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Olive, grass or both? Molecular diagnosis for the allergen immunotherapy selection in polysensitized pollinic patients

C. Moreno[1], J. L. Justicia[2], J. Quiralte[3], Á. Moreno-Ancillo[4], A. Iglesias-Cadarso[5], M. Torrecillas[6], N. Labarta[7], M. A. García[2] & I. Dávila[8]

[1]Hospital Universitario Reina Sofía, Cordoba; [2]Stallergenes Ibérica, Barcelona; [3]Hospital Virgen del Rocío, Sevilla; [4]Hospital Nuestra Señora del Prado, Talavera de la Reina; [5]Hospital Puerta de Hierro, Majadahonda; [6]Complejo Universitario Hospitalario de Albacete, Albacete; [7]Policlínica Alto Aragón, Hospital San Jorge, Huesca; [8]Instituto Biosanitario de Salamanca (IBSAL), Hospital Universitario de Salamanca, Salamanca, Spain

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Correspondence
Mario A. García, Stallergenes Ibérica S.A., Paseo de Yeserias, 33, CP 28005 Madrid, Spain.
Tel.: +34 91 354 50 63
Fax: +34 91 354 60 61
E-mail: mgarciag@stallergenes.com

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Abstract

Background: Grass and olive are the most frequently pollens that induce seasonal allergic rhinitis in Spain. Cross-reactivity due to panallergens shared by them and overlapping pollination complicates the recognition of allergy-causing agents, making it difficult to identify the most appropriate allergen immunotherapy (AIT) to use. The aim of this study was to determine the sensitization pattern to major grass and olive pollen allergens using component-resolved diagnostics in patients with seasonal allergic rhinitis (SAR) and positive skin prick test to grass and olive pollens and evaluate how knowledge of the sensitization patterns might influence AIT prescription.

Methods: After informed written consent, a total of 1263 patients were recruited. A serum determination of specific IgE levels to Ole e 1 and Phl p 1 + 5 was performed to all patients. A comparison was made before and after obtaining the specific IgE results, and differences in diagnosis were stated.

Results: At the 0.35 kU/l cut-off point, 71.2% of patients were positive to Ole e 1 and Phl p 1 + 5, 14% were positive only to Phl p 1 + 5 and 12% were positive only to Ole e 1. Based on available clinical data and skin prick test results, 922 (73%) patients would have been indicated for a mixture of grass and olive pollens for AIT. In 56.8% of patients, there was non-coincidence in the composition of AIT that would be selected before and after investigators received the *in vitro* data.

Conclusion: The diagnostic accuracy of the recombinant allergen-specific IgE test could help to improve the selection of specific-allergen immunotherapy in polysensitized patients.

The prevalence of allergic rhinitis (AR) in the general Spanish population ranges from 8.2% in children (1) to 21.5% in adults (2). AR has conventionally been classified as perennial allergic rhinitis (PAR), mainly caused by exposure to house dust mites, moulds or animal dander, or seasonal allergic rhinitis (SAR), related to pollen exposure. In Spain, SAR is the most common reason for allergy consultations in primary care (3), being grass and olive pollens the most frequently involved (3–5). Nowadays, the AR classification according to ARIA guideline is widely used and it classifies AR in terms of frequency (intermittent or persistent) and severity (mild or moderate/severe) (6).

Allergic rhinitis management includes patient education, environmental control, drug therapy and allergen immunotherapy (AIT). The latter reduces symptoms and medication use (7). Moreover, AIT in patients with AR has a long-lasting therapeutic effect and may alter the natural course of allergic diseases that can lead to the development of asthma (6).

Before starting AIT, an aetiological diagnosis of AR is required. Usually, the first diagnostic approach is based on

the patient medical history and skin prick test (SPT) (8–10). The detection of serum-specific IgE (sIgE) is a complementary tool used for clinical diagnosis (11). Diagnostic tests based on allergen extracts use mixtures of various allergens, some of which are specific for the allergen source, while others contain cross-reactive allergens of various unrelated allergen sources. It can be difficult to identify the disease-causing allergen by performing such tests, particularly in patients sensitized to more than one allergen source (12).

Considered a step forward in allergy diagnosis, the development of component-resolved diagnostics (CRD) has made possible to identify potential disease-eliciting molecules, to which patients are sensitized, including primary or species-specific allergens and markers of cross-reactivity or panallergens, enabling specialists to provide patients with the right treatment. The discrepancy between the indication and use of allergens for AIT before and after performing CRD underlines its utility (12, 13). *In vitro* test results should always be evaluated in the context of the medical history, as allergen sensitization does not necessarily imply a clinical reaction.

In southern Europe, there are large numbers of patients with SAR and positive SPT to both grass and olive pollens (4, 10). The percentage of patients with positive SPT to grass pollen is higher than the percentage of patients with IgE against major molecular components of *Phleum* (14). Cross-reactivity phenomenon due to panallergens shared by these pollens may explain this inconsistency (8, 15, 16). In addition, the overlapping olive tree and grass pollination seasons in Spain complicates the recognition of true allergy-causing agents and a correct aetiological diagnosis.

In view of these diagnostic difficulties, the determination of sIgE, using recombinant or purified proteins, enables the detection of *in vitro* sensitization to major allergens and possible cross-reactivity by minor allergens (17, 18). Accurate detection of major allergens from olive and grass pollens as aetiological agents of SAR would facilitate the appropriate choice of AIT composition (12, 14).

Genuine grass pollen sensitization can be diagnosed with a combination of the major grass pollen allergens Phl p 1 and Phl p 5 (19), whereas for olive tree pollen, Ole e 1 is considered the most common sensitizing allergen (14, 19–23).

The aim of this study was to determine the sensitization pattern to major grass and olive pollen allergens (Phl p 1 + 5 and Ole e 1, respectively) using CRD in patients with SAR and positive SPT to grass and olive pollens. Additionally, we evaluated how the knowledge of the sensitization patterns might influence allergen immunotherapy prescribing.

Methods

Study design and selection of patients

This was a multicentre, prospective, epidemiological, observational, cross-sectional study conducted under routine clinical practice conditions over a period of 6 months (June 2009–November 2009) in 87 allergy centres around Spain. Spanish provinces with a minimum of 1000 Ha dedicated to olive grove cultivation were selected (32 provinces) and then stratified into five areas according the olive grove cultivation surface (24) (Fig. 1).

Patients aged 5–65 years with diagnosis of SAR clinically related to grass and olive pollinosis by an allergy specialist that had positive SPT to both pollens were recruited. Patients also had to have both a history of SAR and continuous residence in the geographical area of study for at least the previous 2 years. Patients were excluded from the study if there was potential interference with SPT interpretation (i.e. affecting wheal) or previous administration of AIT.

The SAR diagnosis was established by consistent AR symptoms and confirmed by a positive SPT to both olive and grass pollen. As per the study protocol, skin prick tests were performed only once. The results were recorded after 15–20 min, measuring the mean diameter of the wheal generated. SPTs were performed with a panel of the following extracts: *Alternaria alternata, Artemisia vulgaris, Canis familiaris, Dermatophagoides pteronyssinus, Felis domesticus, Juniperus ashei, Olea europaea, Parietaria officinalis, Phleum pratense, Plantago lanceolata and Salsola kali* (Stallergenes, Antony, France). Histamine (10 mg/ml) and glycerol-saline solution (Stallergenes, Antony, France) were used as controls. An SPT was considered positive if the wheal size was at least 3 mm larger than the wheal of the negative control. Investigators also collected patient sociodemographic data and clinical variables.

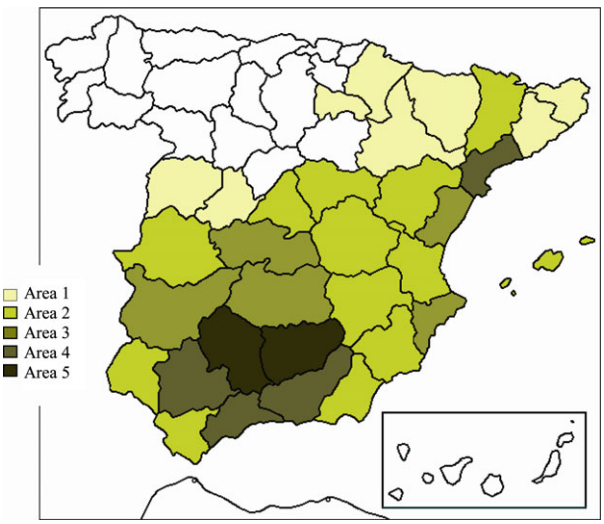

Figure 1 Spanish geographical areas according to province surface area dedicated to olive growth cultivation. Area 1: provinces with <1% of surface area dedicated to olive grove cultivation, with a minimum of 1000 Ha. Area 2: provinces with 1–5% of surface area dedicated to olive grove cultivation. Area 3: provinces with 6–10% of surface area dedicated to olive grove cultivation. Area 4: provinces with 11–20% of surface area dedicated to olive grove cultivation. Area 5: provinces with >20% of surface area dedicated to olive grove cultivation. No colour means <1000 Ha of surface area dedicated to olive grove cultivation.

The study was approved by the Hospital Nuestra Sra. del Prado Ethics Committee (Toledo), and all patients received written information and provided their consent.

Measurements and study variables

The primary study endpoint was to determine sensitization to grass and olive pollens by means of serum determination of sIgE levels to Ole e 1 and Phl p 1 + 5. It was performed using an automated fluoro-enzyme-immunoassay system (UniCAP 250; Phadia, Uppsala, Sweden) following the manufacturer's instructions. Serum samples were aliquoted and frozen immediately after blood extraction. All the samples were analysed centrally in the Faculty of Pharmacy Applications Laboratory, University of the Basque Country, Vitoria, Spain, to guarantee quality and homogeneity of results.

The secondary goal was the evaluation of differences in AIT composition (100% grasses, 100% olive or mixture of the two) that would have been selected for prescription before and after knowing the results of the serum determination of sIgE to Ole e 1 and Phl p 1 + 5.

Statistical analysis

To determine the study sample size under the worst-case scenario, a similar percentage of patients sensitized only to Ole e 1, only to Phl p 1 + 5 and to both allergens (Ole e 1 and Phl p 1 + 5) was assumed. Thus, estimating a proportion for each group of 33.3%, a statistical precision of 5% and an alpha risk of 5%, a sample of 340 patients by area was needed.

For the statistical analysis, four cut-off points were established: 0.35 kU/l, 0.70 kU/l, 2 kU/l and 3.5 kU/l.

Categorical variables were described using absolute and relative frequency. Continuous variables were described using mean, standard deviation, median and minimum and maximum values. Hypothesis tests according to their nature were conducted for comparison between variables. A 5% significance level was set in all statistical tests with outcome variables. Preliminary techniques were performed before doing the tests to ensure compliance with standard statistical assumptions, although nonparametric tests were used when this goal was not achieved. All statistical analyses were performed using SPSS version 17.0 (SPSS, Chicago, IL, USA).

Results

A total of 1263 patients fulfilled the inclusion and exclusion criteria and were analysed.

Sociodemographic and clinical characteristics

Mean patient age was 28.8 years (SD: 12.3 years), and 49.2% were female. According to the modified ARIA classification (25), 78.7% of patients had persistent rhinitis (36.3% mild, 54.6% 'moderate', 9.1% 'severe'). Allergic conjunctivitis (90.4%) and asthma (59%; mainly 'persistent' mild/moderate) were the main comorbidities. The most common co-sensitization to other pollen was to *Salsola/Chenopodium* (43.6%). Table 1 shows a summary of the sociodemographic and baseline clinical data of the patients.

Table 1 Patients sociodemographic and baseline clinical characteristics (n = 1263)

Sociodemographic data	
Age, mean ± SD (years)	28.8 ± 12.3
Age groups, *n* (%)	
5–15 years*	202 (16.0)
16–25 years	354 (28.0)
26–35 years	359 (28.4)
36–45 years	223 (17.7)
46–55 years	93 (7.4)
56–65 years	32 (2.5)
Gender, *n* (%)	
Male	641 (50.8)
Female	622 (49.2)
Caucasian race, *n* (%)	1.242 (98.4)
Urban residence, *n* (%)	914 (72.4)
Time of residence, mean ± SD (years)	22.2 ± 12.5
Clinical data	
Allergic rhinitis classification,† *n* (%)	
Mild intermittent	191 (15.1)
Moderate intermittent	76 (6.0)
Severe intermittent	3 (0.2)
Mild persistent	361 (28.7)
Moderate persistent	542 (42.9)
Severe persistent	90 (7.1)
Time since onset of symptoms, mean ± SD (years)	8.0 ± 6.7
Sensitizations (SPT),‡ *n* (%)	
Pollen	1.263 (100)
Salsola/Chenopodium	551 (43.6)
Platanus	403 (31.9)
Plantago	367 (29.1)
Cupressus	318 (25.2)
Artemisia/Helianthus	252 (20.0)
Parietaria	181 (14.3)
Cat	365 (28.9)
Dog	326 (25.8)
Dermatophagoides	284 (22.5)
Alternaria	175 (13.9)
Wheal diameter, mean ± SD (mm)	
Olive tree pollen	7.8 ± 3.1
Grass pollen	7.6 ± 2.8
Comorbidities, *n* (%)	
Asthma (GINA criteria)§	745 (59.0)
Intermittent	338 (45.4)
Mild persistent	195 (26.2)
Moderate persistent	208 (27.9)
Severe persistent	4 (0.5)
Allergic conjunctivitis	1.142 (90.4)

*Paediatric age in Spanish National Health System.
†Allergic Rhinitis classification based on Valero et al. (25).
‡Skin prick test (SPT) was considered positive if the mean wheal diameter was over 3 mm.
§Asthma classification based on Bateman et al. (31).

In vitro sensitization patterns

At the 0.35 kU/l cut-off point, 899 (71.2%) patients were positive to both Ole e 1 and Phl p 1 + 5, 177 (14%) were positive only to Phl p 1 + 5 and 152 (12%) were positive only to Ole e 1. Thirty-five (2.8%) patients were negative (<0.35 kU/l) to both allergens, although SPTs were positive to grass and olive pollens (see Fig. 2A).

As expected, as the cut-off points for sIgE increased, there was a significant downward trend in the percentage of patients considered to be sensitized to both Phl p 1 + 5 and Ole e 1. In contrast, a remarkable increase was observed in the percentage of patients considered to be sensitized only to Phl p 1 + 5, only to Ole e 1 or not sensitized to any of them. Comparison of *in vitro* sensitization patterns at different cut-off points is shown in Fig. 2.

Results by geographical area

A detailed analysis of *in vitro* sensitization patterns was performed by geographical area according to the two extreme cut-off points: 0.35 and 3.5 kU/l (Table 2).

The largest percentage of patients considered to be sensitized exclusively to major grass pollen allergens (Phl p 1 + 5) was observed in area 1. Sensitization only to Ole e 1 obtained the highest value in the provinces belonging to areas 4 and 5, where the largest extensions of olive grove cultivation were found.

In general, in areas with greater extension of olive grove cultivation (areas 4 and 5), patients suffered from more severe AR. Regarding the classification of asthma, it is observed that the proportion of patients with moderate persistent asthma increases in parallel with the number of hectares of olive grove cultivation, it grows from area 1 to 5, reaching the latter 30% of patients.

Allergen immunotherapy prescription

Based on available clinical data and SPT results at the time of recruitment, 922 (73%) patients would have been prescribed a mixture of grass and olive pollen as the most appropriate extract for AIT. Under the same conditions, 227 (18%) patients would have been indicated 100% grass AIT and 114 (9%), 100% olive AIT.

After investigators received the *in vitro* sensitization pattern data, a change in prescribing criteria was found. Seventy-six of 80 investigators (95%) decided to modify their initial option in at least one of their patients. In this analysis, there was nonagreement in the AIT composition that would have been selected before and after investigators received the allergen–sIgE data in 56.8% of patients (Fig. 3). Also remarkable was that in 20.9% of patients, no AIT was considered as appropriate by investigators after receiving the *in vitro* results.

These outcomes were confirmed in all geographical areas (non-coincidence with initial selected AIT in >45% of patients).

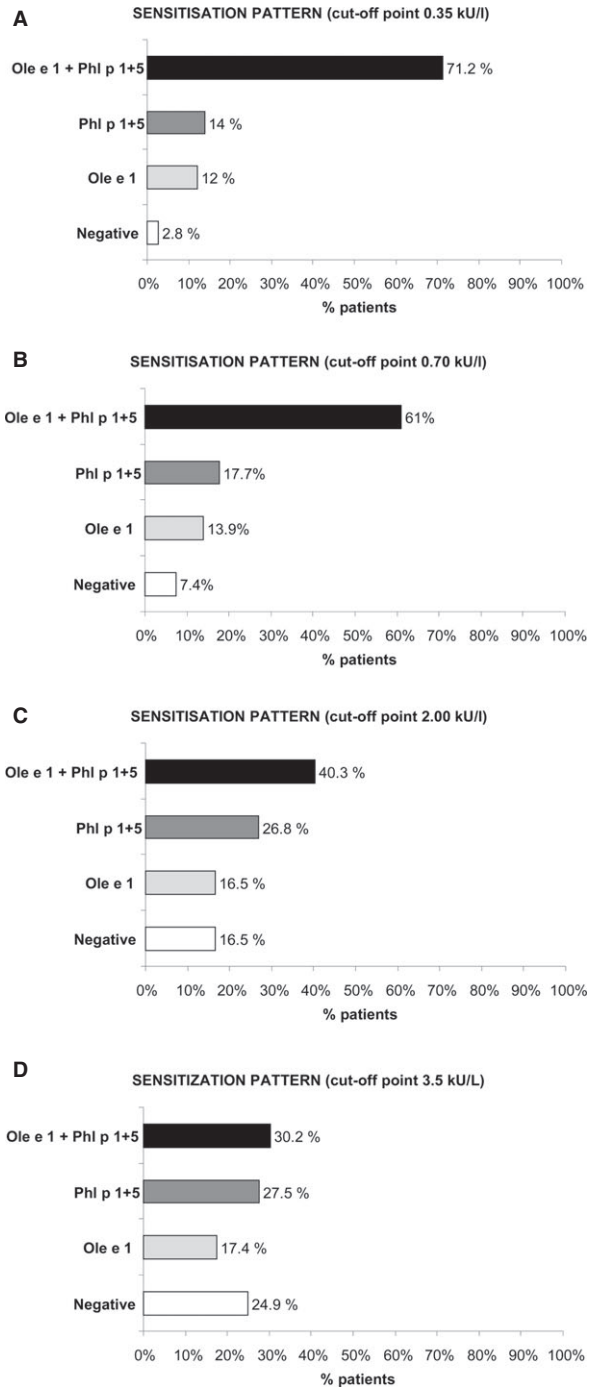

Figure 2 Patterns of *in vitro* sensitisation to Ole e 1 and Phl p 1 + 5 according to cut-off points (0.35 kU/l, 0.70 kU/l, 2.00 kU/l, 3.50 kU/l).

Discussion

A recent analysis of data collected during the ECHRS I study on SPT and sIgE assessments showed that these two measurements are necessary to identify allergen sensitivity

Table 2 *In vitro* sensitization pattern by geographical area

Cut-off points for specific IgE	Area 1 *N* = 264	Area 2 *N* = 230	Area 3 *N* = 252	Area 4 *N* = 247	Area 5 *N* = 270	*P* value
	Percentage of patients					
>0.35 kU/l						
Positivity exclusively to Ole e 1	5.3	15.2	8.7	14.6	16.7	0.0001
Positivity exclusively to Phl p 1 + 5	20.1	11.3	11.5	11.7	14.8	0.0176
Ole e 1 and Phl p 1 + 5	72.7	70.4	78.2	68.8	65.9	0.0304
Negative to Ole e 1 and Phl p 1 + 5	1.9	3.1	1.6	4.9	2.6	0.188
>3.5 kU/l						
Positivity exclusively to Ole e 1	6.5	20.4	13.9	23.1	23.8	0.0000
Positivity exclusively to Phl p 1 + 5	40.9	21.3	33.3	20.2	20.7	0.0000
Sensitization to Ole e 1 and Phl p 1 + 5	29.9	32.6	31.0	33.6	24.4	0.1769
Negative to Ole e 1 and Phl p 1 + 5	22.7	25.7	21.8	23.1	31.1	0.0912

Area 1: provinces with <1% of surface area dedicated to olive grove cultivation, with a minimum of 1000 Ha.
Area 2: provinces with 1–5% of surface area dedicated to olive grove cultivation.
Area 3: provinces with 6–10% of surface area dedicated to olive grove cultivation.
Area 4: provinces with 11–20% of surface area dedicated to olive grove cultivation.
Area 5: provinces with >20% of surface area dedicated to olive grove cultivation.

without being interchangeable (26). Approximately 50% of sensitized subject are polysensitized.

In this study, CRD enabled a more precise diagnosis of sensitization to major allergens of grass and olive pollens responsible for allergic rhinitis. In many cases, results of this test coincide with those obtained *in vivo* (SPT) but, in certain patients, CRD can detect exclusive sensitization to major or minor allergens, or even the absence of sensitization, that may not match SPT results having great repercussions on the subsequent choice of AIT composition for allergic patient treatment.

IgE antibodies to Phl p 1 and Phl p 5 are specific markers for sensitization to Timothy grass. Phl p 7 (calcium-binding protein) and Phl p 12 (profilin) are cross-reactivity markers. Increased IgE to these components and not to Phl p 1 and/or Phl p 5 indicates primary sensitization to another pollen (27).

This is the first large epidemiological study to include CRD of allergy to grasses and olive pollens in real-life clinical practice conditions. The number of patients with positive recombinant sIgE test to grasses and olive changed according to the different cut-off points selected to define the positivity threshold. Thus, at a cut-off point of 0.35 kU/l, 71.2% of patients were positive to Ole e 1 and Phl p 1 + 5, while at a cut-off point of 3.5 kU/l for those allergens, that rate fell to 30.2% (Fig. 2). The potential prescribing of AIT with olive and grass mixture would therefore be lower than initially foreseen.

It has to be noted that there is no consensus on the evaluation of sensitization using cut-off points. Barber et al. (14) used a cut-off point of ≥0.35 kU/l to consider the test as positive, but interpretation of cut-off points for sensitized patients fluctuates from ARIA guideline levels (≥0.35 kU/l) to clinical trial inclusion criteria (0.70 kU/l). Upper cut-offs

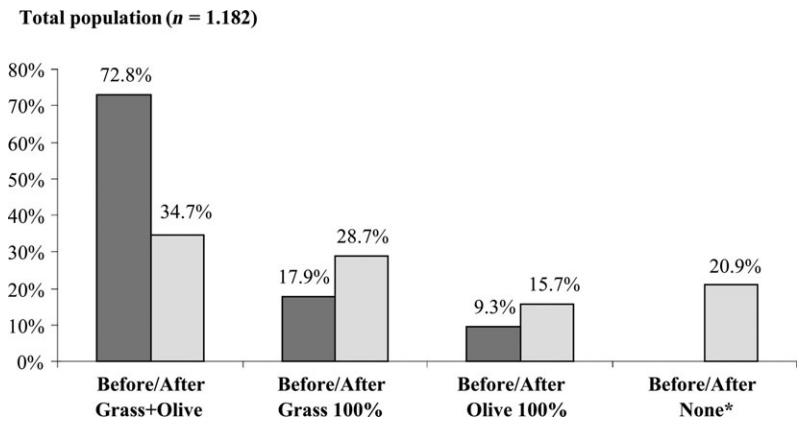

Figure 3 Percentage of allergen immunotherapy considered as the most adequate (in terms of composition) by investigators before and after knowing *in vitro* molecular diagnosis data. *The option 'None' was not available in the questionnaire completed at recruitment.

are widely used in routine clinical practice. Nevertheless, further studies are needed to investigate the clinical response of patients with allergic polysensitization according to sIgE values against each allergen.

A possible relationship between severity of allergic disorder, olive cultivation and pattern of sensitization may be established according to the outcomes obtained. In general, in areas with greater extension of olive grove cultivation, patients suffered from more severe AR and concomitant asthma. Nevertheless, this fact could be influence by concomitant sensitization to Ole e 7 and/or Ole e 9. In this sense, Barber et al (14) observed that in areas with extreme exposure to olive tree pollen (like 4 and 5 areas in our study), patients had an increased sensitization to Ole e 7 and Ole e 9 and this was associated with an increase in asthma symptoms (14).

The problem whether polyallergic patients are best treated with several allergens (28) or one allergen (the most clinically relevant) (29) is a considerable concern among allergists. Calderon et al. (30) concluded that simultaneous delivery of multiple unrelated allergens can be clinically effective, but for more than two allergens, it has to be further investigated. Our patients were sensitized to both olive an grass pollens, but taking into account the 0.35 kU/l cut-off point, only 65.9–78.2% (depending of the area) were polyallergic, the rest being monoallergic or had a negative result to both Ole e 1 and Phl p 1 + 5. If the selection of the extract for immunotherapy would have been exclusively based in the result of SPT, approximately one-third of patients would have been treated with an allergen to which they were not allergic. In this sense, when the prescribing physicians known the results of molecular diagnosis, a notable change in the extract composition was observed, with a nonagreement with the initial selection in more than a half of the patients. In a study about how molecular diagnosis can influence AIT prescription, Sastre et al. (12) observed an agreement in the indication of AIT before and after molecular diagnostic in only 46% of patients, which is a figure similar to the one presented in this study.

Patients were also sensitized to other allergens, like *Salsola*/*Chenopodium, Platanus, Plantago* and *Cupressus*. This sensitization has also been observed in other studies (8, 14). In these cases, CRD has an essential role in the differentiation of a true sensitization from cross-reactivity and in the selection of AIT.

Conclusion

Double sensitization to pollen from olive and grasses is common in Spain and other Mediterranean areas. Nevertheless, a significant percentage of patients are allergic to only one of them; when prescribing AIT based exclusively in the result of skin prick tests, a significant percentage of patients can be treated with an extract to which they are not allergic. It seems clear that the component-resolved diagnosis could help improve the selection of AIT in polysensitized patients. Nevertheless, more studies are needed to investigate the clinical response of patients with allergic polysensitization according to sIgE values against each allergen.

Author contributions

Carmen Moreno, Joaquín Quiralte, Álvaro Moreno-Ancillo and Alfredo Iglesias-Cadarso have directly participated in design, acquisition and interpretation of data, revising the article and final approval of the version to be published. José Luis Justicia and Mario A. García have directly participated in the conception, design, analysis, interpretation of data, drafting and revising the article and final approval of the version to be published. Miguel Torrecillas and Natividad Labarta has directly participated in acquisition of data, revising the article and final approval of the version to be published. Ignacio Dávila has directly participated in the design, acquisition and interpretation of data, drafting and revising the article and final approval of the version to be published.

Conflicts of interest

Carmen Moreno, Álvaro Moreno-Ancillo, Alfredo Iglesias-Cadarso, Miguel Torrecillas, Natividad Labarta and Ignacio Dávila have received personal fees from Stallergenes Ibérica, S.A., during the conduct of the study. José Luis Justicia and Mario A. García was a Stallergenes Ibérica, S.A., employees at the time of the study. Joaquín Quiralte has nothing to disclose.

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