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A Multicenter Study of Sensitization Profiles in an Allergic Pediatric Population in an Area With High Allergen Exposure

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Abstract

Background and *objective*: In areas with a high number of allergens and high allergen concentrations, it is essential to identify the main causes of allergy, especially in pediatric patients. This study was conducted in allergic patients aged 14 or less to identify sensitization profiles during an initial phase, and to then evaluate changes in these profiles after 3 years of follow-up. This article describes the first phase of our investigation.
Methods: A total of 187 patients aged between 2 and 14 years were included by 5 allergy units; all the children had symptoms suggestive of allergic disease (rhinoconjunctivitis and/or asthma). Allergy diagnosis was confirmed by evaluation of clinical history, allergen exposure, and in vivo or in vitro tests. Specific immunoglobulin E (sIgE) to major allergens was tested.
Results: Patients were sensitized to both seasonal (especially grass, olive, cypress and *Cynodon dactylon*) and perennial allergens (*Alternaria alternata*) and to panallergens (especially profilin and lipid transfer protein). Almost 60% of the patients included were polysensitized. Sensitization to certain major allergens such as Cup s1, Phl p1, or Sal k1 seems to increase with age. Patients sensitized to profilin had a higher number of sensitizations than non-profilin-sensitized patients. This panallergen is a diagnostic confounding factor.
Conclusions: A high percentage of allergic pediatric patients living in an area with high exposure levels to a large number of allergens are polysensitized and have a high percentage of sensitization to panallergens. The implementation of new diagnostic tools such as component-resolved diagnosis is crucial.

Key words: Allergen exposure. Component resolved diagnosis. Childhood. Allergen sensitization profile.

Resumen

Antecedentes y *objetivo*: En áreas geográficas donde el paciente está expuesto a un elevado número y altas concentraciones de alérgenos, es importante identificar los principales causantes de la enfermedad alérgica, especialmente en pacientes pediátricos. El presente estudio se ha realizado en pacientes alérgicos ≤14 años con el objetivo de tratar de averiguar, en una primera fase, el perfil de sensibilización y, en una segunda fase, la evolución de dicho perfil tras 3 años de seguimiento. En esta publicación se presenta la primera fase.
Métodos: Se incluyeron en primera visita 187 pacientes por 5 Unidades de Alergia, con edad entre 2 y 14 años y síntomas sugestivos de enfermedad alérgica (rinoconjuntivitis y/o asma). El diagnóstico alergológico fue posteriormente confirmado por prueba cutánea y/o IgE específica. Posteriormente se analizó la IgE a los alérgenos principales.
Resultados: Los pacientes mostraron sensibilización tanto a alérgenos estacionales (gramíneas, olivo, ciprés, Cynodon) como perennes (Alternaria) y panalérgenos (especialmente profilina y proteínas transportadoras de lípidos). Un 60% estaban polisensibilizados. La sensibilización a ciertos alérgenos (Cup s1, Phl p1 o Sal k1) parece aumentar con la edad. Los pacientes sensibilizados a profilina mostraron un mayor número de sensibilizaciones que los negativos al panalérgeno, siendo un posible factor de confusión diagnóstica
Conclusiones: Pacientes pediátricos que viven en áreas de alta exposición a numerosos alérgenos son polisensibilizados, estando un alto porcentaje de ellos sensibilizados a panalérgenos, por lo que la implementación de nuevas herramientas diagnósticas como el diagnóstico por componentes se hace imprescindible para su correcto diagnóstico.

Palabras clave: Exposición alergénica. Diagnóstico por componentes.Infancia. Perfil de sensibilización alergénica.

Introduction

The prevalence of allergic diseases varies widely from one country to another, with up to 20-fold differences for allergic asthma and 30-fold differences for allergic rhinoconjunctivitis according to the International Study of Asthma and Allergies in Childhood [1].

Degree of allergen exposure is one of the main factors responsible for these variations because it affects the prevalence of allergy symptoms through different mechanisms [2]. Depending on the geographic area, there can be significant variations in the number of allergens present and the allergenic pressure exerted on the population. In areas with high allergen numbers and levels, it is essential to identify the main causes of allergic sensitization. This is especially important in areas with a large number of allergens that are an important source of allergy, (eg, seasonal allergens [grass, *Olea europaea*, *Platanus acerifolia*, *Artemisia vulgaris*, *Fraxinus excelsior*, *Plantago lanceolata,* Urticaceae species] and perennial allergens (*Alternaria alternata*) and in which pollen seasons commonly overlap. Such areas are common in Mediterranean countries [3-5] and may be characterized by high rates of sensitization to minority allergens such as panallergens (profilin, polcalcin, lipid transfer proteins [LTPs]). Panallergens are a common cause of cross-reactivity between unrelated species [6-8] and can exert confounding effects if complementary diagnostic techniques such as component-resolved diagnosis are not used [9,10]. These new molecular-based techniques are more specific than routine clinical tests, such as skin prick testing (SPT) and determination of specific immunoglobulin E (sIgE) to whole extract.

The aim of this study was to identify sensitization profiles in allergic patients aged 14 years or less in a Mediterranean region by means of conventional techniques (SPT and sIgE to whole extract) and sIgE to major allergens using component-resolved diagnosis. In a second part of the study, we will study changes in sensitization profiles after 3 years of follow-up. In this article, we report on the first phase of the study.

Material and Methods

Design and Patients

An epidemiological, observational, descriptive, cross-sectional, multicenter study was conducted in patients aged 14 years or less who had clinical manifestations of respiratory allergy (rhinoconjunctivitis and/or asthma). The patients were consecutively enrolled by allergists from 5 allergy units in the south of Madrid, a plateau region with a continental climate and considerable levels of allergens (Figure). The data were collected at a single first visit. All the participants were born in the study area or had been living there for at least 5 years.

Patients who had received immunotherapy were excluded. Informed consent was obtained from all patients or their parents (for children <12 years). The study was approved by the corresponding ethics committees.

Sensitization Profile

Respiratory allergy diagnosis was confirmed according to the routine clinical practice at each allergy unit following evaluation of clinical history, allergen exposure, and in vivo or in vitro tests (SPT and/or sIgE). Sensitization profiles were determined by cutaneous tests and sIgE to the same complete panel of seasonal and perennial allergens used in all patients. Certain perennial allergens, such as house dust mites, were not included due to their extremely low prevalence in the study area.

Sensitization did not necessarily imply the presence of clinical manifestations following exposure to a particular allergen and patients were considered to be polysensitized when sensitized to 2 or more allergens. Food allergy was considered in individuals in whom allergic symptoms appeared within minutes or hours of the ingestion of a specific food on more than 1 occasion [11] and was confirmed with a specific challenge test.

SPT Extracts

Panallergen extracts for SPT, profilin, and polcalcin were prepared according to the technique previously described by the manufacturer [10]. The other SPTs were performed with commercial extracts (ALK-Abelló S.A.) at a concentration of 30 HEP/mL, except for commercial peach extract, which was adjusted to 30 μg/mL. The SPT was considered positive if the mean wheal diameter was larger than 3 mm.

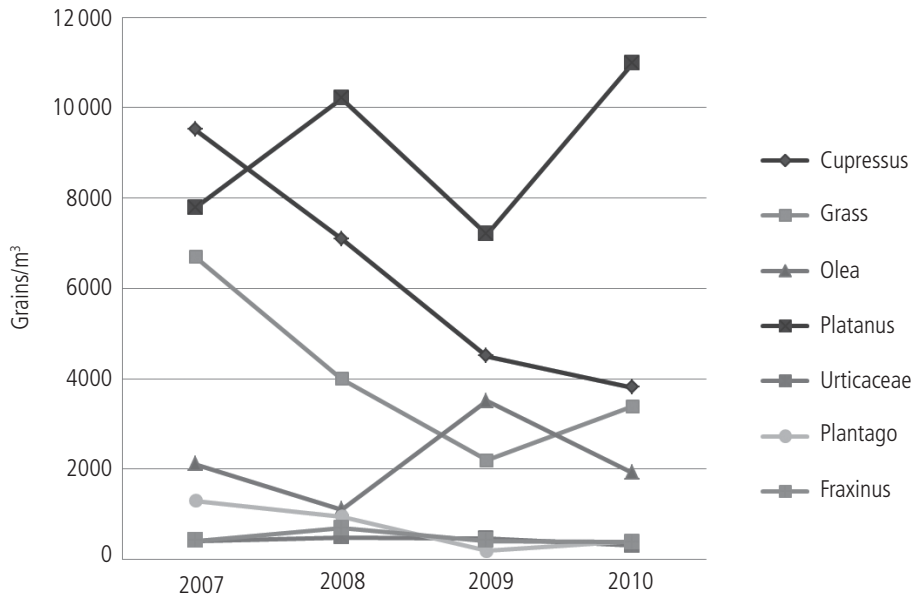

Figure. Global concentrations of pollens (total/year) in the study area between 2007 and 2010.

Purified Allergen Panels for sIgE

We used the following allergen panels to measure sIgE:

Pollens:	*Phleum pratense* nPhl p 1 [12] and nPhl p 5 [13], *A vulgaris* nArt v 1 [14], *O europaea* nOle e 1 [15], *Cupressus sempervirens* nCup s 1 [16], *Salsola kali* nSal k 1 [17], *Cynodon dactylon* nCyn d 1 [18], *P acerifolia* nPla a 1+2 [19].
Panallergens:	Profilin nPho d 2 [20], Polcalcin nPhl p 7 (Biomay AG), and nonspecific LTP from peach rPru p 3 [21,22].
Molds:	*A alternata* nAlt a 1 [23].

sIgE Determination

sIgE to the different allergens was measured with an ADVIA Centaur platform assay (Bayer HealthCare Diagnostics Division), which is based on a reverse sandwich assay. The tests were performed according to previously established methods [9,10,24]. sIgE to the major allergens was considered positive if values were 0.35 kU/L or higher.

Statistical Methods

A descriptive analysis was performed to show the main characteristics of the sample. Agreement between the various diagnostic tests was assessed using the κ index. The Wilcoxon nonparametric test was used to compare panallergen-sensitized and nonsensitized patients in terms of time to disease progression and number of allergens to which they were sensitized. In addition, the sensitization pattern was compared by age groups using the Fisher exact test. Odds ratios were calculated to determine the likelihood of having asthma according to the sensitizing allergen.

In the multivariate analysis, a Cochran-Mantel-Haenszel test was performed to evaluate the association between allergy and panallergens (profilin, polcalcin, and LTP) and each allergen was measured by IgE, adjusted by SPT.

Logistic regression was modeled with sensitization to 1, 2, or 3 panallergens (measured by IgE) for the response variable, and allergy to several allergens, age, and type of allergic disease (presence of rhinitis, conjunctivitis, asthma) as covariables.

Results

Patient Characteristics

In total, 187 patients aged between 2 and 14 years old were included. Mean (SD) age was 8.4 (2.96) years. Table 1 shows the main characteristics of the sample. Thirty-seven patients (19.7%) had food allergy; 19 of these had oral allergy syndrome (OAS) and 9 had anaphylactic reactions. Forty-seven patients (25.1%) had atopic dermatitis. Of the 187 patients, 57.8% were polysensitized and 30% of these were sensitized to both pollens and food.

Sensitization Profiles by SPT Results and sIgE to Major Allergens

Table 1. Patient Characteristics

			No.	%
Age group	2-5 y		34	18.2
	6-8 y		62	33.2
	9-11 y		58	31.0
	12-14 y		31	16.6
Sex	Male		67	35.8
	Female		120	64.2
Diagnosis	Rhinitis or rhinoconjuctivitis (RC)		166	88.8
	Asthma		144	77.0
	Rhinitis/RC and asthma		125	66.8
Diagnosis by age group	Rhinitis/RC	2-5 y	7	17.1
		6-8 y	12	29.3
		9-11 y	17	41.5
		12-14 y	5	12.2
	Asthma	2-5 y	6	31.6
		6-8 y	9	47.4
		9-11 y	4	21.1
		12-14 y	0	
	Rhinitis/RC/Asthma	2-5 y	21	16.8
		6-8 y	39	31.2
		9-11 y	37	29.6
		12-14 y	26	20.8

Table 2. Sensitization Patterns by Skin Prick Testing (SPT) and Specific Immunoglobulin E (sIgE) to Major Allergens

	SPT			sIgE		
		No.	%		No.	%
	Grass	120	64.2	Phl p 1 Phl p 5	86 36	46.0 19.3
Pollens	*Olea europaea*	107	57.2	Ole e 1	68	36.4
	Cynodon dactylon	75	40.1	Cyn d 1	33	17.7
	Platanus acerifolia	58	31.0	Pla a1+2	18	9.6
	Cupressus sempervirens	46	24.6	Cup s 1	39	20.9
	Salsola kali	39	20.9	Sal k 1	19	10.2
	Artemisia vulgaris	22	11.8	Art v 1	5	2.7
Molds	*Alternaria alternata*	43	23.0	Alt a 1	37	19.8
Panallergens	Polcalcin	31	16.6	Phl p 7	3	1.6
	Profilin	26	13.9	Pho d 2	23	12.3
Foods	Peach	21	11.2	Pru p 3	24	12.8
	Peanut	27	14.4			
	Apple	12	6.4			
	Melon	13	6.9			
Epithelia	Cat	52	27.8			
	Dog	32	17.1			

Table 2 shows the sensitization percentages obtained in SPT and sIGE tests. Grass and olive were the most common cause of sensitization, although sensitization to Phl p 1 was much higher than that to Phl p 5 (46% vs 19.3%).

According to sIgE to major allergens, sensitization to *C sempervirens* came third, with a prevalence of about 20%. The allergenic role of *C dactylon* was also notable. However, in this case, there was a remarkable difference in prevalence according to the method used (40% for SPT vs 17% for SPT). Behind these pollens came others such as *S kali* and *P acerifolia*, and there were also clear differences in prevalence according to the test type.

In the case of panallergens, 12.3% of patients were sensitized to profilin (Pho d 2). Prevalence to Pru p 3, the major peach allergen used to represent to Rosaceae fruit sensitization, was 12.8%.

Sensitization to Alt a 1 was also high, at about 20%. No differences were observed between Alt a 1-sensitized and nonsensitized patients with regard to asthma severity. The OR between asthma and positive IgE against Phl p 1 (OR, 0.47;95% CI, 0.23–0.94) indicates that individuals sensitized to this allergen are more likely to develop rhinitis or rhinoconjunctivitis than asthma. In terms of SPT results, asthma was observed only in patients with a positive SPT to grass (OR, 0.39; CI 95%, 0.18–0.88).

Table 3 shows the sensitization profiles by age group, with differences in the proportion of patients sensitized to certain major pollen allergens. There were significantly more patients sensitized to Pru p 3 in the lower age groups (*P*=.02).

Concordance Between Diagnostic Tests: The Role of Panallergens

The relative risk (RR) of having sIgE to profilin and to LTP was calculated and evaluated by the Cochran-Mantel-Haenszel test. This test was not performed for polcalcin because of the small number of patients sensitized to this panallergen. In the case of LTP, the RR was associated with sensitization to Sal k 1 (RR, 3.09;1.1-8.69) and Cup s 1 (RR,2.39; 1.18-4.84). The RR of being sensitized to profilin was associated with positive sIgE to Art v 1 (RR, 4.62; 1.77-2.09), Phl p5 (RR, 4.38; 2.04-9.39), Ole e 1 (RR, 15.43; 3.01-79.18), Pla a 1 (RR, 2.71; 1.4-5.23), Sal k 1 (RR, 3.54; 1.68-7.49), Cup s 1 (RR, 2.28, 1.12-4.64), and Cyn d 1 (RR, 2.34; 1.21-4.52).

Table 3. Sensitization Profile/Patterns by Age Group[a]

Allergen	2-5 y (n=34)	6-8 y (n=62)	9-11 y (n=58)	12-14 y (n=31)	*P* Value[b]
Allergens, No.[c]	2.24 (2.57)	2.47 (2.57)	2.53 (2.59)	3.10 (2.96)	
Alt a 1	4 (11.8)	12 (19.4)	12 (20.7)	8 (25.8)	.5418
Art v 1	1 (2.9)	2 (3.2)	2 (3.4)	0	.8692
Cup s 1	4 (11.8)	10 (16.1)	14 (24.1)	10 (32.3)	.1534
Cyn d 1	8 (23.5)	8 (12.9)	8 (13.8)	9 (29.0)	.1709
Ole e 1	11 (32.4)	24 (38.7)	22 (37.9)	10 (32.3)	.8953
Phl p 1	14 (41.2)	27 (43.5)	26 (44.8)	18 (58.1)	.5184
Phl p5	4 (11.8)	9 (14.5)	17 (29.3)	6 (19.4)	.1397
Pla a 1+2	2 (5.9)	7 (11.3)	5 (8.6)	4 (12.9)	.7858
Sal k 1	2 (5.9)	5 (8.1)	7 (12.1)	5 (16.1)	.5092
Phl p 7	0	1 (1.6)	1 (1.7)	1 (3.2)	.8822
Pho d 2	4 (11.8)	8 (12.9)	7 (12.1)	4 (12.9)	1.0000
Pru p 3	7 (20.6)	12 (19.4)	2 (3.4)	3 (9.7)	.0191

[a]Data are presented as number (%) of patients unless otherwise specified.
[b]Fisher exact test.
[c]Mean (SD) number of allergens (according to specific immunoglobulin E) to which the population studied is sensitized.

On estimating the risk of sensitization to panallergens using multivariate logistic regression analysis, we noted that patients were more likely to be sensitized to profilin if they had a food allergy (OR, 4.23; 95% CI, 1.39-12.88; *P*=.011), or if they were sensitized to grass (OR, 6.7; 95% CI, 1.3-34.67; *P*=.023) or olive (OR, 15.72; 95% CI, 3.18-77.71; *P*<.001). The odds of being sensitized to LTP was higher in patients with food allergy (OR, 38.59; 95% CI, 7.19-207.18; *P*<.001) and in those sensitized to grass (OR, 5.64; 95% CI, 1.48-21.5; *P*=.011). The corresponding risk was lower among patients aged 9 to 11 years (OR, 0.12; 95% CI, 0.02-0.87; *P*=.037).

In view of the differences in prevalence figures for sensitization to certain allergens between SPT and sIgE, the κ index for pollens was calculated for the 2 tests. Considering only allergens in which the index was higher than 0.5 in the total patient sample (grass [0.589], olive [0.537], and profilin [(0.631]), we saw how these figures decreased notably when patients sensitized to profilin were analyzed (0.194 for grasses and 0.312 for olive).

We also noted that in these children, sensitization to panallergens was associated with a higher number of sensitizations (by both SPT and sIgE) (Table 4).

Discussion

The aim of this study was to identify the allergen profile of pediatric patients in an area of high allergen exposure, especially to pollens, combining conventional and molecular diagnostic techniques. Molecular diagnostics has been shown to be a completely valid method for this type of study [9] and is a means of obtaining a more accurate profile in children. The use of more sensitive and specific diagnostic methods not only improves the identification of sensitization profiles in children but is also essential for monitoring changes in

Table 4. Relationship Between Sensitization to Panallergens (Profilin and Polcalcin) and Polysensitization

	SPT (mean ∅ >3 mm)				sIgE[a] >0.35 kU/L			
	No.	Mean[b]	SD	*P* Value	No.	Mean	SD	*P* Value
Profilin –	160	4.5	3.04	<.001	164	1.9	1.9	<.001
Profilin +	26	10.4	3.5		23	7.0	2.6	
Polcalcin –	156	4.5	3.2	<.001	*			
Polcalcin +	31	9.8	3.1					
Profilin – and Polcalcin –	141	3.9	2.6	<.001	*			
Profilin + and Polcalcin +	12	11.2	3.7					

Abbreviations: sIgE, specific immunoglobulin E; SPT, skin prick test.
[a]Data on polcalcin by sIgE are not included due to the low prevalence of this allergen measured by this technique.
[b]Mean number of allergens to which each group of patients is sensitized.

sensitization profiles over time and for implementing more effective preventive and therapeutic measures targeted at the most relevant causes of an individual's allergic disease.

As expected, the most prevalent allergens were olive and grasses. However, despite the clear dominance of these allergens in the study area, the percentage of monosensitized patients was low. As with the aforementioned allergens, the percentage of patients sensitized to other pollens, such as *C dactylon*, *P acerifolia*, *S kali*, and *A vulgaris*, dropped considerably when diagnosis was made with sIgE to the major allergen rather than SPT. However, regardless of the method used, there was a higher percentage of polysensitized than monosensitized patients. This clearly has significant implications in terms of the implementation of preventive and therapeutic measures.

Panallergens, and profilin in particular, are particularly prevalent in the study area [35] and have a close relationship with sensitization to grasses [9]. Especially remarkable is the high rate of sensitization to profilin from the early years of life. It is essential to include panallergens as a routine part of the diagnostic process because of their potential as a diagnostic confounder. This confounding effect might explain the difference in percentages obtained using SPT and sIgE. In our study, 12% of patients had positive IgE to Pho d 2, despite the fact that they only had a short disease history and that panallergens are minority allergens. We also noted that patients sensitized to Pho d 2 had a much higher percentage of sensitization to other allergens than non-profilin-sensitized patients. Although this has already been demonstrated [9,10], as mentioned earlier, it is noteworthy that this parameter is as significant in patients with such a short disease history as it is in adults. When the RR of being sensitized to profilin was assessed, it was found to be associated with a significant number of pollens. Furthermore, the differences between profilin-sensitized and nonsensitized patients were also demonstrated when we compared agreement between SPT and sIgE using the κ index. Agreement was much higher in nonsensitized than in sensitized patients. This emphasizes the importance of including panallergens (profilin in our population) in routine diagnostic tests in order to correctly interpret results.

In Mediterranean countries, food allergy is mainly associated with Rosaceae fruits. LTP is the main trigger and is also associated with greater disease severity [25-29]. In our study, quite a substantial proportion of patients (almost 13%) were sensitized to Pru p 3, and 62.5% of this group (15/24 patients) had food allergy. It should be noted that 24% of patients with food allergy had an anaphylactic type–reaction. Of the 27 patients sensitized to peanuts (14.4%), 15 (55.6%) had food allergy. As occurred with profilin, the rate of sensitization to Pru p 3 in young children was especially remarkable.

We observed a low prevalence of sensitization to perennial allergens, except in the case of *A alternata*, for which it was about 20%. Studies have shown *A alternata* to be a significant allergen. In 1991, O'Hollaren et al [30] analyzed 11 patients aged 11 to 25 years and found that exposure to this allergen was a risk factor for respiratory arrest in asthma patients. Another study published in 1998 [31] noted the importance of *A alternata* as an independent risk factor for the development of asthma among schoolchildren in different locations in the United States. Later, in a study based on a sample of 1235 preschool children aged 5 to 6 years, dampness and molds such as *A alternata* were found to be associated with a higher rate of sensitization [32]. Sensitization to this allergen has also been described as a risk factor for intensive care admission due to asthma exacerbations [33], and also associated with increased airway hyperresponsiveness, wheezing, and bronchodilator use in children [34]. In our study we did not find a significant association between sensitization and asthma severity (data not shown), although it would be very interesting to monitor changes over time in this subgroup and determine whether sensitization patterns might be influenced by other factors such as age, sex, or place of residence.

In summary, we have used both component-resolved and in vivo tests to analyze the sensitization profiles of a very large series of pediatric patients with respiratory allergy in a geographical area with high exposure levels to a large number of allergens. We observed a surprisingly high rate of polysensitization, as well as a high prevalence of sensitization to panallergens, although the clinical significance was unclear in some cases. Finally, component-resolved diagnostics proved to be a useful tool for accurate diagnosis in these patients.

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Conflict of Interests

Lucía Jimeno and Fernando de la Torre are employees of ALK. Angélica Feliu, David González-de-Olano, Emma González, Beatriz Rodríguez, and Javier Ruiz-Hornillos declare no conflict of interests.

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