ORIGINAL ARTICLE

Contribution of Molecular Diagnosis of Allergy to the Management of Pediatric Patients With Allergy to Pollen

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Abstract

Background: Component-resolved diagnosis based on recombinant allergens facilitates treatment of multiple sensitization and/or cross-reactivity in allergic patients.
Objective: To assess the usefulness of molecular diagnosis in childhood allergies.
Methods: A total of 162 children aged 4-16 years diagnosed with allergic rhinitis or asthma/rhinitis caused by pollen were referred for recombinant allergen–based diagnosis in 2006. Specific immunoglobulin (Ig) E against pollen allergens and purified recombinant *Phleum pratense* pollen allergens were measured using an in vitro quantitative assay, and considering the recombinant allergens Phl p 1+Phl p 5 as *P pratense*–specific allergens and Phl p 7+Phl p 12 as cross-reacting allergens. Conditional probability was calculated to determine the relationship between values for specific IgE against major allergens and those for cross-reacting allergens.
Results: Specific IgE antibodies against *P pratense* were detected in 99.4% of serum samples, and cross-reacting allergens in 46%. Multiple sensitization to pollen was documented in 38% of patients, with *Plantago lanceolata* as the main cause. Conditional probability calculations showed that patients with specific IgE values of 75-80 kU_A/L to Phl p 1+Phl p 5 were 75% (95% confidence interval) more likely to present values ≥2 kU_A/L to Phl p 7+Phl p 12.
Conclusions: Our results show that recombinant DNA technology can help diagnose allergy in cases of multiple sensitization and cross-reactivity, and is therefore a promising option for improving prognosis and management of allergic pediatric populations.

Key words: Allergens/immunology. Immunologic tests/methods. Recombinant proteins/immunology. Immunoglobulin E/analysis. Cross-reactions.

Resumen

Antecedentes: El diagnóstico por componentes utilizando alérgenos recombinantes contribuye a resolver problemas de diagnóstico derivados de la polisensibilización y/o de la reactividad cruzada en pacientes alérgicos.
Objetivo: Evaluar la utilidad del diagnóstico por componentes en una población de niños alérgicos.
Métodos: Se analizaron 162 sueros pertenecientes a niños diagnosticados de rinitis o asma/rinitis durante el año 2006. Mediante ImmunoCap 250 se cuantificó la IgE específica a pólenes y a los alérgenos recombinantes de *Phleum pratense*, considerando a Phl p 1+Phl p 5 alérgenos específicos de grupo y Phl p 7+Phl p 12 alérgenos de reactividad cruzada. Mediante cálculos de probabilidad condicionada se determinó la relación existente entre los valores de IgE específica obtenidos frente a Phl p 1+Phl p 5 y los obtenidos frente a Phl p 7+Phl p 12.
Resultados: El 99,4% de las muestras fueron positivas frente a los alérgenos específicos de *Phleum*, mientras que el 46% fueron positivas para los alérgenos de reactividad cruzada. El 38% de los pacientes estaban polisensibilizados a pólenes, siendo el polen de *Plantago lanceolata* el principal responsable de la polisensibilización. Los cálculos de probabilidad condicionada demostraron que pacientes con valores de IgE específica de 75-80 kU/L frente a Phl p 1+Phl p 5 tenían una probabilidad mayor del 75% de presentar valores ≥2 kU/L frente a Phl p 7+Phl p 12.
Conclusiones: La utilización de alérgenos recombinantes permite definir el diagnóstico etiológico de la alergia en casos de polisensibilización y reactividad cruzada, siendo por lo tanto una prometedora herramienta de ayuda en el pronóstico y el seguimiento de la población pediátrica alérgica.

Palabras clave: Alérgenos/inmunología. Análisis/métodos inmunológicos. Proteínas recombinantes/inmunología. Inmunoglobulina E/análisis. Reactividad cruzada.

Introduction

Type I allergy is a major health problem affecting more than 25% of the population in industrialized countries [1], and specific immunotherapy is one of the few therapeutic approaches available. It is generally administered using crude allergen extracts, which consist of a mixture of allergenic and nonallergenic components. These components, however, are difficult to standardize and cannot be tailored to an individual reactivity profile. The rapid development of molecular biological techniques in the late 1980s made it possible to clone the first allergen [2], and the subsequent advent of recombinant allergens has provided us with a large panel of allergenic molecules [3-5].

Recombinant allergen–based testing in clinical practice has enabled the systematic study of the principal allergens and cross-reactivity processes involved in allergic reactions [6,7]. Thus, we can now classify tree pollen and related allergies using major allergen molecules instead of botanical relationships [8]. The application of these recombinant allergens in in vitro tests has led to new forms of component-resolved diagnosis that reveal the antibody reactivity profile of allergic patients and identify the disease-eliciting allergen molecules. Accordingly, progress in this field will make it possible to establish individual patient reactivity profiles and tailor immunotherapy [9].

The aim of our study was to assess the usefulness of molecular diagnosis in the decision-making process for the clinical management and prognosis of allergic pediatric patients and also to discriminate between cross-reactivity and multiple sensitization.

Methods

Study Population

Serum samples for molecular diagnosis were obtained from 162 children (59.9% boys/40.1% girls) aged 4-16 years diagnosed with allergic rhinitis or asthma/rhinitis caused by pollen on the basis of their clinical history and skin prick test results. These patients were attended in the outpatient clinic of the Pediatrics Department of Complejo Hospitalario de León, León, Spain during 2006 and were referred for an extensive work-up. We used a standard panel of allergens including house dust mite (*Dermatophagoides pteronyssinus, Dermatophagoides farinae*), pollen (grasses, *Artemisia vulgaris, Ambrosia elatior, Salsola kali, Plantago lanceolata, Olea europaea, Cupressus arizonica*, and *Platanus acerifolia*), moulds (*Alternaria alternata, Aspergillus fumigatus, Cladosporium herbarum* and *Penicillium chrysogenum*), epithelia (dog and cat), milk fractions, and egg fractions. All patients were sensitized to grass pollens and revealed seasonal symptoms. Only 16% of patients were sensitized to multiple allergens from pollens and other nonplant allergens (mites and/or epithelia and/or moulds). A total of 81% of patients sensitized to grass pollen also had positive skin prick test results to other pollen species, and they all had higher specific immunoglobulin (Ig) E values for grass pollen than those revealed by other pollen species.

Symptoms for all the study patients were compatible with grass pollen allergy, although we could not rule out subclinical sensitization to other pollen species.

The study was performed in accordance with the Declaration of Helsinki (1964) and informed consent was obtained from all the patients.

Procedure

We measured specific IgE against pollen allergens from different species according to requests from the patients' pediatricians. The specific IgE assay was carried out using ImmunoCAP 250 Specific IgE (Phadia AB, Uppsala, Sweden). We used complete allergens from different grasses (*Cynodon dactylon, Dactylis glomerata, Lolium perenne, Phleum pratense* and *Poa pratensis*), weeds (*Artemisia absinthium, A elatior, Chenopodium album, P lanceolata*, and *S kali*), and trees (*O europaea*), as well as recombinant allergens of *P pratense* (rPhl p 1, expansin family; Phl p 5, ribonuclease family; Phl p 7, polcalcin family; and Phl p 12, profilin family). Recombinant allergens were grouped as Phl p 1+Phl p 5 (*P pratense*–specific allergens) and Phl p 7+ Phl p 12 (cross-reacting allergens of pollens and/or vegetables).

Statistical Analysis

Data are expressed as the mean and 95% confidence interval (CI). Means were compared using an unpaired *t* test. *P* values of <.05 were considered statistically significant. The conditional probability was calculated to determine the relationship between specific IgE values against major allergens and against cross-reacting allergens. The results are presented as a 3D-scatter plot using the NCSS Statistical Analysis and Graphics program for Microsoft Windows (NCSS, PASS, and GESS; NCSS, Kaysville, Utah, USA).

Results

Grass species revealed higher specific IgE values than other pollen or nonplant species. The mean values obtained were 30.5 kU_A/L for *C dactylon*, 70.0 kU_A/L for *D glomerata*, 77.6 kU_A/L for *L perenne*, 67.3 kU_A/L for *P pratense*, and 71.6 kU_A/L for *P pratensis*. The differences demonstrated were not statistically significant except for *C dactylon* (*P*<.0001, with a 95% CI).

Specific IgE antibodies (>0.1 kU_A/L) against major allergens from *P pratense* were detected in 161 (99.4%) serum samples, whereas 75 (46%) proved positive for cross-reacting allergens.

Figure 1 shows the results for specific IgE against the complete allergen extract of *P pratense* and its individualized allergens (Phl p 1+Phl p 5, major allergens; Phl p 7+Phl p 12, cross-reacting allergens). Statistically significant differences between *P pratense* and its major allergens were not found, although there were significant differences between cross-reacting allergens and both the complete allergenic extract and the individualized major allergens of *P pratense*. The sum of the mean values of the major allergens and cross-reacting allergens of *P pratense* accounted for 97% of the mean value

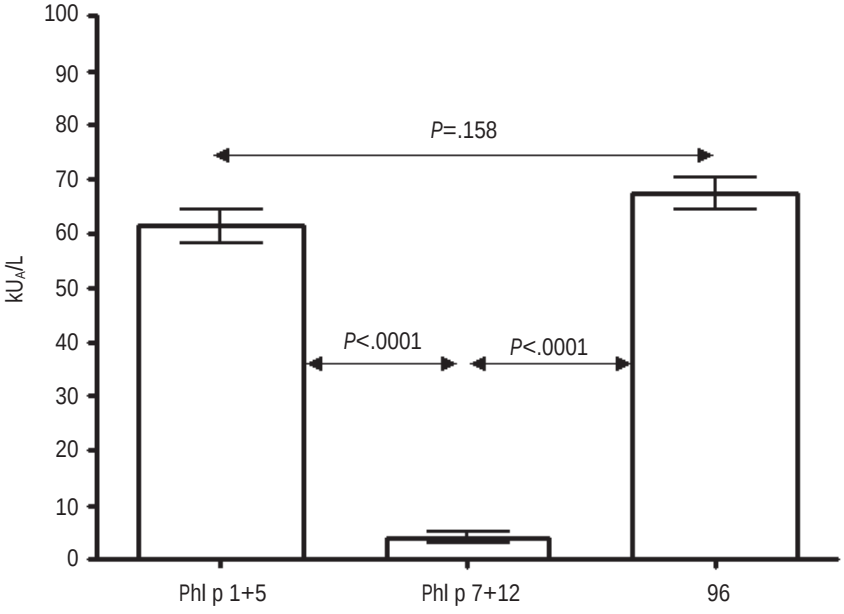

Figure 1. Specific immunoglobulin E to *Phleum pratense* allergenic extract and its individualized allergens (Phl p 1+Phl p 5, major allergens; Phl p 7+Phl p 12, cross-reacting allergens).

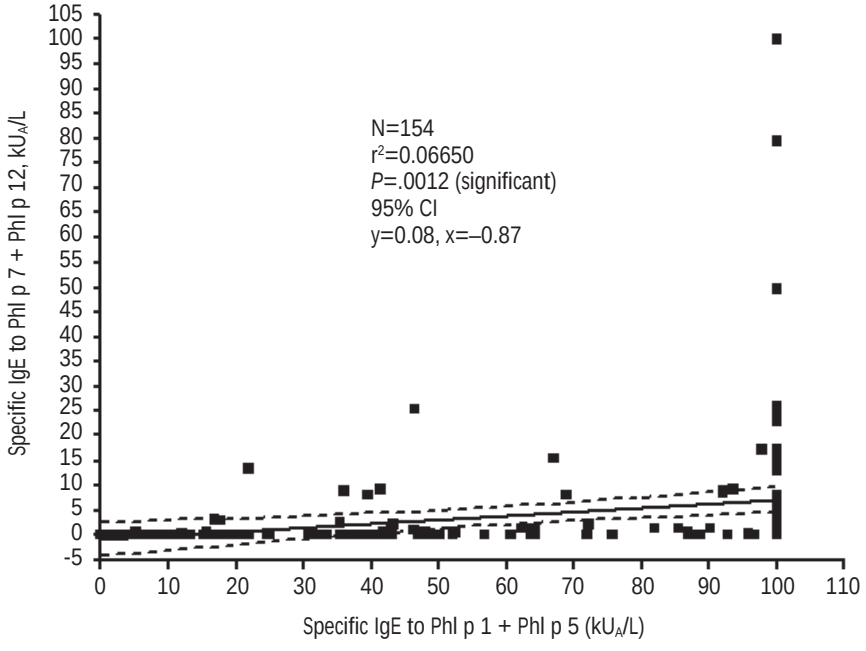

Figure 2. Linear regression between quantitative values of specific immunoglobulin (Ig) E to Phl p 1+Phl p 5 (specific grass allergens) and specific IgE to Phl p 7+Phl p 12 (cross-reacting allergens).

obtained for the complete allergen of the same species.

Positive IgE results to cross-reactive allergens and/or nongrass pollen species were observed in 133 patients, and 29 patients (18%) showed positive specific IgE values for Phl p 1+Phl p 5 only.

Of the 162 sera studied, 62 (38%) showed positive specific IgE values to grass pollens and other pollen species; 58 (36%) revealed positive specific IgE values to Phl p 1 + Phl p 5 and other nongrass pollen species, and exhibited negative specific IgE values to Phl p 7+ Phl p 12.

Fifty-four patients (33%) had lower specific IgE values against cross-reacting allergens (Phl p 7+Phl p 12) than those generated by pollen from other nongrass species (Table 1). The nongrass species that yielded the highest values was *P lanceolata*, whereas the lowest values corresponded to *A absinthium.* Both revealed statistically significant differences ($P<.05$) when compared with cross-reacting allergens (Phl p 7+Phl p 12). *A vulgaris*, *C album*, and *O europaea* showed no significant differences when compared with the same cross-reacting allergens mentioned above (Phl p 7+Phl p 12) (Table 2).

Figure 2 shows how the correlation between quantitative values of specific IgE to Phl p 1+Phl p 5 and specific IgE to Phl p 7+Phl p 12 was statistically significant ($P=.05$; Pearson r=0.258; $r^2=0.0665$; $P=.0012$ [2-tailed]; 95% CI, 154 XY pairs.

When the values of specific IgE against the major allergens were compared with the values of the cross-reacting allergens according to patient subgroups defined by different cutoff values, it was observed that, for negative values of IgE to cross-reacting allergens, the mean (SD) value of IgE to the major allergens corresponding to the same sera was 48.3 (38.7) kU$_A$/L. This group showed significant differences with the other subgroups, defined as those patients with IgE to cross-reacting allergens >1 kU$_A$/L. The different subgroups with IgE to cross-reacting allergens >1 kU$_A$/L showed no significant differences (Figure 3).

Calculation of conditional probabilities between the values corresponding to anti-Phl p 1+Phl p 5 IgE and the values of anti-Phl p 7+Phl p 12 IgE showed the presence of specific IgE to Phl p 1+Phl p 5 (75-80 kU$_A$/L) that can be associated with another anti-Phl p 7+Phl p 12 IgE value (>2 kU$_A$/L), with a probability greater than 75% (95% CI) (Figure 4).

Table 1. Specific IgE to Phl p 1+Phl p 5, Phl p 7+ Plh p 12 and Nongrass Pollen in Patients Exhibiting Positive Values to More Than 1 Allergenic Source[a]

Serum	rPhl p 1+5	rPhl p 7+12	*Artemisia*	*Ambrosia*	*Plantago*	*Chenopodium*	*Salsola*	*Olea*
1	1	*17*	**26**	0	12	0	**29**	2
2	90	*1*	0	1	**77**	0	0	0
3	100	*8*	3	0	**14**	6	0	3
4	100	*2*	0	0	**60**	**45**	0	**59**
5	100	*0*	**4**	0	**13**	**36**	0	**11**
6	75	*0*	0	0	**23**	**1**	0	0
7	17	*0*	0	0	**61**	**1**	0	0
8	25	*0*	1	1	**34**	0	0	**1**
9	7	*0*	0	0	**8**	0	0	0
10	100	*2*	1	1	**3**	1	2	**6**
11	33	*1*	1	1	**7**	0	1	1
12	100	*3*	2	1	**11**	6	3	**10**
13	100	*4*	**20**	1	2	2	1	1
14	61	*0*	**1**	**1**	**12**	**7**	**1**	**1**
15	17	*3*	2	0	**58**	**8**	0	**6**
16	43	*2*	**6**	0	1	0	0	1
17	100	*0*	**1**	1	**5**	**2**	0	**1**
18	85	*1*	**23**	1	**60**	**12**	0	**5**
19	52	*1*	**7**	1	**38**	1	0	1
20	50	*0*	0	0	**4**	0	0	0
21	41	*9*	1	0	**11**	6	0	**11**
22	3	*0*	0	0	**18**	0	0	0
23	100	*15*	2	0	**18**	8	0	9
24	100	*0*	0	0	**15**	0	0	0
25	46	*25*	0	0	**31**	0	0	0
26	87	*1*	**16**	0	1	0	0	**2**
27	36	*8*	0	0	**25**	0	0	0
28	8	*0*	0	0	**4**	0	0	0
29	100	*0*	**48**	0	**1**	0	0	0
30	43	*0*	0	0	**42**	0	0	0
31	10	*0*	1	0	**52**	0	0	0
32	5	*0*	0	0	**10**	**15**	0	0
33	63	*1*	0	0	1	1	0	0
34	100	*0*	0	0	3	0	0	0
35	100	*17*	0	0	0	11	0	0
36	100	*0*	0	0	1	**1**	0	0
38	100	*0*	0	0	4	0	0	0
39	40	*0*	0	0	0	**1**	0	0
40	100	*0*	0	0	**1**	**1**	0	0
41	62	*0*	0	0	**2**	0	0	0
42	100	*0*	0	0	**15**	0	0	**1**
43	87	*0*	0	0	**33**	0	0	0
44	97	*2*	0	0	**17**	**1**	0	0
45	100	*0*	0	0	**1**	0	0	0
46	100	*3*	0	0	**12**	6	0	0
47	100	*0*	0	0	**2**	0	0	0
48	19	*0*	0	0	**2**	8	0	0
50	0	*0*	0	0	**18**	**8**	0	0
51	36	*0*	0	0	12	13	0	0
52	82	*0*	0	0	**24**	0	0	0
53	100	*1*	0	0	**15**	0	0	0
54	25	*7*	0	0	2	0	0	0
55	64	*25*	0	0	1	0	0	0
56	22	*1*	0	0	1	**2**	0	0
57	100	*0*	0	0	**2**	0	0	0
58	100	*0*	0	0	**4**	0	0	0
59	45	*9*	0	0	5	0	0	0
60	35	*0*	0	0	**1**	0	0	0
61	69	*0*	0	0	0	6	0	0
62	25	*0*	0	0	**1**	0	0	0

Abreviations: Values for immunoglobulin E to nongrass species that proved higher than the corresponding values against cross-reacting allergens are shown in bold. Values for immunoglobulin E to recombinant cross-reacting allergens are shown in italics.

Table 2. Comparison of the Means Generated by the Pollen Allergens in Sera Giving Rise to Anti–Cross-reacting Allergen Immunoglobulin E Values (Phl p 7+Phl p 12: profilins plus polcalcins) Lower Than Those Recorded for 1 or More of the Other Tested Nongrass Species[a]

	7+12	*Artemisia*	*Ambrosia*	*Plantago*	*Chenopodium*	*Salsola*	*Olea*
1+5[b]	<.001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
7+12[c]		.3489	.0092	<.0001	.3714	.1807	.6604
Artemisia			.0116		.9599	.0645	.6885
Ambrosia					.0116	.2922	.0491
Plantago					<.0001	<.0001	<.0001
Chenopodium						.0679	.7217
Salsola							.1807
Olea							

[a] The results were analyzed using the *t* test, with a 95% confidence interval. Statistical significance was set at *P*<.05.
[b] 1+5: Phl p 1+Phl p 5 (major allergens).
[c] 7+12: Phl p 7+Phl p 12 (cross-reacting allergens).

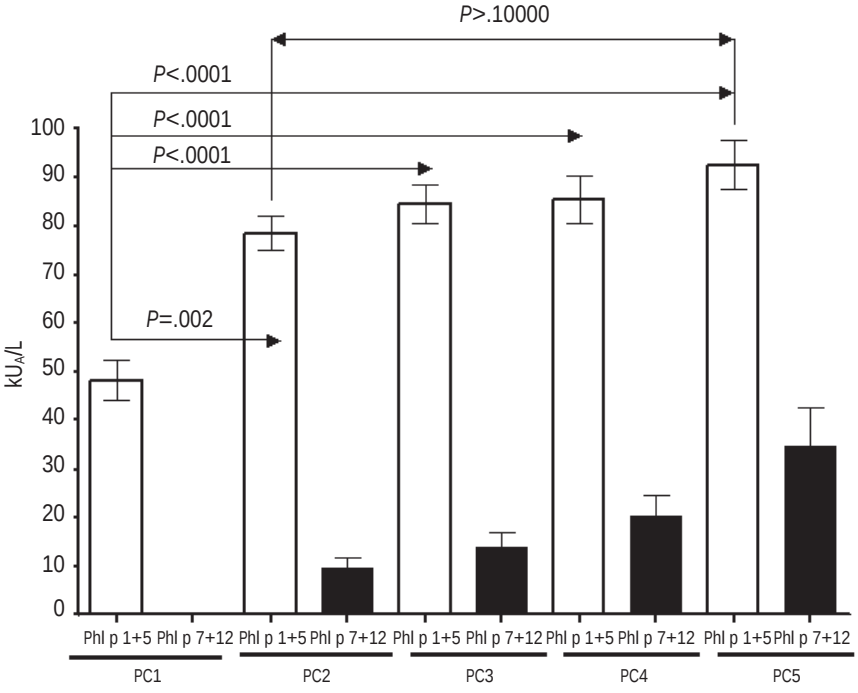

Figure 3. Specific immunoglobulin E values to major *Phleum pratense* allergens (Phl p 1+Phl p 5) in different subgroups of patients according to cross-reacting allergen IgE reference values (PC1, <0.1 kU_A/L; PC2, >1 kU_A/L; PC3, >2 kU_A/L; PC4, >7 kU_A/L; PC5, >15 kU_A/L).

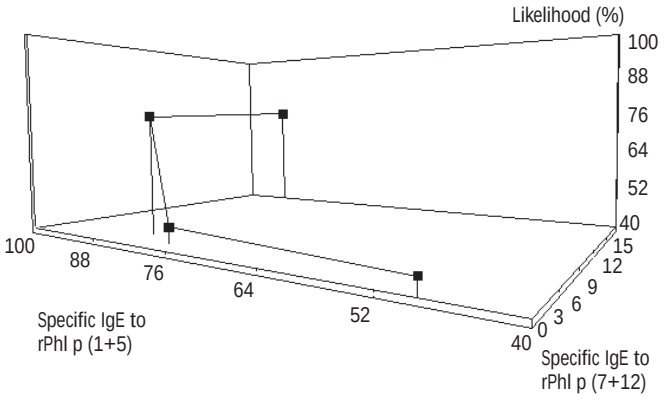

Figure 4. Percentage probability of the specific immunoglobulin (Ig) E values (kU_A/L) to cross-reacting allergens according to the specific IgE values (kU_A/L) targeting the corresponding major allergens.

Discussion

Until less than a decade ago, assessment of specific IgE levels against extracts of different allergens was the cornerstone of laboratory diagnosis of allergy and the only option for confirming IgE-mediated hypersensitivity. However, the shortcomings of specific IgE antibody levels in distinguishing between multiple sensitization, cosensitization, and cross-reactivity, which are key mechanisms of atopy, have been extensively recognized. The development of recombinant allergens and their routine application in the diagnosis of allergic diseases is a crucial advance in diagnostic accuracy and in the optimization of therapy, particularly immunotherapy [7]. This enables us to progress from mere taxonomy-based diagnostic approximations to the molecular classification of allergens [8], and thus to well-founded clinical decisions and tailored therapy [6].

Airborne grass pollen belonging to the Cupressaceae and Poaceae families and house dust mite are major sources of hypersensitivity reactions in the area where this study was performed [10-13]. According to the International Study of Asthma and Allergies in Childhood (ISAAC) [14], the prevalence of asthma symptoms was 7.1%-12.4% for 6–7-year-olds and 7.1%-13.7% for 13–14-year-olds, and the prevalence of allergic rhinoconjunctivitis was 6.1%-11.1% and 10.5%-18.7% for 6–7-year-olds and 13–14-year-olds, respectively. In addition, the prevalence of allergic diseases may increase in Europe in the coming years as more people become sensitized to allergens. These data reinforce the huge importance of grass pollen–induced hypersensitivity phenomena in our setting, and indicate that a correct diagnosis may help improve the quality of life of patients with allergic disease. Although an extensive panel of recombinant grass allergens and recommendations for its application in clinical diagnosis have been available for some years [15], there is little information on

the use of individualized pollen allergens in our environment [16]. In fact, the few studies that have been published refer to the relationship between pollen allergy and fruit allergy and are limited to populations from the southern part of Spain and the Mediterranean basin [17,18].

The present study has shown that the grass species tested generated very similar specific IgE values, with the exception of *C dactylon*, for which the values were significantly lower (around 50%). This finding, however, is not unexpected, because *C dactylon* belongs to a subfamily (family Poaceae, subfamily Chlorodoideae), that is different from the other grass species analyzed (family Poaceae, subfamily Pooideae) and group 5 major allergen appears to be exclusive to the subfamily Pooideae.

A total of 99.4% of the study patients classified as sensitized to grass pollen yielded positive values (>0.1 kU_A/L) for the recombinant major allergens (Phl p 1+Phl p 5), while 46% proved positive for the cross-reacting allergens (Phl p 7+Phl p 12).

The patient with negative results for the recombinant major allergens of grass pollen revealed positive values for *Plantago* and *Chenopodium* pollens and cross-reacting allergens. These results could justify the labeling of grass pollen allergy in this patient, because the cross-reacting allergens can produce a false-positive value to grasses and the overlap between Plantago and Pooideae pollinization does not allow us to make a differential clinical diagnosis.

Taking into account that 29 patients were only positive to Phl p 1+Phl p 5 and 71 patients were positive to Phl p 1+Phl p 5 and Phl p 7+Phl p 12, we can consider 100 patients as monosensitized to grasses, even though the 71 patients with positive results to cross-reactive allergens can give positive results to other nongrass pollen species by skin prick test or specific IgE to crude extracts.

Only 58 patients (36%) had specific positive IgE values to nongrass pollen species with no specific IgE values to cross-reactive allergens. Thus, these 58 patients should be considered sensitized to several allergens without intervention of cross-reactivity. In this study, the real figure for multiple sensitization is 36%.

The results demonstrated that most of the patients in the multiple sensitization group were sensitized to grass and *Plantago* allergens (41 out of 62 patients).

As mentioned above, symptoms due to *Plantago* allergens may be disguised by the pressure of grass allergens and the overlap between *Plantago* and Pooideae pollinization.

Establishing a definitive diagnosis in patients sensitized to one or more allergens has important implications not only for patient management, but also when defining the composition of hyposensitizing extracts [7,19-21]. Furthermore, the presence of specific IgE against cross-reacting allergens, in particular profilins, means that pollen-sensitized patients should be monitored to rule out cross-reactivity phenomena that could be indicative of sensitization to pollen-fruit or pollen-vegetables, and which, in some cases, may originate oral allergy syndrome [17,18,22-24]. In such cases, including sensitization to food allergens of vegetable origin in molecular allergen characterization could be highly relevant for diagnosis and prognosis.

The presence of Cupressaceae pollen in the air of the region of Castile-León leads us to assume that this group may also cause multiple sensitization, and therefore should be included in diagnostic approaches. However, the difference in pollen seasons for Cupressaceae and Poaceae enables a differential clinical diagnosis to be established.

As expected, sensitization to major allergens is correlated with sensitization to cross-reacting allergens. The specific IgE values for the cross-reacting allergens are significantly lower than those of the major allergens, which could be regarded as the cause of primary sensitization to grasses. When the relationship between sensitization to major allergens and to cross-reacting allergens was analyzed on the basis of specific IgE levels, anti-major allergen IgE concentrations proved to be associated with probability values indicating different levels of sensitization by cross-reacting allergens. These data suggest that certain specific IgE concentrations to primary sensitizing allergens could trigger the specific IgE response to cross-reactive allergens. They also reinforce the concept of specific IgE quantification [25] extended to molecular diagnosis, which allows us to objectively establish associations between groups of allergens for diagnostic purposes.

In summary, our results show that component-resolved diagnosis based on recombinant allergens may help to refine allergy diagnosis in cases of multiple sensitization and cross-reactivity. Therefore, it holds great promise for improving the prognosis and management of allergic pediatric populations.

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