ORIGINAL ARTICLE

Enhanced Diagnosis of Pollen Allergy Using Specific Immunoglobulin E Determination to Detect Major Allergens and Panallergens

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In memory of our colleague Prof. Dr. José Conde Hernández

Abstract

Background: Pollen is one of the main causes of allergic sensitization. It is not easy to make an etiological diagnosis of pollen-allergic patients because of the wide variety of sensitizing pollens, association with food allergy, and increasing incidence of polysensitization, which may result from the presence of allergens that are common to different species, as is the case of panallergens.
Objective: To compare the results of skin prick tests (SPT) using whole pollen extract with specific immunoglobulin (Ig) E determination for several allergens (purified panallergens included) in the diagnosis of polysensitized pollen-allergic patients.
Methods: The study sample comprised 179 pollen-sensitized patients who underwent SPT with pollen extract and allergen-specific IgE determination against different allergens.
Results: The level of concordance between the traditional diagnostic test (SPT) and IgE determination was low, especially in patients sensitized to the panallergens profilin and polcalcin. In the case of SPT, the results demonstrated that patients who are sensitized to either of these panallergens present a significantly higher number of positive results than patients who are not. However, IgE determination revealed that while patients sensitized to polcalcins are sensitized to allergens from a higher number of pollens than the rest of the sample, this is not the case in patients sensitized to profilins. On the other hand, sensitization to profilin or lipid transfer proteins was clearly associated with food allergy.
Conclusions: Sensitization to panallergens could be a confounding factor in the diagnosis of polysensitized pollen-allergic patients as well as a marker for food allergy. However, more studies are required to further investigate the role of these molecules.

Key words: Panallergens. Profilin. Lipid transfer protein. Polcalcin. Skin test. Molecular diagnosis.

Resumen

Introducción: el polen es una de las principales causas de sensibilización alérgica. No es fácil a veces hacer un diagnóstico etiológico de los pacientes polínicos debido a la gran variedad de pólenes alergénicos, asociaciones con alergias a alimentos, y al incremento de la polisensibilización. Éste último factor parece deberse fundamentalmente a la existencia de alérgenos comunes en diferentes especies, como es el caso de los panalérgenos.
Objetivos: comparar los resultados de los test cutáneos intraepidérmicos usando extractos completos de pólenes con la determinación de IgE específica a diferentes alérgenos (incluido panalérgenos purificados) en el diagnóstico de pacientes alérgicos polisensibilizados a pólenes.
Métodos: se incluyeron 179 pacientes sensibilizados a pólenes. Se les realizó test cutáneos con extractos de pólenes y se determinó la IgE específica frente a diferentes alérgenos.
Resultados: el nivel de concordancia entre el test diagnóstico tradicional y el diagnóstico molecular fue bajo, especialmente en pacientes sensibilizados a profilina y polcalcina. En el caso de las pruebas cutáneas, los resultados demostraron que los pacientes que estaban sensibilizados a alguno de esos panalérgenos presentaban un número significativamente mayor de resultados positivos que los pacientes no sensibilizados a panalérgenos. Sin embargo, en la determinación de IgE específica a alérgenos, se observó que mientras los pacientes

sensibilizados a polcalcina estaban sensibilizados a alérgenos de un mayor número de pólenes que el resto de la muestra, esto no ocurría con los sensibilizados a profilinas. Por otro lado, la sensibilización a profilina o LTP se asoció claramente con alergia alimentaria.
Conclusiones: la sensibilización a panalérgenos podría ser un factor de confusión en el diagnóstico de los pacientes polínicos polisensibilizados, así como un marcador de alergia a alimentos. En cualquier caso, se requieren más estudios para investigar más a fondo el papel de estas moléculas.

Palabras clave: Panalérgenos. Profilina. Proteína de transferencia de lípidos. Polcalcina. Test cutáneos. Diagnóstico molecular.

Introduction

Recent epidemiological studies show that the prevalence of allergic diseases is increasing [1]. In addition, the association between atopic sensitization and respiratory allergic disease has increased in more economically developed countries [2], and pollen is one of the principal factors in allergic sensitization. A correct diagnosis of pollen allergy depends on 2 factors: the higher prevalence of associated food allergy [3] and an increase in polysensitization, particularly in high-prevalence areas [4]. These factors not only hinder diagnosis, but also limit therapeutic options, particularly allergen-specific immunotherapy. Consequently, we must determine whether current diagnostic techniques (skin tests and/or allergen-specific immunoglobulin [Ig] E determination against a whole extract) are sufficient and we must establish which factors affect the onset of polysensitization and associated food allergy. Sensitization to panallergens could play an important role, because these molecules are present in different plant pollens and plant foods and have marked structural similarity in different species. In addition, sensitization to panallergens would lead to the onset of cross-reactivity, with the subsequent possibility of false positives. The aim of this study was to evaluate the role of sensitization to panallergens in the diagnosis of pollen-allergic patients.

Methods

Patient Selection

The small number of previous studies on this subject made it hard to calculate the sample size. However, estimating the prevalence of profilin sensitization at about 20% [5] and aiming to obtain precision with a confidence interval of 6%, 171 patients needed to be included. A total of 179 patients were consecutively recruited over a period of 6 months. The inclusion criteria were clinical symptoms compatible with pollinosis and no previous immunotherapy. All the patients included in the sample lived in an area in which sensitization to specific pollens (especially *Olea* and grass) was the most relevant cause of respiratory disease. The study was approved by the Ethics Committee of Hospital Virgen Macarena (Sevilla, Spain) and oral informed consent was obtained from all patients.

Skin Tests

Prick tests (using the reference of the histamine equivalent prick [HEP]) were performed with the following test battery (ALK-Abelló, S.A., Madrid, Spain): grass mix (*Dactylis, Festuca, Lolium, Phleum, Poa*, 30 HEP, Group 5 allergens: 60 µg/mL), *Olea europaea* (30 HEP: Ole e 1, 180 µg/mL; Ole e 9, 6 µg/mL), *Artemisia vulgaris* (30 HEP: Art v 1, 35 µg/mL), *Platanus acerifolia* (30 HEP), *Cupressus sempervirens* (30 HEP), *Chenopodium album* (30 HEP), *Salsola kali* (30 HEP), *Plantago lanceolata* (30 HEP: Pla l 1, 30 µg/mL), *Parietaria judaica* (30 HEP; Par j 1, 20 µg/mL), purified profilin from date palm (Pho d 2, 50 µg/mL), and peach skin extract (Pru p 3, 30 µg/mL). Histamine was used as the positive control and saline solution as the negative control. The technique followed the directives of the European Academy of Allergy and Clinical Immunology [6].

Clinical Questionnaire

The data collected for each patient were as follows: age, gender, profession, family history of atopy, sensitization to allergens other than pollen, type and period of manifestation of symptoms, diagnosis of rhinitis using the ARIA classification [7], and diagnosis of asthma using the GINA criteria [8], as well as whether the clinical history was compatible with food allergy. Type of clinical reaction due to food allergy was also recorded.

Specific IgE Determination

Specific IgE was determined using the ADVIA-Centaur platform [9] (Bayer HealthCare Diagnostics Division, Tarrytown, New York, USA) against the following pollen allergens: Phl p 1, Phl p 5, Cyn d 1, Ole e 1, Ole e 7, Ole e 9, Art v 1, Sal k 1, Che a 1, Pla l 1, Cup s 1, Pla a 1, and Pla a 2. IgE was also determined to 3 profilins (Ole e 2, Pho d 2, and Mal d 4), polcalcin (Che a 3), and 4 lipid transfer proteins (LTP) (Pru p 3, Art v 3, Ole e 7, and Par j 1).

Statistical Analysis

The association between qualitative variables was analyzed using the Pearson χ^2 test or Fisher exact test, depending on the sample characteristics. The concordance analysis between the different skin tests was performed by calculating the κ statistic. To check whether there was an association between the 2 qualitative variables when controlled by a third variable, the Cochran–Mantel–Haenszel test was applied, calculating the common relative risk in one group against another through a logit model.

The association between the quantitative variables was analyzed using the Spearman correlation coefficient. In order to detect differences between the groups, the Mann-Whitney or Kruskal-Wallis tests were used.

The association between a qualitative ordinal variable and a dichotomous variable was analyzed using the Cochran–Armitage test.

Logistic regression models were applied to determine risk factors in sensitization to panallergens.

Results

Sample Characteristics

The study sample comprised 179 patients with a mean (SD) age of 30.1 (10.8) years (range, 9-69 years); 57% were women and 43% were men. With regard to diagnosis, 29% had rhinoconjunctivitis, 1% asthma, and the remaining 70% rhinoconjunctivitis and asthma. Seasonal symptoms occurred mainly in April and May.

The sensitization profiles are summarized in Table 1. A high prevalence of sensitization was found to olive and grass, measured both by SPT and by specific IgE to major allergens. However, for other pollens, such as *Plantago* (Pla l 1) or *Chenopodium* (Che a 1), different percentages were obtained using IgE determination.

Of the total number of patients included, 55 (30.7%) were monosensitive (or they were sensitized to allergens from a single pollen, according to the determination of principal allergens by Advia-Centaur), mainly to grasses (26, 47.3%), *Olea* (10, 18.2%), and *Artemisia* (10, 30.7%). On the contrary, almost 60% of patients presented positive SPT results to 4 or more pollens.

Association Between Sensitization to Panallergens and Sensitization to Other Pollen Allergens

The major grass allergen Phl p 5 was identified as a clear risk factor of profilin sensitization (odds ratio [OR] 8.3; 95% confidence interval [CI], 2.4-29.1). The remaining allergens did not present a statistical association with profilin. No statistical association was found between sensitization to LTPs and polcalcins and sensitization to other major or minor allergens, probably due to the small sample size.

Concordance of Results Between Skin Tests and Molecular Diagnosis

In order to determine the degree of concordance between the 2 diagnostic techniques, we focused on the results obtained with 1 profilin (Pho d 2) and 1 LTP (Pru p 3), because the SPTs with profilin and LTP only contain the allergens Pho d 2 and Pru p 3, respectively, as allergenic compounds, unlike the other prick tests which contain several major and minor allergens. Concordance was 0.7 and 0.77, respectively. Therefore, we could assume that values ranging between 0.7 and 0.8 would indicate an adequate level of concordance between each pollen extract and its corresponding major allergen. For

Table 1. Sensitization Profiles As Estimated by Skin Prick Testing or Specific Immunoglobulin E Determination

SPT-Positive			IgE-Positive		
Allergen	n	%	Allergen	n	%
Grass mix	139	77.7	Phl p 5	65	36.3
			Phl p 1	118	65.9
Olea europaea	134	74.9	Ole e 1	108	60.3
			Ole e 7	19	10.6
			Ole e 9	11	6.2
			Ole e 2	32	17.9
Chenopodium album	118	65.9	Che a 1	4	2.2
			Che a 3	12	6.7
Plantago lanceolata	94	52.5	Pla l 1	8	4.5
Artemisia vulgaris	66	36.9	Art v 1	33	18.4
			Art v 3	16	8.9
Parietaria judaica	42	23.5	Par j 1	6	3.4
Platanus acerifolia	67	37.4	Pla a 1	16	8.9
Cupressus sempervirens	7	3.9	Cup s 1	21	11.3
Salsola kali	51	28.5	Sal k 1	22	12.3
Palm profilin (Pho d 2)	42	23.5	Pho d 2	32	17.9
Peach skin (Pru p 3 marker)	26	14.5	Pru p 3	24	13.4
			Mal d 4	28	15.6
			Cyn d 1	86	48.0

Abbreviations: Ig, immunoglobulin; SPT, skin prick test.

grasses (pollen extract/Phl p 1 or Phl p 5) and *Olea* (pollen extract/Ole e 1), the level of concordance was acceptable (0.69 and 0.65, respectively). For *Plantago, Chenopodium*, and *Platanus*, the level of concordance was low (0.08, 0.02, and 0.11, respectively). In the cases mentioned above, sensitization to panallergens does not appear to significantly affect concordance. However, in cases of sensitization to *Artemisia, Salsola,* and, to a lesser extent, *Cupressus*, the level of concordance increased in the global sample from 0.53, 0.49, and 0.32, respectively, to 0.74, 0.74, and 0.44 when patients not sensitized to panallergens were analyzed individually (Figure 1).

Assessment of the Degree of Diagnostic Confusion in Patients Sensitized to Panallergens

To assess the degree of possible diagnostic confusion in the case of a patient who may or may not be sensitized to a panallergen, we analyzed the number of allergens to which both types of patients presented a positive result. Tables 2 and 3 show the results for profilins and polcalcin, respectively. In the case of SPTs, the results demonstrated that patients sensitized to either of these panallergens present a significantly higher number of positive results than those who are not. However, IgE determination revealed that, while patients sensitized to

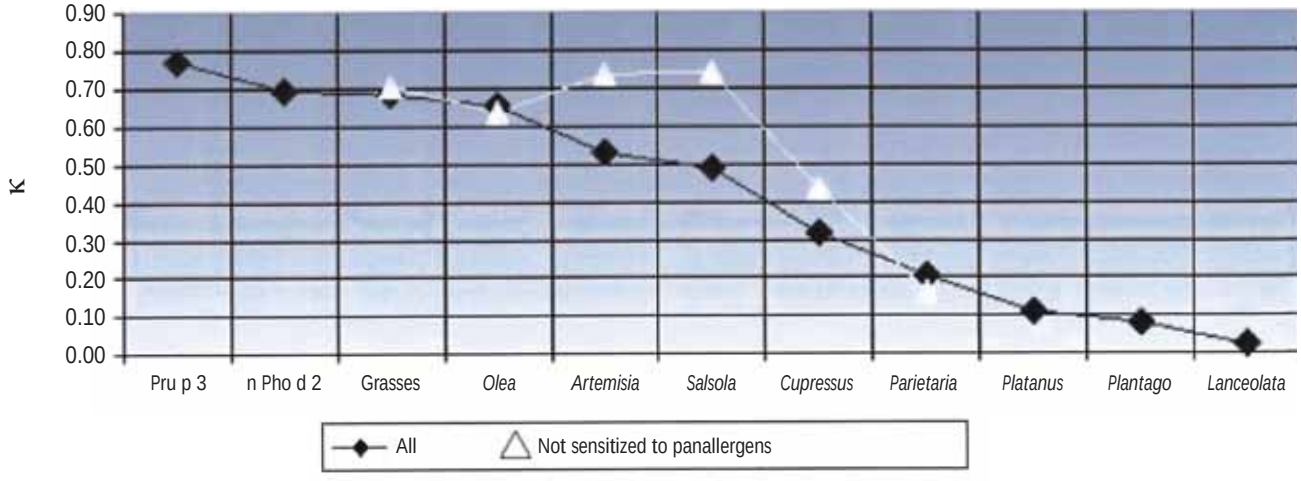

Figure 1. Degree of concordance (κ statistic) between SPT (pollen extract) and sIgE to major allergens in patients sensitized or not sensitized to panallergens. Only statistically significant values have been included.

Table 2. Number of Positive Results to Pollen Measured by Skin Prick Test or Specific Immunoglobulin E in Patients Sensitized or Not Sensitized to Profilins

	Specific Immunoglobulin E		Skin Prick Test	
	Median	95% CI	Median	95% CI
Profilin-positive (n=36)	2	2.3-3.14	6	5.22-6.39
Profilin-negative (n=143)	2	2.03-2.35	3	3.21-3.91
P Value	*P*<.0187		*P*<.0001	

Abbreviation: CI, confidence interval.

Table 3. Number of Positive Results to Pollen (Median) Measured by Skin Prick Test or Specific Immunoglobulin E in Patients Sensitized or Not Sensitized to Polcalcin

	Specific Immunoglobulin E		Skin Prick Test	
	Median	95% CI	Median	95% CI
Polcalcin-positive (n=12)	3.5	2.42-4.25	8	7.47-8.20
Polcalcin-negative (n=167)	2	2.07-2.37	4	3.43-4.05
P Value	*P*<.0187		*P*<.0001	

Abbreviation: CI, confidence interval.

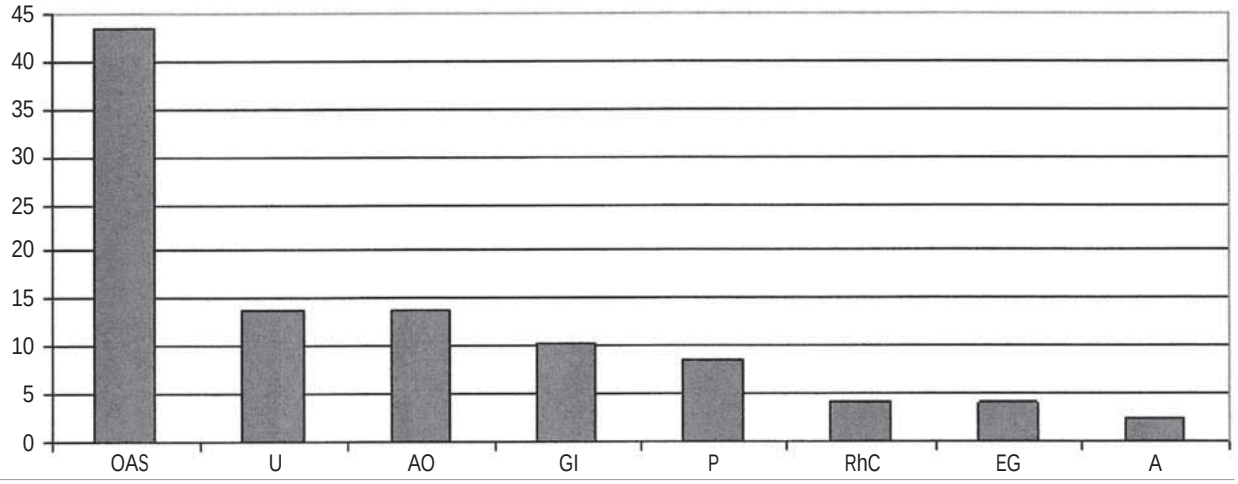

Figure 2. Type of clinical symptoms in pollen-allergic patients with associated food allergy. OAS indicates oral allergy syndrome; U, urticaria and/or angioedema; AO, anaphylaxis; GI, gastrointestinal symptoms (dyspepsia, vomiting); P, pruritus; RhC, rhinoconjunctivitis; EG, edema of the glottis; A, asthma.

Table 4. Level of Association Between Food Allergy And Positive Immunoglobulin E To Panallergens[a]

	Allergens									
	Positive				Negative				Relative Risk	*P* Value
	Food Allergy				Food Allergy					
	Yes		No		Yes		No			
	n	%	n	%	n	%	n	%		
Art v 3	11	68.8	5	31.3	39	23.9	124	76.1	2.87	.0004
Pho d 2	14	43.8	18	56.3	36	24.5	111	75.5	1.79	.0484
Ole e 2	14	43.8	18	56.3	36	24.5	111	75.5	1.79	.0484
Mal d 4	13	46.4	15	53.6	37	24.5	114	75.5	1.89	.0226
Pru p 3	19	79.2	5	20.8	31	20.0	124	80.2	3.96	<.0001

[a] Only statistically significant values have been included.

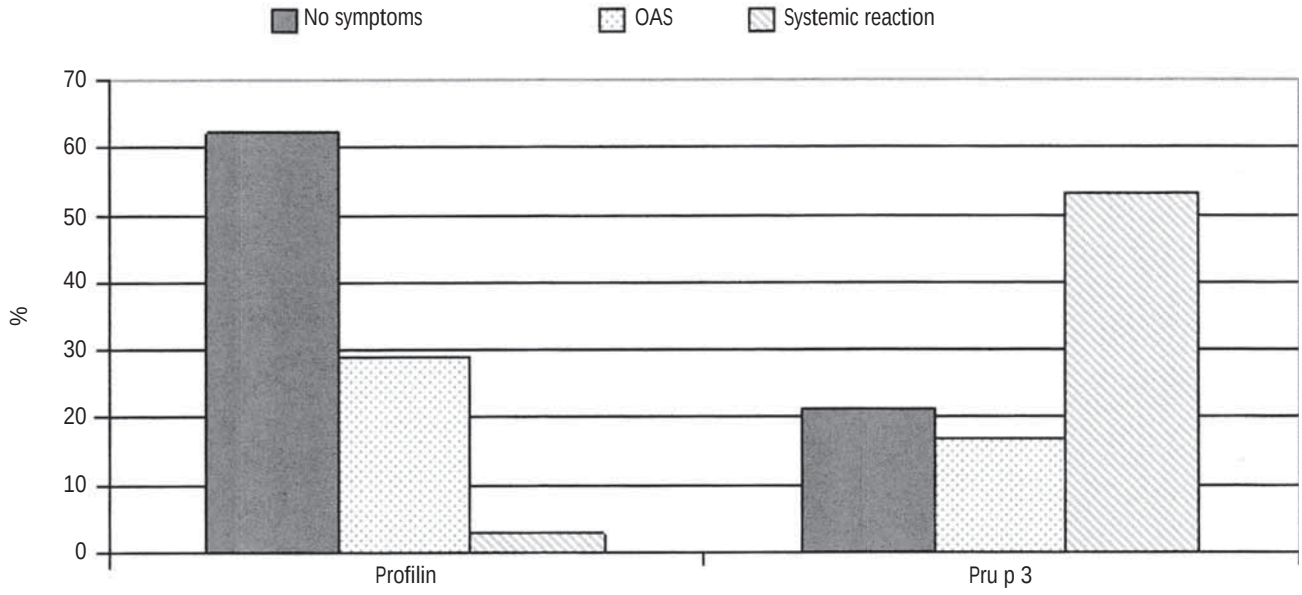

Figure 3. Clinical characteristics of food allergy in patients sensitized to profilin or Pru p 3. Sensitization to Pru p 3 is statistically significantly associated with a higher frequency and greater severity of food allergy than sensitization to profilins (*P*=.0004). OAS indicates oral allergy syndrome.

polcalcins are sensitized to allergens from a higher number of pollens than in the rest of the sample, this is not the case in patients sensitized to profilins. No significant differences were observed for patients sensitized to LTPs.

Food allergy: Sensitization to Panallergens as a Marker of Severity

Clinical symptoms of allergy to 1 or more plant foods were observed in 49 patients (27.4%) (Figure 2). Peach was the most common food involved (46.9%), followed by melon (24.5%) and, to a lesser extent, kiwi (14.3%), walnut (12.2%), and peanut and banana (10.2%). Other foods were some way behind the frequency of allergic symptoms to the above foods.

The potential link between food allergy and sensitization (specific IgE) to each panallergen was analyzed (Table 4). Patients sensitized to profilins (Mal d 4, Ole e 2, and Pho d 2) and LTPs (Pru p 3 and Art v 3) presented a significantly higher frequency of reactions to plant foods than nonsensitized patients. No significant differences were observed for the remaining allergens. Moreover, in patients with food allergy and sensitization to profilin, clinical symptoms were less severe than in patients sensitized to Pru p 3 (Figure 3), who showed a higher frequency of systemic reactions, including anaphylaxis (Cochran–Armitage test, P=.0004).

Discussion

Panallergens can be common to different pollens and/or foods and show high structural similarity between species [10]. In the case of profilins, which were the most prevalent in our study, we observed 87% concordance in sensitization in these 3 allergens (Ole e 2, Pho d 2, and Mal d 4), which seems to confirm the high immunological similarity between them. Thus, any of the 3 could be indistinctly used as a sensitization marker.

The first aim of this study was to find out whether pollen-allergic patients could be accurately diagnosed with currently available resources. In view of the lack of concordance in the results obtained between SPT and determination of IgE, a certain percentage of patients could be misdiagnosed. It would be logical to think that the specificity of a molecular technique is superior to that of SPT; however, the prevalence levels that we found with Pho d 2 and Pru p 3 indicate slightly higher sensitivity in SPT. The concordance levels between the 2 diagnostic tests found with these 2 molecules indicated that values of between 0.7 and 0.8 could be considered good. However, we only found values close to this level in the case of grass pollen and olive. The clinical relevance of these 2 pollens is clear, given the higher frequency and severity of symptoms, occurrence of symptoms during the period of peak exposure for grass and olive, and the number of patients monosensitized to these pollens (15% grass, 6% *Olea*, and 22% both). This was not so in the case of the other pollens we tested, where lack of concordance between SPT and specific IgE to major allergens, absence of symptoms in most patients during the pollen season, and the low number of monosensitized patients may be an indication of their limited clinical relevance despite a positive SPT result. This finding could have several explanations. For example, SPT is considered positive when mean wheal diameter is ≥3 mm [11,12]. However, in our study, mean wheal size was considerably higher (data not shown) when there was concordance between the 2 tests. We could speculate that the higher the wheal size, the higher the concordance. Furthermore, in some pollens, the minor allergens may play a more relevant role than was previously thought. Thus, some patients with a positive SPT result to olive pollen have no IgE to the major allergen (Ole e 1) and positive IgE to a minor allergen (Ole e 7). Although this occurs in a small percentage of patients, the result has clear implications for diagnosis and therapy. Our most relevant finding was that concordance between the 2 tests was low in patients sensitized to panallergens (mainly profilin) and much higher in patients not sensitized to panallergens.

Patients sensitized to panallergens (profilin and polcalcin) show a significantly higher mean number of cutaneous sensitizations than nonsensitized patients. Thus, we could conclude that sensitization to panallergens is a confounding factor, because it leads to increased pollen recognition in skin tests (polysensitization), which does not correspond to real sensitization (in our study profilin accounted for approximately 33% of these cases and polcalcin for 7%). However, in contrast with profilin-sensitized patients, polcalcin-sensitized patients also present a higher number of positive specific IgE results to major and minor allergens. Furthermore, polcalcin-sensitized patients presented a significant longer disease course than the other patients (data not shown). We might speculate that sensitization to profilin occurs sooner than sensitization to polcalcin, and it seems that sensitization to polcalcin serves as a marker in the long-term development of allergic disease. Furthermore, sensitization to polcalcin probably marks a sensitization process, not only through major allergens, but also through some minor ones. In any case, further studies are required to confirm these data, since molecules that we did not study, such as glycoproteins or Ole e 10, show great similarity and cross-reactivity between different species [13].

Of note, sensitization to the most prevalent panallergen (profilin) is only statistically significantly associated with sensitization to Phl p 5. Patients sensitized to this allergen present an 8-fold greater risk of sensitization to profilin than to other allergens, including some of the most prevalent, such as Phl p 1 or Ole e 1. This finding may indicate that sensitization to profilin is secondary to sensitization to the main pollen allergen in the area studied. For this reason, in other geographical areas with different pollen profiles, the risk profilin sensitization may lie with other nongrass pollens. Only 1 patient was monosensitized to profilin in our study. Due to the prevalence of certain panallergens, such as profilin, studies to determine their possible clinical relevance in respiratory allergic disease would be of great scientific interest.

Finally, as for food allergy, the prevalence of plant food allergy in our study sample (27.4%) was much higher than the estimated prevalence in the general population, which is usually under 3% [14]. Overall, 65% of our patients were sensitized to profilin or Pru p 3, suggesting that these proteins may be responsible, at least in a high percentage of cases, for the association between food allergy and pollinosis in our patients. Furthermore, a higher frequency of severe systemic reactions (including anaphylaxis) is observed in patients sensitized to Pru p 3 than in patients sensitized

to profilin. It is remarkable that most patients with systemic reactions and sensitization to Art v 3 were also sensitized to Pru p 3, a finding that has been reported elsewhere [15,16]. This statistically significant association could be an indicator of cross-reactivity between the 2 allergens. In Spain, food allergy related to LTPs is the most frequent plant food allergy in adults [17].

We observed that the diagnostic approach to pollen-allergic patients involves aspects that have not been sufficiently studied to date, are essential for clear identification of the real cause of the disease, and make it possible to improve both the efficacy and safety of etiological treatment where indicated (allergen-specific immunotherapy). However, more studies are required so that we can investigate more closely the role played by molecules such as panallergens in order to significantly increase the reliability of diagnostic tests and interpret results more accurately.

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