Is the performance of ImmunoCAP ISAC 112 sufficient to diagnose peach and apple allergies?

Food allergies constitute a public health issue, with a reported overall estimated prevalence of 6% in Europe[1] and *Rosacea* as the main allergenic fruits among adults.[2] The commercial microarray ImmunoCAP ISAC 112 (Thermofisher, Uppsala, Sweden) is a semiquantitative and reproducible in vitro diagnostic tool used for the determination of specific IgE (sIgE).[3] However, its panel of allergens does not have the best accuracy when it comes to determining fruit allergies in the Mediterranean area: the inclusion of the thaumatin-like protein (TLP) Pru p 2 or the apple lipid transfer protein (LTP) Mal d 3 has been proposed to improve the diagnosis of peach[4] and apple[5] allergies, respectively, in the Mediterranean basin. We sought to determine the usefulness of a component-resolved microarray for the diagnosis of peach and apple allergies in the Mediterranean area.

Ninety-five patients with a consistent history of an IgE-dependent peach (n=86) and/or apple (n = 34) allergy, positive skin prick test results (ALK-Abelló, Madrid, Spain), and positive sIgE (ImmunoCAP; ThermoFisher) to the symptomatic fruit were prospectively enrolled. Eighty control patients (37 atopic, dust mite allergic individuals without sensitization to plant allergens [grass, birch, plane tree, cypress, *Salsola*, or *Parietaria* pollens] and 43 nonatopic controls) from 14 Spanish hospitals were also enrolled. A detailed clinical history was compiled for all participants. The research ethics committee of the participating hospitals approved the study. The study and control patient data are summarized in Table 1.

Determination of sIgE against recombinant (r) peach (rPru p 1, rPru p 3) and apple components (rMal d 1), among other allergens, was performed by ImmunoCAP ISAC 112 in all participants (the sIgE test result was considered positive when the ISAC standardized units value was $\geq$ 0.3), following the manufacturer's recommendations. sIgE to common panallergens from other plant sources (LTPs, TLPs, PR-10 proteins, and profilin) was assessed in ISAC-negative patients to its fruit components.

In apple or peach allergic patients who did not have sIgE to its components in ISAC, determination of sIgE to rPru p 1, rPru p 3, rPru p 4, and rMal d 3 and of sIgE to nPru p 2.2 was performed by fluorescence enzyme immunoassay ImmunoCAP (the sIgE test result was considered positive when $\geq$0.35 kU_A/L) and direct enzyme-linked immunosorbent assay, respectively, as previously described.[6]

Among the 86 peach allergic patients, 4 (5%) had sIgE to Pru p 1 and 76 (88%) to Pru p 3 by ISAC. Sensitization to both components was observed in 3 patients (3%). sIgE was not detected in 9 patients (10%) using this method. One control subject had positive sIgE test results to Pru p 3 but none to Pru p 1.

One of the 9 ISAC-negative peach allergic patients (11%) had sIgE to Pru p 1 and 5 (56%) to Pru p 3 by ImmunoCAP (3 of them presented with systemic symptoms). Enzyme-linked immunosorbent assay and ImmunoCAP methods were used to determine the sIgE against the unavailable components in ISAC, enabling the diagnosis of 4 patients (44%) sensitized to Pru p 2 and 3 (33%) to Pru p 4, respectively.

Moreover, 3 patients (33%) were sensitized to LTPs other than Pru p 3 and 2 (22%) to profilin. One patient was sensitized to TLP and PR-10 proteins, respectively. Sensitization to panallergens was not detected in 2 of the ISAC-negative peach allergic patients.

In the apple allergic group, 3 of 34 patients (9%) were sensitized to Mal d 1, whereas 31 (91%) did not have sIgE in ISAC. Among these ISAC-negative apple allergic patients, 28 (90%) had sIgE to Mal d 3 by ImmunoCAP (19 of them presented with systemic symptoms). However, none of these techniques was able to detect the presence of sIgE to the apple components in 3 patients (9%). Twenty-nine patients (94%) had sensitization to LTPs, 4 (13%) to profilin, and 3 (10%) to TLP. Two patients (6%) were sensitized to both LTP and profilin. All of the ISAC-negative patients who had sIgE to LTP in ISAC also had sIgE to Mal d 3 by ImmunoCAP.

According to our results, most of the peach allergic patients had sIgE to its components in ISAC; however, the presence of sIgE was not detected in 10% of them. In our sample, Pru p 2 was a minor allergen (5%) compared with other data published in Spain.[4] Thus, we believe its inclusion would not significantly improve the diagnosis of peach allergy in our region.

Despite the fact that all patients sensitized to peach components had their conditions diagnosed by ImmunoCAP or ISAC, the performance of ImmunoCAP enabled the detection of sIgE to Pru p 4 in the 2 patients sensitized to profilin, despite having a negative response to Pru p 4 in ISAC, hence suggesting the improvable sensitivity of this component.

In the apple allergic group, less than 9% had sIgE to the unique apple component included in ISAC (Mal d 1), whereas sensitization to Mal d 3 was detected in 90% by CAP. The determination of sensitization to panallergens provided helpful information in apple allergic patients because sIgE to LTPs was detected in more than 90% (all the patients were sensitized to Pru p 3). In our opinion, the said result improved the diagnosis in this group of patients because Mal d 3, the major allergen of our population, is not included in ISAC. However, the presence of sIgE against Pru p 3 in LTP-sensitized patients can be due to cross-reactivity and should therefore not be used to predict clinical symptoms.[7]

Table 1
Clinical and demographic characteristics of the study and control patients

Characteristic	Peach allergic patients	Apple allergic patients	Nonatopic controls	Atopic controls
No. of patients	86	34	43	37
Age, mean (SD), y	29.7 (9.1)	29.6 (8.7)	47.3 (15.4)	39 (14.2)
Male sex, %	28.2	27.7	25.6	37.8
Symptoms with the triggering fruit, No. (%)				
OAS	34 (40)	13 (38)		
SS	31 (36)	15 (44)		
Anaphylaxis	21 (24)	6 (18)		
sIgE to fruit, median (IQR), kU_A/L	5.51 (1.83–10.1)	2.65 (1.28–4.95)		
Patients wth ISAC sIgE Pru p 1 >0.3 ISU, No. (%)	4 (5)	3 (9)	0	0
ISAC sIgE Pru p 1, median (IQR), ISU	6.41 (0.92–13.53)	11.85 (1.04–24.37)		
Patients with ISAC sIgE Pru p 3 >0.3 ISU, No. (%)	76 (88)	30 (88)	0	1 (3)
ISAC sIgE Pru p 3, median (IQR), ISU	2.55 (1.21–5.46)	2.71 (1.27–5.32)		0.36
Patients with ISAC sIgE Mal d 1 >0.3 ISU	4 (5)	3 (8)	0	0
ISAC sIgE Mal d 1, median (IQR), ISU	10.26 (3.63–18.51)	21.49 (4.76–43.77)		

Abbreviations: IQR, interquartile range; ISAC, ImmunoCAP ISAC 112; ISU, ISAC standardized units; OAS, oral allergy syndrome; SPT, skin prick test; SS, systemic symptoms.

Our results suggest that although the sensitivity of the peach components in ISAC is improvable, it can be sufficient in our region. However, regarding the diagnosis of apple allergy, we suggest that Mal d 3 be included in the allergen panel to identify the recognition pattern of the allergic patients and its correlation with the clinical manifestations[5] in the Mediterranean population. In addition, we believe that considering the sIgE to Pru p 3 would improve the performance of ISAC in the diagnosis of apple allergy in the Mediterranean area.

Finally, the limitations of our study include the lack of oral food challenge test due to financial constraints. Further studies are needed to evaluate the usefulness of ISAC in the diagnosis of other fruits allergies.

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References

[1] Nwaru BI, Hickstein L, Panesar SS, et al. The epidemiology of food allergy in Europe: a systematic review and meta-analysis. *Allergy*. 2014;69(1):62–75.
[2] Fernandez Rivas M. Food allergy in Alergologica-2005. *J Investig Allergol Clin Immunol*. 2009;19(suppl 2):37–44.
[3] Martinez-Aranguren R, Lizaso MT, Goikoetxea MJ, et al. Is the determination of specific IgE against components using ISAC 112 a reproducible technique? *PloS One*. 2014;9(2):e88394.
[4] Palacin A, Tordesillas L, Gamboa P, et al. Characterization of peach thaumatin-like proteins and their identification as major peach allergens. *Clin Exp Allergy*. 2010;40(9):1422–1430.
[5] Gomez F, Aranda A, Campo P, et al. High prevalence of lipid transfer protein sensitization in apple allergic patients with systemic symptoms. *PloS One*. 2014;9(9):e107304.
[6] Diaz-Perales A, Lombardero M, Sanchez-Monge R, et al. Lipid-transfer proteins as potential plant panallergens: cross-reactivity among proteins of Artemisia pollen, Castanea nut and Rosaceae fruits, with different IgE-binding capacities. *Clin Exp Allergy*. 2000;30(10):1403–1410.
[7] Javaloyes G, Goikoetxea MJ, Garcia Nunez I, et al. Pru p 3 acts as a strong sensitizer for peanut allergy in Spain. *J Allergy Clin Immunol*. 2012;130(6):1432–1434.e3.