multiplex platforms due to the high number of molecular components that present, establish the sensitization profiles to primary allergens as well as cross-reactivity among allergens belonging to common allergen families such as the non-specific lipid-transfer proteins (nsLTPs) and pathogenesis related-10 protein (PR10) [12].

In nut allergy both the primary sensitization and cross-reactivity are dependent on differences between age groups (pediatric and adult populations), geographic regions, and environmental factors among others [2]. Mediterranean area represents a particular issue in nut allergy, being the nsLTPs shared in several nuts the main allergen source [13,14]. For this, the ALEX assay represents a suitable tool in nut allergy based on the high number of recombinant allergens and whole extracts that presents (walnut, hazelnut, peanut, almond, pistachio and sunflower seed among others allergen sources). In patients with nut allergy from the Mediterranean area, quantification of sIgE levels using the ALEX platform has been barely studied focusing solely on allergy to walnut, hazelnut and peanut but without taking into account other nuts such as almond, pistachio and sunflower seed [15,16]. Accordingly, the propose of this study has been to investigate in a Mediterranean population with nut allergy the sensitivity and agreement of the ALEX multiplex technique respect to ImmunoCAP-singleplex assay as the gold standard method. In order to establish in this group of patients the clinical performance of the ALEX platform.

2. Material and methods

2.1. Patients

In this study were enrolled a total of 58 patients suffering from allergic reactions to nuts. The diagnosis was made at the Allergy Outpatient Clinics of the Ciudad Real University General Hospital (Spain) in accordance with the clinical history and symptoms upon the ingestion and/or contact with the offending food as well as skin tests made in the group of patients [17].

The cohort of patients was formed by 27 females and 31 males, with a mean age at diagnosis of 25.36 ± 15.45 years (mean $\pm$ SD), 22 patients were under 18 years old. The clinical symptoms recorded were the following: a) 37 patients had anaphylactic reactions, b) 14 patients exhibited only oral allergy syndrome (OAS), and lastly c) 7 patients presented urticaria. In supplementary Table 1 the demographic and clinical data from the 58 patients upon suffering the allergic reactions to nuts are summarized. Skin prick tests (SPTs) using whole extracts from walnut, hazelnut, peanut, pistachio, almond and sunflower seed as well as the recombinant Pru p 3 were used for the study of *in vivo* sensitization. All extracts employed as well as Pru p 3 were provided by ALK-Abelló (S.A., Madrid, Spain). A SPT with a wheal of average diameter ≥ 3 mm after subtracting the negative control (saline) was considered as positive in accordance with the international guidelines [17]. Equally, a histamine solution as positive control was used (histamine dihydrochloride 10 mg/mL). The means obtained from the average diameters using whole extracts and Pru p 3 were the following: a) walnut: 4.5 mm (range 3–11 mm); b) hazelnut: 4.4 mm (range 3–6.5 mm); c) peanut: 4.9 mm (range 3–10 mm); d) almond: 4.7 mm (range 3–10.5 mm); e) pistachio: 4.4 mm (range 3–5.5 mm); f) sunflower seed: 5.4 mm (range 3–6.5 mm); and g) Pru p 3: 6.8 mm (range 3–10.5 mm).

Medical records from patients were obtained in accordance with the ethical standards of the institutional and/or national research committee, the 1964 Declaration of Helsinki, and its later amendments or comparable ethical standards. In addition, because for diagnosis all laboratory interventions were part of routine clinical practice, formal

approval by the Ethics Committee was no needed and was not requested.

2.2. Analysis of sIgE levels by multiplex and singleplex assays

2.2.1. ALEX-multiplex technique

Arrays from the ALEX platform (version 2) (MacroArray Diagnostics GmbH, Lemböckgasse, Wien) were incubated with serum samples obtained from the 58 patients with nut allergy. A serum sample of 100 μ L was mixed with 400 μ L of sample diluent during 2 h in constant shaking at room temperature according to kit's instructions (MacroArray). The next steps of the technique consisted in successive washing cycles and then an incubation step for 30 min with an anti-IgE antibody conjugated with alkaline phosphatase. Next, upon 5 cycles of washing, it was added the substrate solution (MacroArray) with an incubation step of 8 min and finally the stop solution (MacroArray). The colored spots from the arrays were read in the Image Explorer (MacroArray) and the signals were transformed in quantitative data using the Raptor software (MacroArray). The sIgE levels both for molecular components and whole extracts were estimated as positive with values above 0.3 kUA/L. In the case of allergy to walnut, hazelnut, peanut and pistachio which are composed of several recombinant allergens (Table 1) were considered as positive the biological sources when at least one of their components was higher than 0.3 kUA/L (Table 1 and Table 2).

2.2.2. ImmunoCAP-singleplex technique

Given the positivity of SPTs in the group of patients with nut allergy (N = 58), it was determined in parallel the serum levels of sIgE (Table 2). Quantification of sIgE both to whole extracts and Pru p 3 was made in an ImmunoCAP Phadia 250 analyzer (Thermo Fisher, Uppsala, Sweden) by fluorescent enzyme immunoassay according to the manufacturer's instructions. The following sIgE levels to whole extracts from nuts were determined: a) F256 to walnut, b) F17 to hazelnut, c) F13 to peanut, d) F20 to almond, e) F203 to pistachio, and f) k84 to sunflower seed (Thermo Fisher). In addition, F420-sIgE (Thermo Fisher) to Pru p 3 was determined (Thermo Fisher). Quantitative levels of sIgE with values ≥ 0.35 kU/L were considered as positive.

Table 1

Frequency of allergen components detected by ALEX technique in patients with allergy to nuts.

Allergen source	ALEX-Component	Function	Frequency N (%)
Walnut (N = 26)	Jug r 1	2S albumin	N = 9 (34.61 %)
	Jug r 2	7/8S globulin	N = 6 (23.07 %)
	Jug r 3	nsLTPs	N = 24 (92.30 %)
	Jug r 4	11S globulin	N = 3 (11.53 %)
	Jug r 6	7/8S globulin	N = 5 (19.23 %)
Hazelnut (N = 29)	Cor a 1.0401	PR-10	N = 4 (13.79 %)
	Cor a 8	nsLTPs	N = 27 (93.10 %)
	Cor a 9	11S globulin	N = 2 (6.89 %)
	Cor a 11	7/8S globulin	N = 3 (10.34 %)
	Cor a 14	2S albumin	N = 2 (6.89 %)
Peanut (N = 27)	Ara h 1	7/8S globulin	N = 4 (14.81 %)
	Ara h 2	2S albumin	0
	Ara h 3	11S globulin	N = 3 (11.11 %)
	Ara h 6	2S albumin	N = 2 (7.4 %)
	Ara h 8	PR-10	0
	Ara h 9	nsLTPs	N = 25 (92.59 %)
	Ara h 15	Oleoin	0
	Pis v 1	2S albumin	N = 3 (75 %)
Pistachio (N = 4)	Pis v 2	11S globulin subunit	0
	Pis v 3	7/8S globulin	N = 2 (50 %)

Abbreviations: N, number of patients; nsLTPs, non-specific lipid-transfer proteins; PR10, pathogenesis related-10 protein.

Table 2

Comparison and agreement between ALEX and ImmunoCAP techniques in patients suffering from nut allergy.

Positive allergens in SPTs (N) (%)	ALEX ^a	ImmunoCAP ^b	ALEX-S vs ImmunoCAP-S	PPA	NPA	k-index (95 % CI)
	N, S (%)	N, S (%)				
Pru p 3 (N = 47) (81.03 %)	N = 44 93.61 % Component	N = 45 95.74 % F420 ^d	$p = 0.647$	98.11 %	66.67 %	0.791 (0.394–1)
Walnut (N = 38) (65.52 %)	N = 26 68.42 % Components ^c	N = 33 86.84 % F256 ^c	$p_c = 0.053$	75.75 %	33.33 %	0.386 (0.065–0.707)
Hazelnut (N = 38) (65.52 %)	N = 29 76.31 % Components ^c	N = 34 89.47 % F17 ^f	$p_c = 0.127$	85.29 %	44.44 %	0.550 (0.221–0.878)
Peanut (N = 34) (58.62 %)	N = 27 79.41 % Components ^c	N = 31 91.17 % F13 ^g	$p_c = 0.170$	87.09 %	42.85 %	0.544 (0.170–0.917)
Almond (N = 44) (75.86 %)	N = 4 9.09 % Whole extract	N = 39 88.63 % F20 ^h	$*** p_c < 0.00001$	10 %	10 %	0.020 (-0.007–0.047)
Pistachio (N = 27) (46.55 %)	N = 4 14.81 % Components ^c	N = 19 70.37 % F203 ⁱ	$*** p_c = 0.000037$	21.05 %	34.78 %	0.136 (-0.010–0.283)
Sunflower seed (N = 27) (46.55 %)	N = 5 8.51 % Whole extract	N = 24 88.88 % k84 ^j	$*** p_c < 0.00001$	20.83 %	13.65 %	0.055 (-0.021–0.131)

Abbreviations: CI, confidence interval; N, number of patients; NPA, negative percent agreement; PPA, positive percent agreement; S, sensitivity; SPT, skin prick test. p_c , p-value corrected with Yates' correction.^a Patients with positive sIgE levels (>0.3 kUA/L).^b Patients with positive sIgE levels (>0.35 kU/L).^c At least one of the components is positive.^d sIgE to Pru p 3.^e sIgE to whole extract from walnut.^f sIgE to whole extract from hazelnut.^g sIgE to whole extract from peanut.^h sIgE to whole extract from almond.ⁱ sIgE to whole extract from pistachio.^j sIgE to whole extract from sunflower seed. $*** p < 0.0001$.

2.3. Statistical analysis

The frequency of qualitative variables was expressed as percentage and the comparison of sensitivity between techniques (ALEX vs ImmunoCAP) was made with the Chi-Square test and Yates' correction. For the agreement determination, Cohen's kappa index (k) was used according to the following k-values: a) < 0, no agreement; b) 0–0.2 poor agreement; c) 0.21–0.4, fair agreement; d) 0.41–0.6, moderate agreement; e) 0.61–0.8, substantial agreement; and f) 0.81–1.00 almost perfect agreement [18].

Differences between means of quantitative variables were determined by the *T*-test and Mann-Whitney *U* test for parametric and non-parametric variables respectively. The association study was performed with the Pearson correlation expressing the association degree with the correlation-coefficient (*r*).

All the statistical determinations were analyzed using the Statistical Package for the Social Sciences version 18.0 (SPSS Inc., Chicago, Ill., USA). The differences were considered statistically significant at *p* values of <0.05.

3. Results

3.1. Analysis of biological sources and allergen components in patients with nut allergy

The group of 58 patients with symptomatic reactions to nuts, mainly presented anaphylactic reactions (63.79 %), followed by OAS (24.14 %) and urticaria (12.07 %). All patients included in this cohort had at least a positive SPT to one of the 6 allergen sources involved in nut allergy (walnut, hazelnut, peanut, almond, pistachio and sunflower seed as biological sources) (Fig. 1). In addition, SPTs to Pru p 3 were included as it is the main source of sensitization described in patients from the Mediterranean area with allergy to nuts (Fig. 1) [14].

Fifty-eight arrays from the ALEX-multiplex platform were performed to investigate their usefulness as *in vitro* test in the diagnosis of nut allergy. For this, arrays from the ALEX platform are composed of several allergen components for walnut, hazelnut, peanut and pistachio as biological sources (Table 1). In addition, they contain only whole extracts to almond and sunflower seed. Table 1 summarizes the number of positive patients according to molecular components from allergen sources of walnut (N = 26), hazelnut (n = 29), peanut (N = 27) and pistachio (N = 4). Only a few number of patients presented positivity to whole extracts from almond (N = 4) and sunflower seed (N = 5) (Table 2). When the frequency of positive molecular components was analyzed, it was found that >92 % of them had positive sIgE levels to Jug

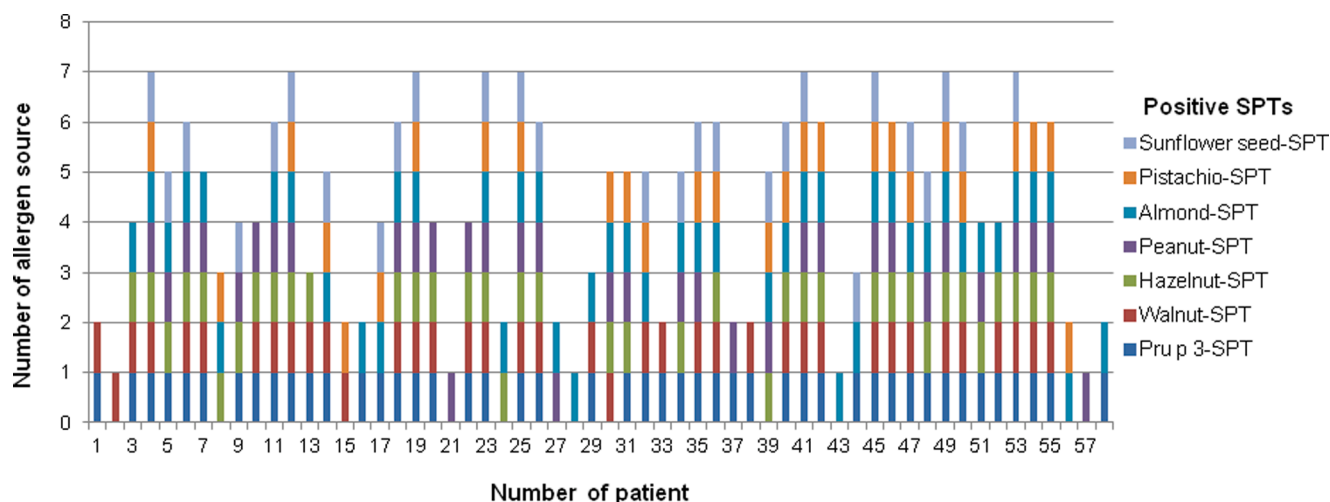


Fig. 1. Co-sensitization profiles to nut allergens and Pru p 3 based on positive SPTs in the group of patients with nut allergy (N = 58). SPTs, skin prick tests.

r 3, Cor a 8 and Ara h 9 corresponding to the common family of nsLTPs from walnut, hazelnut and peanut respectively (Table 1). Moreover, almost all these patients with positivity to walnut, hazelnut and peanut by the ALEX platform had in parallel positive results to Pru p 3 included in the ALEX array (93.61 %) (Table 2).

Among patients suffering from nut allergy with sensitization to a different allergen source apart from the nsLTPs, it was found co-sensitization of the nsLTPs with storage proteins to walnut (38.46 %), hazelnut (13.79 %) and peanut (14.81 %) (Table 3). In addition, nsLTPs together with PR10 were present in allergy to hazelnut (6.89 %) (Table 3). Among patients with negativity to nsLTPs, two patients had sensitization to storage proteins from walnut and peanut (Table 3), and two more patients presented positivity to PR10 (Table 3).

3.2. Comparative study between ALEX-multiplex and ImmunoCAP-singleplex techniques in nut allergy

In patients with positive SPTs to whole extracts and Pru p 3 were determined in parallel sIgE levels by ImmunoCAP-singleplex assay as the *in vitro* reference method. The results obtained were compared with values from the ALEX-multiplex platform to calculate the sensitivity of both methods regarding *in vivo* skin tests (SPTs). The results of sensitivity obtained from the comparative analysis are recorded in Table 2. No significant differences were found between both techniques in sIgE

levels to walnut, hazelnut, peanut and Pru p 3 (Table 2). Conversely, the sensitivity detected by ALEX platform to almond, pistachio and sunflower seed was significantly lower than ImmunoCAP assay (9.09 % vs 88.63 %; 14.81 % vs 70.37 %; and 8.51 % vs 88.88 %; respectively) (Table 2).

The concordance between both techniques showed only a substantial agreement for Pru p 3 ($k = 0.791$) with a positive percent agreement (PPA) of 98.11 %. Moreover, agreement was moderate for hazelnut and peanut ($k = 0.550$ and $k = 0.544$, respectively) with a PPA for both around 85 %; and walnut had a fair agreement ($k = 0.386$) (Table 2). However, for almond, pistachio and sunflower seed the agreement was from none to poor ($k < 0.2$) and with only a PPA ranging from 10 % to 21 % (Table 2).

3.3. Quantitative analysis between ALEX-multiplex and ImmunoCAP-singleplex platforms in patients with positivity to allergen sources

For the quantitative study of differences between means and correlation between variables using ImmunoCAP and ALEX techniques were taken into account only those patients with positive results simultaneously, that is, positive with both methods.

When mean values of the allergen sources were compared between both techniques, sIgE levels to Pru p 3, hazelnut, almond and pistachio did not show significant differences (Fig. 2). However, values of sIgE to walnut, peanut and sunflower seed presented means by ImmunoCAP higher than ALEX platform (walnut: 14.33 ± 18.78 vs 4.32 ± 4.84 ; peanut: 14.98 ± 20.08 vs 6.86 ± 8.98 ; sunflower seed: 15.74 ± 15.28 vs 1.83 ± 0.62 ; mean $\pm$ SD) (Fig. 2).

Correlation analysis of sIgE levels between ImmunoCAP and ALEX platforms showed a positive association for Pru p 3, walnut, hazelnut, and peanut with high correlation coefficients (Fig. 3). Conversely, the allergen sources from almond, pistachio and sunflower seed did not present any correlation (almond: $r = 0.121$, $p = 0.879$; pistachio: $r = -0.877$, $p = 0.123$; and sunflower seed: $r = 0.876$, $p = 0.051$).

4. Discussion

Geographic differences and environmental local factors represent in nut allergy an important issue to take into consideration in the clinical and laboratory management of patients [6]. In European countries the nut most prevalent in food allergy is hazelnut, followed by peanut, walnut, almond, pistachio and lastly cashew as the least frequent [5–6,19]. In similar way, hazelnut, peanut and walnut represent the first, second and third allergen source respectively in adult population with nut allergy from Spain [19]. Almond represents the fourth

Table 3

Frequency of patients with allergy to walnut, hazelnut and peanut with other positive allergen components apart from nsLTPs.

Biological source	nsLTPs + storage proteins (N) (%)	Storage proteins (N) (%)	nsLTPs + PR10(N) (%)	PR10 (N) (%)
Walnut (N = 26)	N = 10 (38.46 %) (Jug r 1, Jug r 2, Jug r 4, and Jug r 6)*	N = 2 (7.69 %) (Jug r 1)*		
Hazelnut (N = 29)	N = 4 (13.79 %) (Cor a 9, Cor a 11, and Cor a 14)*		N = 2 (6.89 %)	N = 2 (6.89 %)
Peanut (N = 27)	N = 4 (14.81 %) (Ara h 1, Ara h 3, and Ara h 6)*	N = 2 (7.4 %) (Ara h 1 and Ara h 6)		

Abbreviations: N, number of patients; nsLTPs, non-specific lipid-transfer proteins; PR10, pathogenesis related-10 protein.

+, Plus.

* Molecular components.

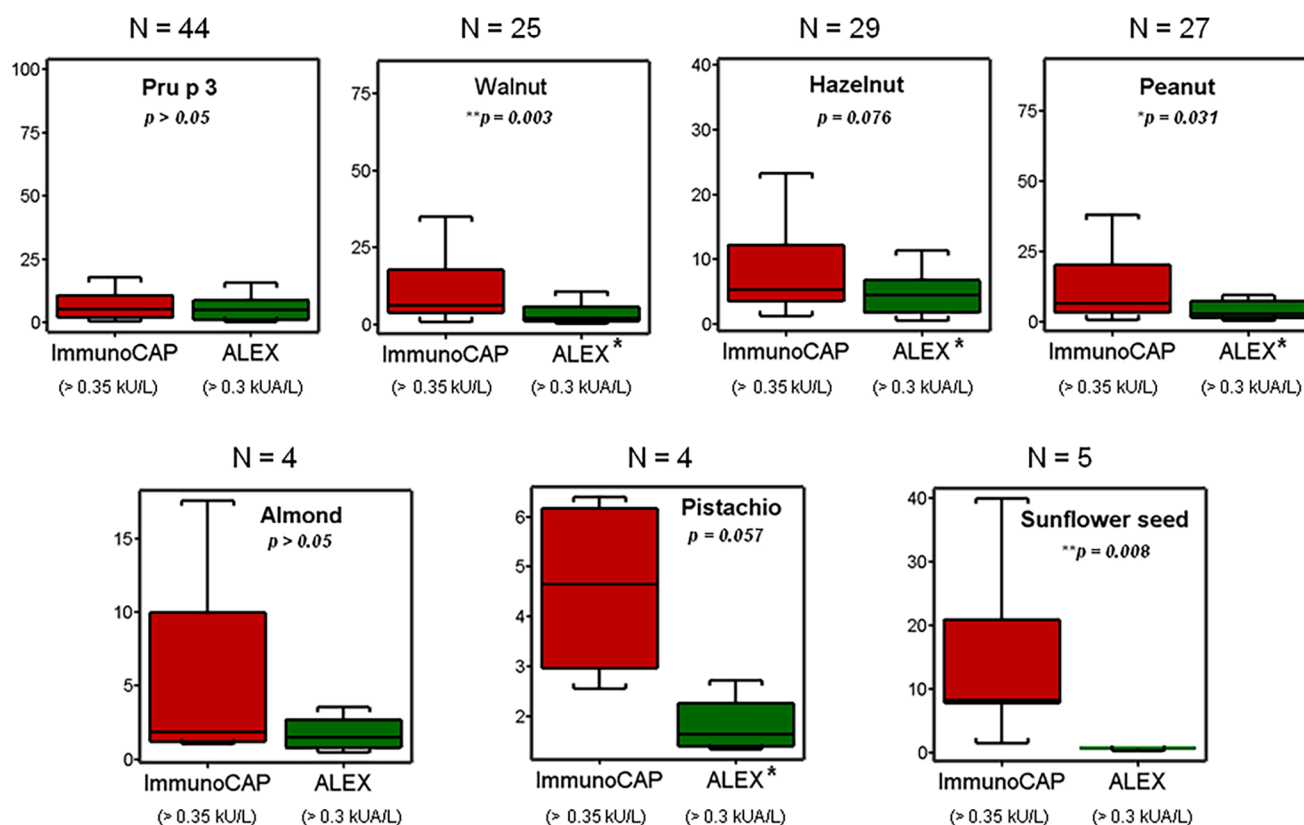


Fig. 2. Box and whisker plots showing the differences between means (p-value) of sIgE levels from allergen sources measured by ImmunoCAP and ALEX techniques. N, number of patients with positivity to whole extracts by both techniques or at least to one of the molecular components; *sum of molecular components; (* $p < 0.05$; ** $p < 0.001$).

biological source in the prevalence ranking of nut allergy in European regions [20]. In a recent multicenter study on Spanish pediatric population with nut allergy, almond was found as the sixth allergen involved in positive SPTs [21]. In allergy to sunflower seed even though its high consumption as sunflower oil in Europe the studies on prevalence are scarce [3], similarly to what happens with the pistachio nut allergy [22].

Currently, between the two multiplex platforms available for clinical routine in allergy diagnosis (ISAC and ALEX), ALEX assay applied to nut allergy represent a new tool to analyze the co-sensitization profile to different nuts in the Mediterranean area. Indeed, when are compared both multiplex platforms, ALEX platform contains a higher number of allergen sources than ISAC [10]. Specifically, it presents recombinant allergens to pistachio (Pis v 1, Pis v 2 and Pis v 3) and whole extracts to almond and sunflower seed which are not present in ISAC platform. Since the ALEX arrays commercialization, only a limited number of studies have been published, mainly comparing them with ISAC arrays [11,23–24]. ALEX platform is presented as a quantitative technique in contrast to ISAC platform which is semi-quantitative [10]. Therefore to corroborate the clinical validation of the ALEX platform it is mandatory to perform a qualitative and quantitative study respect to a singleplex technique as the quantitative gold standard.

In the current study it has been analyzed the diagnostic performance of the ALEX platform comparing it with ImmunoCAP technique in a cohort of patients with nut allergy ($N = 58$) from a Mediterranean region (Fig. 1). Only two previous studies comparing ALEX platform with ImmunoCAP technique have focused on nut allergy triggered by walnut, hazelnut and peanut but without studying other nuts [15,16]. Striking results have been found in this report regarding the low sensitivity obtained by ALEX platform to whole extracts from almond and sunflower seed as well as the sum of components from pistachio. Thus, it has been detected a sensitivity <10 % both for almond and sunflower seed as well as <15 % for pistachio, with a poor agreement for all of them respect to

ImmunoCAP technique ($k\text{-index} < 0.2$). Conversely, the ALEX platform was able to detect correctly the sIgE levels to whole extracts from walnut, hazelnut and peanut with a moderate agreement for hazelnut and peanut respect to ImmunoCAP technique, although with a fair agreement for walnut. These results are in consonance with the sensitivity detected by Quan et al. [16] in Spanish population with allergy to hazelnut and peanut when they compared both platforms using whole extracts. However, they found that for whole extract from walnut the ImmunoCAP assay had higher sensitivity than ALEX (92.9 % vs 60.7 %, respectively; $p = 0.01$), though they studied a smaller number of patients ($N = 28$) [16]. In the first study performed on food allergy using ALEX platform, Buzzulini et al. [15] investigated a reduced number of patients with co-sensitization to walnut and hazelnut ($N = 19$) finding a non agreement and poor agreement respectively to whole extracts.

In the Mediterranean area the major allergens involved in cross-reactivity among nuts are the nsLTPs from walnut (Jug r 3) [25], hazelnut (Cor a 8) [26], peanut (Ara h 9) [27], almond (Pru du 3) [28], and sunflower seed (Hel a 3) [3]. In this study the ALEX platform was able to detect nsLTPs from walnut, hazelnut and peanut with a frequency of >90 %. In agreement with the study published by Quan et al. [16], in this report the sensitivities obtained for Pru p 3 by ALEX and ImmunoCAP platforms were similar ($p > 0.05$). Equally, in this study, the agreement detected between both quantitative techniques for Pru p 3 was substantial ($k = 0.791$), nevertheless Buzzulini et al. [15] found a close to perfect agreement.

At quantitative level when the ALEX platform was compared with ImmunoCAP assay for whole extracts only were found differences between mean values of sIgE for walnut, peanut and sunflower seed. However the allergen sources from walnut and peanut presented a positive correlation of sIgE levels with high correlation coefficients (Fig. 3). This is in consonance with data published by Hoang et al. [29] employing regression models to compare the ALEX platform with

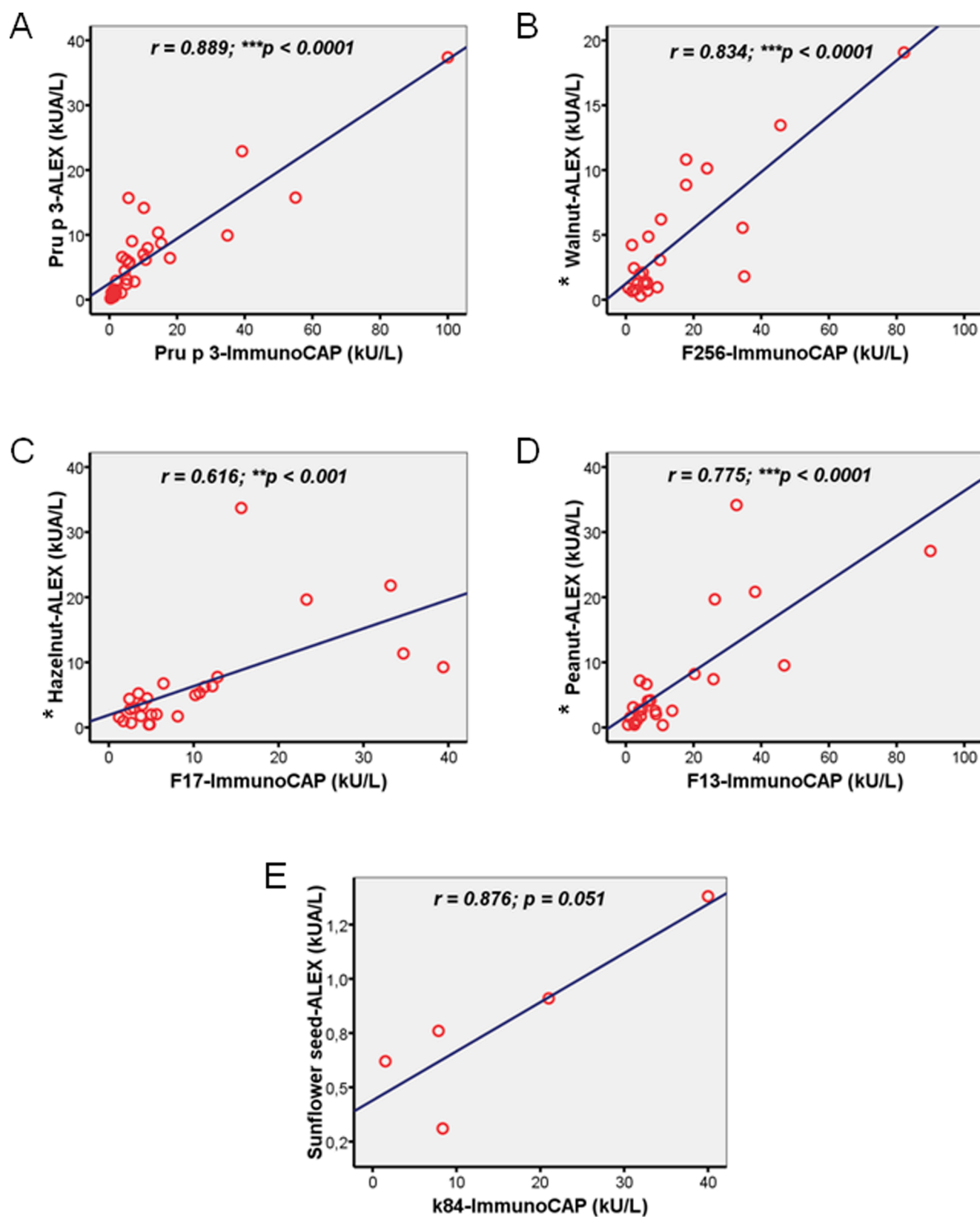


Fig. 3. Relationships of sIgE levels to Pru p 3 (A) and whole extracts (B, C, D and E) between ImmunoCAP and ALEX platforms. *sum of molecular components; (* $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$). Correlation-coefficient (r).

ImmunoCAP technique for whole extracts [29]. Moreover, in this study it has been found significant correlations between both techniques for the whole extract from hazelnut as well as for Pru p 3 except to almond, pistachio and sunflower seed (Fig. 3). Similarly, Quan et al. [16] studying Spanish population with nut allergy found a significant correlation for Pru p 3 between ALEX and ImmunoCAP platforms [16].

5. Conclusions

Taken together, these findings, the technical validation of the ALEX platform in patients with nut allergy from a Mediterranean area can be established except to those suffering from allergy to almond, pistachio and sunflower seed.

One of the possible causes explaining the low sensitivity detected for the whole extracts from almond and sunflower seed as well as the sum of recombinant allergens from pistachio could be attributed to inhibition effect on cross-reactive carbohydrate determinants (CCDs). In this sense, Heffler et al. [11] demonstrated this effect assayed two serum samples by ALEX arrays with or without CCD inhibitor, upon the inhibition both the number and intensity of spots on arrays were reduced. Therefore, the presence in at least whole extracts of glycoproteins with CCDs could explain it.

A second reason that could be argued is the interference produced by specific IgG (sIgG) including the IgG4 subclass against food allergens which prevents the binding of sIgE on solid-phase of arrays [8]. Indeed, although the sIgG4 antibodies to food antigens are not clinically relevant, are produced during the normal immunization process against foods in the intestinal lamina propria [30]. However, the ImmunoCAP-singleplex system does not present interference mediated by IgG antibodies [31] in contrast to multiplex assays such as the ALEX platform [10].

Furthermore, intrinsic defects of whole extracts in the case of almond and sunflower seed as well as in the case of pistachio the absence of reactivity against a recombinant allergen does not present in the ALEX array could be involved in the low sensitivities found. Therefore, in nut allergy due to the influence of geographic location and environmental factors the ALEX-multiplex platform should be clinically validated from different populations.

The sIgE levels and positive SPTs do not always indicate clinical food allergy, which can only be confirmed by the occurrence of specific allergic symptoms after food exposure [2]. Therefore, in those patients who did not experience anaphylactic reactions in this study (21 out of 58) it should have carried out an oral food challenge (OFC) test to confirm the diagnosis of nut allergy. This fact represents a weakness of this study.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cca.2022.08.022>.

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