ORIGINAL ARTICLE

Is the ISAC 112 Microarray Useful in the Diagnosis of Pollinosis in Spain?

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Abstract

Background: Multiple sensitization is frequent among pollen-allergic patients. The goal of this study was to determine the diagnostic accuracy of the ImmunoCAP ISAC 112 (ISAC112) microarray in allergy to pollen from several taxa and its clinical utility in a Spanish population.
Methods: Specific IgE was determined in 390 pollen-allergic patients using the ISAC112 microarray. Diagnostic accuracy (sensitivity, specificity, predictive values, and area under the ROC curve) was calculated for the diagnosis of allergy to pollen from grass (n=49), cypress (n=75), olive tree (n=33), plane tree (n=63), and pellitory of the wall (n=17) and compared with that of the singleplex ImmunoCAP immunoassay.
Results: The sensitivity of the ISAC112 microarray ranged from 68.2% for allergy to plane tree pollen to 93.9% for allergy to grass pollen. The specificity was >90%. The AUC for the diagnosis of allergy to plane tree pollen was 0.798, whereas the AUC for the remaining cases was ≥0.876. The accuracy of ISAC112 was higher than that of ImmunoCAP for plane tree pollen and similar for the remaining pollens. The frequency of sensitization to most species-specific allergenic components and profilins varied between the different geographical regions studied. A total of 73% of pollen-allergic patients were sensitized to species-specific components of more than 1 pollen type.
Conclusions: The ISAC112 microarray is an accurate tool for the diagnosis of allergy to pollen from grass, cypress, olive tree, plane tree, and pellitory of the wall. The features of the ISAC112 microarray are similar or superior (in the case of plane tree pollen) to those of ImmunoCAP. This microarray is particularly useful for the etiologic diagnosis of pollinosis in patients sensitized to multiple pollen species whose pollination periods overlap.

Key words: Microarray. Pollen allergy. Molecular diagnosis. Accuracy.

Resumen

Introducción: La sensibilización a múltiples pólenes es frecuente entre los pacientes alérgicos a polen. El objetivo de este estudio fue determinar la exactitud diagnóstica de la micromatriz ImmunoCAP ISAC 112 (ISAC112) en alergia a polen de diversos taxones y su utilidad clínica en una población española.
Métodos: Se determinó IgE específica mediante ISAC112 en 390 pacientes polínicos. Se calculó su exactitud diagnóstica (sensibilidad, especificidad, valores predictivos y área bajo la curva ROC) para el diagnóstico de alergia a polen de gramíneas (n=49), ciprés (n=75), olivo (n=33), plátano de sombra (n=63) y parietaria (n=17) y se comparó con la de ImmunoCAP monocomponente (CAP).
Resultados: La sensibilidad de ISAC112 osciló entre 68,2% para alergia a polen de plátano de sombra y 93,9% a polen de gramíneas. La especificidad se situó por encima del 90% en todos los casos. El área bajo la curva (AUC) de la curva ROC para diagnóstico de alergia a polen de plátano fue de 0,798. El resto de AUC fueron ≥ 0,876. La exactitud diagnóstica de ISAC112 fue superior a la de CAP para la alergia a polen de plátano de sombra y similar para el resto de pólenes estudiados.
La frecuencia de sensibilización a la mayoría de componentes alergénicos genuinos y a profilinas varió entre las diferentes zonas. El 73 % de los pacientes polínicos estaban sensibilizados a componentes genuinos de más de un tipo polínico.
Conclusiones: ISAC112 es una herramienta exacta para el diagnóstico de alergia al polen de gramíneas, ciprés, olivo, plátano de sombra y parietaria, con prestaciones similares o superiores, en el caso de alergia a polen de plátano de sombra, a las de CAP. Es especialmente útil para el diagnóstico etiológico de la polinosis en pacientes con sensibilizaciones a múltiples pólenes con periodos de polinización solapados.

Palabras clave: Microarray. Alergia a polen. Diagnóstico molecular. Exactitud.

Introduction

Pollen allergy is the main cause of seasonal rhinoconjunctivitis and/or asthma, affecting up to 40% of the European population [1]. The diagnosis of pollinosis is based on the presence of typical symptoms during the pollination period, along with confirmation of IgE-mediated sensitization to pollen, which has traditionally been based on allergenic extracts obtained from the biological source. Consequently, many pollen-allergic patients are sensitized to several species [2], whose pollination periods often overlap, with no difference established between co-allergy or cross-sensitization caused by panallergens. The recent advent of purified allergenic components has opened the door to molecular diagnostic techniques that make it possible to differentiate between both situations.

In vitro diagnosis based on purified allergenic components can be performed both individually and using multiplex systems, such as the ImmunoCAP ISAC112 microarray (ISAC112). The expectation generated by this platform since the first version was launched has had the paradoxical effect that its incorporation into clinical practice [3-6] and recommendation as a guide as to whether or not immunotherapy will be suitable [7-11] have frequently preceded validation of its technical and diagnostic accuracy [12,13]. Despite published assessments of previous versions of this test (ISAC CRD 103) [6,14,15], the adequate reproducibility of ISAC112, a version with significant technical differences compared with the previous models (including a new calibration system), has only recently been reported [16]. Neither its validity for the diagnosis of pollinosis (in terms of sensitivity and specificity) nor its safety (in terms of negative and positive predictive values) has been assessed until now.

The main goal of this study was to determine the diagnostic accuracy of ISAC112 in allergy to pollen from grass, olive tree, cypress, birch, plane tree, prickly saltwort, and pellitory of the wall and its clinical utility in a Spanish population. The specific goals of the study were as follows: (*1*) To calculate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the receiver operating characteristic (ROC) curve (AUC) of the ISAC112 microarray for each of these pollens; (*2*) To compare the diagnostic performance of ISAC112 with that of the singleplex ImmunoCAP test; and (*3*) To analyze the results of application of ISAC112 in a broad sample of pollen-allergic patients from different regions of Spain.

Materials and Methods

Patients

A total of 390 patients were recruited from 13 clinics distributed throughout 10 Spanish provinces. All patients had experienced the typical symptoms of pollinosis for at least 2 years, had a positive skin prick test (SPT) result to any number of pollen types, and had resided in the same region for the previous 5 years.

The study protocol was approved by the Ethics Committee of the coordinating center (045/2011), and all participants provided their written informed consent.

In order to study the sensitivity of ISAC112 for the diagnosis of allergy to pollen from each botanical family, we chose patients who had experienced the typical symptoms of rhinoconjunctivitis and/or asthma during the pollination period of the relevant taxon over the 2 years that preceded their recruitment and who had a positive SPT result for that pollen type. Patients with skin sensitization to pollen from another taxon with the same pollination period or *Alternaria* species were excluded from this part of the study (n=190). The pollination calendar of each recruitment area was determined based on the pollen counts of the Aerobiology Committee of the Spanish Society of Allergology and Clinical Immunology (www.polenes.com). The sensitivity of ISAC112 was calculated for each taxon based on the species-specific components of each pollen type available in the microarray.

Two control groups were included in the study of the specificity of ISAC112, as follows: control group 1 comprised nonatopic patients (n=51) and atopic patients not sensitized to plant allergens (other pollens or plant foods) (n=39); control group 2 comprised patients (n=52-164) who were allergic to pollen from a taxon other than that considered in each case and with a negative SPT result to this pollen. The specificity of the ISAC112 test was calculated for each taxon based on control patients not sensitized to plant allergens (control group 1) and based on patients with allergies to other pollens (control group 2).

Finally, after assessing the diagnostic accuracy of ISAC112, we analyzed the results obtained from its application in the overall sample of 390 pollen-allergic patients, including the 190 patients with skin sensitization to more than 1 pollen species with the same pollination period and/or *Alternaria* species.

Skin Tests

A series of SPTs were performed with a mix of grass pollens and pollens from birch (*Betula verrucosa*), olive tree (*Olea europea*), plane tree (*Platanus acerifolia*), cypress (*Cupressus arizonica*), pellitory of the wall (*Parietaria judaica*), prickly saltwort (*Salsola kali*), goosefoot (*Chenopodium album*), and mugwort (*Artemisia vulgaris*). SPT was also performed with date palm (*Phoenix dactylifera*) profilin and polcalcin (ALK-Abelló). Saline solution (0.9%) and histamine hydrochloride (10 mg/mL, ALK-Abelló) served as negative and positive controls, respectively. Wheals with a diameter of ≥3 mm were considered to be positive, as recommended by the guidelines of the European Academy of Allergy and Clinical Immunology [17].

Multiplex Specific IgE Assay

Specific IgE to allergens of grass (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7, Phl p 11, Phl p 12, and Cyn d 1), *Olea europea* (Ole e 1 , Ole e 7, and Ole e 9), *Cupressus arizonica* (Cup a 1), *Betula verrucosa* (Bet v 1, Bet v 2, and Bet v 4), *Platanus acerifolia* (Pla a 1, Pla a 2, and Pla a 3), *Salsola kali* (Sal k 1), and *Parietaria judaica* (Par j 2) were measured in all patients by means of ISAC112 (Thermo Fisher). Values of over 0.30 ISU were considered positive.

Singleplex Specific IgE Assay

Specific IgE to the major allergen components of each relevant pollen available for fluorescence enzyme immunoassay (ImmunoCAP, Thermo Fisher) was determined in all patients chosen to participate in the study of sensitivity to any of the taxa and in 35 control patients (12 atopic and 23 healthy persons). Therefore, Phl p 1 and Phl p 5 were determined as the major allergens of *Phleum pratense*, as were Ole e 1, Cup a 1, Pla a 1, and Par j 2 as the major allergens of *Olea europea, Cupressus arizonica, Platanus acerifolia*, and *Paretaria judaica*, respectively. Values of over 0.35 kU_A/L were considered positive.

Statistical Analysis

Given that the variables did not have a normal distribution (Shapiro-Wilk test), quantitative values were described as median (IQR). Qualitative values were described as frequencies. Proportions were compared using the χ^2 test. Concordance between variables was analyzed using the κ index and was interpreted to be poor (<0.2), weak (0.21-0.4), moderate (0.41-0.6), good (0.61-0.8), or very good (0.8-1) according to the Altman model [18].

ROC curves were used as a graphic expression of sensitivity against 1–specificity of the patients studied with both ISAC112 and singleplex ImmunoCAP. The AUC for each component and for the sum of the components of each pollen type with both techniques was calculated. According to Ebell [19], a test was considered to be perfect if the AUC was 1.0, excellent if it ranged from 0.9 to 0.99, good from 0.8 to 0.89, sufficient from 0.7 to 0.79, mediocre from 0.51 to 0.69, and void if its value was ≤0.5.

The correlation between the quantitative variables was evaluated using the Spearman correlation coefficient. The entire statistical analysis was performed using Stata/IC 12.0. Differences of $P<.05$ were considered to be statistically significant.

Results

Of the 390 pollen-allergic patients studied, 49, 75, 33, 63, and 17 patients who met the specified criteria for allergy to pollen from grass, cypress, olive tree, plane tree, and pellitory of the wall, respectively, were included in the technical analysis of the microarray. Only 7 and 8 patients met the strict criteria established for them to be considered cases of allergy to birch and prickly saltwort pollen, respectively. Since this is an insufficient number to provide valid results, the performance of ISAC112 in the diagnosis of allergies to birch and prickly saltwort pollen could not be assessed. Table 1 sets out the clinical and demographic data of the patients included in the study and of the subgroups of cases and control patients for each pollen type.

Table 2 describes the diagnostic accuracy of ISAC112 for the diagnosis of allergy to pollen from grass, cypress, olive tree, plane tree, and pellitory of the wall in terms of its sensitivity, specificity, positive and negative predictive values, and AUC. The sensitivity of ISAC112 ranged from 68.2% for allergy to plane tree pollen to 93.9% for allergy to grass pollen. The NPV ranged from 90% to 97.9% for these pollen types. Specificity fluctuated between 93.3% for olive tree pollen and 100% for pellitory of the wall pollen among control patients with no plant allergies and between 90.2% and 98.7%, respectively, in control patients with plant allergy. Moreover, the PPV ranged from 64.5% for olive tree pollen to 86.8% for grass pollen. Except for plane tree allergy, whose AUC was 0.798, the AUC of the remaining allergies exceeded 0.876, reaching a maximum value of 0.945 in the case of allergy to grass pollen.

The results of the analysis of the diagnostic accuracy of the singleplex ImmunoCAP test for the determination of sIgE to Phl p 1, Phl p 5, Ole e 1, Cup a 1, Pla a 1, and Par j 2 (Table 3) were similar to those obtained for ISAC-112. Table 4 shows the results of the comparison of the diagnostic performance of both techniques for the determination of sIgE to the major allergens of the pollens studied. Concordance between the singleplex ImmunoCAP and ISAC112, assessed using the κ index [8], proved to be very good for the determination of sIgE to Phl p 1, Phl p 5, Cup a 1, and Par j 2 and good for that

Table 1. Clinical Characteristics of the Sample of Pollen-Allergic Patients as a Whole, of the Subgroups of Patients Allergic to Each Pollen Type (Cases) and of the Control Patients

	Total No. of Pollen-Allergic Patients	Control Patients[a]	Grasses		Cypress		Olive Tree		Plane Tree		Pellitory of the Wall	
			Cases	Control Patients[b]	Cases	Control Patients[b]	Cases	Control Patients[b]	Cases	Control Patients[b]	Cases	Control Patients[b]
No.	390	90	49	56	75	122	33	73	63	107	17	141
Gender, No. (%)												
Male	153 (39)	25 (28)	20 (41)	18 (32)	25 (33)	55 (45)	8 (24)	26 (36)	23 (37)	43 (40)	5 (29)	53 (38)
Female	237 (61)	65 (72)	29 (59)	38 (68)	50 (67)	67 (55)	25 (76)	47 (64)	40 (63)	64 (60)	12 (71)	88 (62)
Median (IQR) age, y	30 (22-38)	43 (31-55)	32 (25-40)	34 (25-46)	31 (25-39)	33 (25-40)	32 (25-36)	33 (25-33)	33 (22-40)	31 (22-39)	31 (27-38)	33 (25-41)
Symptoms, No. (%)												
Rhinitis	330 (87)	31 (34)	47 (96)	51 (91)	74 (99)	108 (89)	31 (94)	64 (88)	61 (97)	101 (97)	16 (94)	134 (98)
Conjunctivitis	249 (66)	19 (21)	40 (82)	41 (73)	64 (55)	93 (76)	22 (67)	56 (77)	47 (75)	83 (79)	13 (76)	117 (86)
Asthma	145 (39)	7 (8)	18 (37)	21 (38)	41 (55)	46 (37)	10 (30)	24 (33)	24 (38)	44 (42)	7 (41)	53 (38)
Multiple sensitization, No. (%)	344 (88)	3 (3)	45 (92)	27 (48)	69 (92)	100 (82)	23 (70)	50 (69)	61 (97)	78 (73)	14 (82)	115 (82)
Food allergy, No. (%)	258 (87)	0 (0)	37 (76)	31 (55)	46 (61)	66 (54)	20 (61)	45 (62)	52 (83)	50 (47)	15 (88)	79 (56)

[a]Patients without allergies to plant allergens.
[b]Patients with allergies to other plant allergens.

Table 2. Diagnostic Performance of the ImmunoCAP ISAC112 Test

		ImmunoCAP ISAC112					
Pollen Component		Se, %	Sp 1, %	Sp 2, %	PPV	NPV	AUC
Grasses	Phl p 1	91.8	99.9	97.3	91.8	97.3	0.945
	Phl p 2	42.9	100	98.0	87.5	83.6	0.704
	Phl p 4	51.0	100	98.0	89.3	85.6	0.745
	Phl p 5	32.6	100	98.0	84.2	81.2	0.653
	Phl p 6	26.5	100	98.6	86.7	80.0	0.626
	Phl p 11	14.3	100	100	100.0	77.7	0.571
	Cyn d 1	85.7	96.7	96.5	89.4	95.3	0.911
	Total	93.9	96.7	95.8	86.8	97.9	0.945
Cypress	Cup a 1	88	97.8	92.9	81.5	95.6	0.905
Olive tree	Ole e 1	78.8	93.3	93.3	70.3	95.6	0.860
	Ole e7	18.2	100	97.6	60.0	85.5	0.578
	Ole e 9	0	100	99.4	0.0	83.1	0.497
	Total	87.9	93.3	90.2	64.5	97.4	0.890
Plane tree	Pla a 1	23.8	98.9	99.0	88.3	80.3	0.614
	Pla a 2	34.9	97.8	93.4	62.3	81.8	0.642
	Pla a 3	42.8	100	97.5	84.4	84.2	0.702
	Total	68.2	97.8	91.4	71.7	90.0	0.798
Pellitory of the wall	Par j 2	76.5	100	98.7	81.3	98.3	0.876

Abbreviations: AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; Sp 1, specificity calculated with control patients without plant allergies; Sp 2, specificity calculated including patients with plant allergies.

of sIgE to Ole e 1 and Pla a 1. No significant differences were found between the AUC of each technique for detection of any of the components, and the shape of the ROC curves of both techniques was similar for all the major allergens studied. Furthermore, for both techniques, a significant correlation was observed between the concentration of sIgE to each component, with correlation coefficients ranging from 0.71 for Pla a 1 to 0.89 for Phl p 1 and Par j 2.

Table 3. Diagnostic Performance of the ImmunoCAP ISAC112 Test

	ImmunoCAP ISAC112				
	Component	Se, %	Sp 1, %	PPV	NPV	AUC
Grasses	Phl p 1	91.5	97.3	97.7	90.0	0.944
	Phl p 5	36.2	100.0	100.0	55.2	0.681
	Total	91.5	97.3	97.7	90.0	0.944
Cypress	Cup a 1	81.4	100.0	100.0	72.9	0.907
Olive tree	Ole e 1	70.0	82.9	75.0	79.1	0.764
Plane tree	Pla a 1	21.8	94.3	85.7	43.4	0.581
Pellitory of the wall	Par j 2	73.3	100.0	100.0	89.4	0.867

Abbreviations: AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; Sp 1, specificity calculated with control patients without plant allergies.

Table 5 sets out the results of the application of ISAC112 in pollen-allergic patients from different parts of Spain who are sensitized to 1 or multiple pollens. Overall, the most frequent positive results were observed for species-specific components of grass pollen (70%), olive tree pollen (55%), and plane tree pollen (44%). However, the frequency of sensitization to the different species-specific allergenic components varied greatly between regions, except for Ole e 7 and Ole e 9, for which the frequency of sensitization was low. Overall, 73% of pollen-allergic patients were sensitized to species-specific allergenic components of more than 1 pollen type. ISAC112 detected sensitization to profilin(s) in 88 patients (23%), once again with major geographical variations, reaching 44% of patients in some regions. The frequency of sensitization to polcalcins was less variable, with an average sensitization rate of 7%. In the case of the subgroup of 190 patients sensitized to more than 1 taxon whose pollination periods overlapped with the symptomatic period, the results of ISAC112 were positive for the genuine components of more than 1 pollen type in 72% of cases. The frequency of sensitization to profilins and polcalcins in this subgroup of patients was 30.5% and 9%, respectively.

Table 4. Comparison of the ImmunoCAP ISAC112 and Singleplex ImmunoCAP Tests

	No. of Observations	Concordance		Correlation		AUC		
		κ	*P* Value	r	*P* Value	ISAC112	ImmunoCAP	*P* Value
Phl p 1	84	0.952	<.001	0.900	<.0001	0.939	0.948	.858
Phl p 5	84	0.887	<.001	0.741	<.0001	0.670	0.677	.852
Cup a 1	105	0.923	<.001	0.909	<.0001	0.943	0.925	.268
Ole e 1	71	0.797	<.001	0.830	<.0001	0.796	0.800	.908
Pla a 1	90	0.760	<.001	0.689	<.0001	0.611	0.596	.732
Par j 2	50	1.000	<.001	0.885	<.0001	0.867	0.887	.503

Abbreviation: AUC, area under the curve.

Discussion

The performance or accuracy of a diagnostic tool can be described as its ability to correctly classify participants into clinically relevant subgroups according to health status (eg, allergic or not allergic to a specific pollen) [20]. The accuracy of a diagnostic test must be known and compared with that of other existing procedures before the clinical utility of the new tool and its role in clinical practice are established.

ISAC112 is a powerful molecular diagnostic tool that enables semiquantitative determination of sIgE to 112 allergenic components. Its accuracy in the diagnosis of pollinosis has been studied for grass pollen [21], but not for pollen from olive tree, cypress, plane tree, or pellitory of the wall. Its intrinsic properties are achieved by comparing the results obtained by the subgroups of allergic and nonallergic patients for each of the pollen types studied. Therefore, the patients included in the technical analysis of the accuracy of ISAC112 met strict selection criteria, which enabled us to avoid the potential diagnostic confusion that sensitization to panallergens can cause when complete extracts are used during the diagnostic process [22]. Singleplex ImmunoCAP was considered a reference technique in the comparative analysis.

The results of our study proved that ISAC112 is a very accurate technique for the diagnosis of allergies to pollen from grass, olive tree, cypress, plane tree, and pellitory of the wall, even when applied to individuals with allergies to other pollen sources. Its sensitivity is good for the diagnosis of allergy to pollen from grass, cypress, and olive tree and moderate for the diagnosis of allergy to pollen from plane tree and pellitory of the wall.

The AUC quantifies the diagnostic accuracy of the tool with a single number [23]. Based on the usual interpretation of AUCs [19], we obtained an excellent value (>0.90) for the diagnosis of allergy to grass, cypress, olive tree, and pellitory of the wall and a good value for allergy to plane tree.

When comparing the efficacy of the ISAC112 with that of the singleplex ImmunoCAP test for the major allergens studied here, we obtained very good or good concordance values [18]. Additionally, despite the fact that ISAC112 is a semiquantitative technique, while the singleplex ImmunoCAP is a quantitative technique, the correlation between sIgE to the major allergens of grass, cypress, olive tree, plane tree, and pellitory of the wall was good, as reported elsewhere for grass allergens [24]. Likewise, the overall estimate of the diagnostic accuracy of both techniques for each of the

Table 5. Molecular Sensitization by Geographical Region

	Total[a] 390	Coruña[b] 16	Bilbao[b] 10	Pamplona[b] 31	Barcelona[b] 38	Madrid[b] 103	Cáceres[b] 34	C Real[b] 24	Alicante[b] 42	Málaga[b] 67	Almería[b] 25	*P* Value[c]
Phl p 1, No. (%)	257 (65.9)	8 (50.0)	5 (50.0)	29 (93.5)	15 (39.5)	84 (81.5)	26 (76.5)	16 (66.7)	18 (42.9)	41 (61.2)	15 (60.0)	<.001
Phl p 2, No. (%)	136 (34.9)	5 (31.2)	3 (30.0)	13 (41.9)	7 (18.4)	50 (48.5)	23 (67.6)	11 (45.8)	6 (14.3)	14 (20.9)	4 (16.0)	<.001
Phl p 4, No. (%)	161 (41.3)	7 (43.7)	4 (40.0)	22 (71.0)	9 (23.7)	46 (44.6)	26 (76.5)	12 (50.0)	10 (23.8)	19 (28.4)	6 (24.0)	<.001
Phl p 5, No. (%)	126 (32.3)	6 (37.5)	2 (20.0)	18 (58.1)	6 (15.8)	46 (44.6)	18 (52.9)	9 (37.5)	6 (14.3)	12 (17.9)	3 (12.0)	<.001
Phl p 6, No. (%)	89 (22.8)	5 (31.2)	0 (0.0)	15 (48.4)	3 (7.9)	32 (31.1)	14 (41.2)	7 (19.2)	3 (7.1)	8 (11.9)	2 (8.0)	<.001
Phl p 7, No. (%)	26 (6.7)	1 (6.1)	0 (0.0)	2 (6.4)	2 (5.3)	6 (5.8)	2 (5.9)	4 (16.7)	4 (9.5)	4 (6.0)	1 (4.0)	.766
Phl p 11, No. (%)	37 (9.5)	3 (18.75)	2 (20.0)	3 (9.7)	1 (2.6)	10 (9.7)	9 (26.5)	1 (4.2)	1 (2.4)	6 (8.9)	1 (4.0)	.015
Phl p 12, No. (%)	76 (19.5)	4 (25.0)	0 (0.0)	11 (35.5)	2 (5.3)	224 (23.3)	13 (28.2)	7 (29.2)	4 (9.5)	8 (11.9)	3 (12.0)	.001
Cyn d 1, No. (%)	235 (60.3)	6 (37.5)	3 (30.0)	29 (93.5)	16 (42.1)	77 (74.7)	26 (76.5)	15 (62.5)	14 (33.3)	38 (56.7)	11 (44.0)	<.001
Phl p _ , No. (%)	274 (70.3)	9 (56.2)	6 (60.0)	30 (96.8)	18 (47.4)	85 (82.5)	29 (85.3)	16 (66.7)	19 (45.2)	46 (68.6)	16 (64.0)	<.001
Ole e 1, No. (%)	193 (49.5)	2 (12.5)	1 (10.0)	6 (19.3)	11 (28.9)	55 (53.4)	16 (47.1)	19 (79.2)	25 (59.5)	39 (58.2)	19 (76.0)	<.001
Ole e 7, No. (%)	47 (12.1)	2 (12.5)	0 (0.0)	4 (12.9)	5 (13.2)	9 (8.7)	2 (5.9)	3 (12.5)	3 (7.1)	16 (23.9)	3 (12.0)	.140
Ole e 9, No. (%)	20 (5.1)	0 (0.0)	0 (0.0)	1 (3.2)	0 (0.0)	5 (4.8)	2 (5.9)	2 (8.3)	0 (0.0)	8 (11.9)	2 (8.0)	.148
Ole e_ , No. (%)	214 (54.9)	4 (25.0)	1 (10.0)	11 (35.5)	14 (36.8)	57 (55.3)	18 (52.9)	19 (79.2)	27 (64.3)	43 (64.2)	20 (80.0)	<.001
Par j 2, No. (%)	32 (8.2)	3 (18.7)	0 (0.0)	0 (0.0)	7 (18.4)	1 (1.0)	2 (5.9)	0 (0.0)	2 (4.7)	13 (19.4)	4 (16.0)	<.001
Bet v 1, No. (%)	25 (6.4)	4 (25.0)	0 (0.0)	3 (9.7)	0 (0.0)	6 (5.8)	3 (8.8)	0 (0.0)	2 (4.7)	7 (10.4)	0 (0.0)	.027
Bet v 2, No. (%)	88 (22.6)	4 (25.0)	0 (0.0)	11 (35.5)	3 (7.9)	29 (28.1)	15 (44.1)	7 (29.2)	5 (11.9)	10 (14.9)	4 (16.0)	.001
Bet v 4, No. (%)	27 (6.9)	2 (12.5)	1 (10.0)	2 (6.4)	2 (5.3)	5 (4.8)	2 (5.9)	4 (16.7)	4 (9.5)	4 (6.0)	1 (4.0)	.716
Bet v _, No. (%)	25 (6.4)	4 (25.0)	0 (0.0)	3 (9.7)	0 (0.0)	6 (5.8)	3 (8.8)	0 (0.0)	2 (4.7)	7 (10.4)	0 (0.0)	.027
Sal k 1, No. (%)	77 (19.7)	0 (0.0)	0 (0.0)	2 (6.4)	2 (5.3)	16 (15.5)	2 (5.9)	12 (50.0)	16 (38.1)	11 (16.4)	16 (64.0)	<.001
Pla a 1, No. (%)	26 (6.7)	0 (0.0)	0 (0.0)	1 (3.2)	12 (31.6)	10 (9.7)	0 (0.0)	0 (0.0)	0 (0.0)	3 (4.5)	0 (0.0)	<.001
Pla a 2, No. (%)	98 (25.1)	1 (6.2)	0 (0.0)	3 (9.7)	16 (42.1)	29 (28.2)	10 (29.4)	6 (25.0)	12 (28.6)	17 (25.4)	4 (16.0)	.031
Pla a 3, No. (%)	101 (25.9)	4 (25.0)	2 (20.0)	8 (25.8)	16 (42.1)	16 (15.5)	3 (8.8)	2 (8.3)	14 (33.3)	25 (37.3)	11 (44.0)	<.001
Pla a _, No. (%)	172 (44.1)	4 (25.0)	2 (20.0)	10 (32.2)	30 (78.9)	37 (35.9)	13 (38.2)	8 (33.3)	24 (57.1)	31 (46.3)	13 (52.0)	<.001
Multiple sensitization, No. (%)[d]	285 (73.0)	7 (43.7)	1 (10.0)	19 (41.3)	31 (47.0)	80 (69.6)	23 (67.5)	21 (87.5)	36 (58.0)	51 (62.2)	20 (80.0)	<.001

Abbreviations: Bet v _, sensitization to ≥1 molecular components of *Betula verrucosa*, excluding Bet v 2 and Bet v 4; Phl p _, sensitization to ≥1 molecular components of *Phleum pratense*, excluding Phl p 7 and Phl p 12; Ole e _, sensitization to one or more molecular components of *Olea europea*; Pla a _, sensitization to ≥1 molecular components of *Platanus acerifolia*.

[a]Number of sensitized patients and their percentage in relation to the total number of pollen-allergic patients included in the study.

[b]Number of sensitized patients per geographical region and their percentage in relation to the total number of patients included per region.

[c]Comparison of the frequency of cases of molecular sensitization between different geographical regions using the Pearson chi-square test.

[d]Number and percentage of patients sensitized to genuine molecular components of more than 1 taxon.

major allergens based on ROC curves was found to be similar, with AUC values that enabled both techniques to be classified as excellent tools for the diagnosis of grass and cypress pollen allergies and as good tools for the diagnosis of allergy to *Parietaria* pollen. Consistent with the findings of Bokanovic et al [21], our results prove that determination of sIgE to Phl p 1 with the singleplex technique is sufficient for diagnosis of grass pollen allergy. In the case of allergy to olive tree pollen in this sample of patients, singleplex ImmunoCAP was also very accurate for the determination of Ole e 1 as the only allergen, with a similar AUC to both that obtained for the same allergen with ISAC112 and that obtained when the 3 olive tree allergens included in the microarray (Ole e 1, Ole e 7, and Ole e 9) were analyzed together. Although Ole e 7 belongs to the lipid transfer protein (LTP) family, we believe that it should be considered a genuine pollen allergen, as it was described as the only sensitizing component of olive tree pollen in 5% of patients from high-exposure areas [25]. The diagnostic accuracy of the determination of Pla a 1 alone with the ImmunoCAP test (the only plane tree allergen available for this technique) or ISAC112 is mediocre; however, when all 3 allergenic components of plane tree pollen are studied together, ISAC112 does prove to have good diagnostic accuracy. The role of Pla a 3, an LTP, as a species-specific pollen allergen is supported by its description [26] as a minor allergen (27.3%) in patients who are allergic to it but who do not have food allergy and as a major allergen in patients who are allergic to both plane tree pollen and peach with a sensitization prevalence of 64%. Additionally, cases of sensitization to Pla a 3 without reactivity to Pru p 3 are possible.

After determining the diagnostic accuracy of ISAC112 and comparing it with that of the singleplex ImmunoCAP test, we proceeded to apply the test in a nonselected sample of pollen-allergic patients with a high frequency of skin sensitization to multiple allergens (80%). Such a high percentage is seen daily in allergy clinics. The determination of both sIgE to the main allergens of the sensitizing pollens and panallergens could clarify the diagnosis of these patients. We confirmed high rates of cosensitization to genuine allergen components from several taxa, with an average of 75% of patients showing genuine sensitization to more than 1 pollen type, and revealed major geographical variations in terms of the pollinosis-causing pollens of all taxa. The results obtained when the SPTs were positive to more than 1 taxon with pollination periods that overlapped both with one another and with the patients' symptomatic period were of particular clinical interest. This circumstance could correspond both to actual cosensitization and to mere cross-sensitization whose immunological basis is the sensitization to pollen panallergens (eg, profilins and polcalcins). ISAC112 distinguishes between both situations in such a way that the analysis of its results for this subgroup of patients shows true cosensitization in 73% of the patients, with 30.5% and 9% of them being sensitized to profilins and polcalcins, respectively.

To conclude, it is safe to say that ISAC112 is an accurate tool for the diagnosis of allergy to pollen from grass, cypress, olive tree, plane tree, and pellitory of the wall. Its features are similar to those of the singleplex ImmunoCAP test and, in the case of allergy to plane tree pollen, superior to those of the singleplex ImmunoCAP test used for the same purpose. Given the associated costs and volume of samples required to simultaneously determine multiple allergen components, the maximum clinical utility of ISAC112 for the etiologic diagnosis of pollinosis would be in the subgroup of patients with skin sensitization to multiple pollen types and overlapping pollination periods, especially in the event of a suspected clinical allergy to plane tree pollen and potential indication for immunotherapy.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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