

Molecular diagnosis and immunotherapy selection in a Portuguese population sensitized to grass and olive pollens

Diagnóstico molecular e seleção de imunoterapia numa população portuguesa sensibilizada ao pólen de gramíneas e de oliveira

Joana Cosme¹, Amélia Spínola Santos¹, Manuel Branco Ferreira^{1,2},
Manuel Pereira Barbosa^{1,2}, Maria Conceição Pereira Santos^{2,3}

ABSTRACT

Introduction: *Phleum pratense* (*Phl p*) and *Olea europaea* (*Ole e*) are common allergenic pollen. **Objectives:** To describe the sensitization patterns to *Phl p* and *Ole e* allergens in a subset of allergic rhinitis patients with positive skin prick tests (SPTs) to these pollens and compare the allergen immunotherapy (AIT) choice before and after determination of molecular components. **Methods:** Candidates to pollen immunotherapy with positive SPTs to both *Phl p* and *Ole e* were recruited. All of them underwent an SPT with a panel of aeroallergens and measurements of serum specific IgE (sIgE) to *Phl p*, *Ole e*, Phl p1, Phl p5, Phl p7, Phl p12, Ole e1, Ole e7, and Bet v2. **Results:** Forty adults were included. Of these, 83% and 65% were sIgE-positive to *Phl p* and *Ole e*, using the 0.35 kUA/L and 0.70 kUA/L cut-offs, respectively. Moreover, 42.5% of patients had positive sIgE to Phl p1 and/or Phl p5, 2.5% only to Ole e1, and 47.5% to both (0.35 kUA/L cut-off). By increasing the cut-off to 0.7 kUA/L, 55% of patients were sensitized to Phl p1 and/or Phl p5, and no patient was sensitized only to Ole e1. After component-resolved diagnosis, AIT choice was changed in 15 (37.5%) patients, with a decrease in the number of prescriptions of AIT with both grass and olive pollens and with olive alone, together with an increase in the prescriptions of AIT with grass pollen alone. **Conclusion:** Genuine sensitization to *Olea europaea* was reduced, and the sensitization patterns were heterogeneous. Knowledge of pattern of sensitization to molecular components changed immunotherapy prescription in more than one third of the patients.

Keywords: Allergic rhinitis, component resolved diagnosis, grass pollen, immunotherapy, olive tree pollen.

RESUMO

Introdução: Os polens de *Phleum pratense* (*Phl p*) e de *Olea europaea* (*Ole e*) são fontes alergênicas comuns. **Objetivos:** Descrever os padrões de sensibilização aos alergênicos destes dois polens num subconjunto de pacientes com rinite alérgica polínica e comparar a escolha de imunoterapia, antes e depois da determinação de alergênicos moleculares para *Phl p* e *Ole e*. **Métodos:** Foram recrutados candidatos para imunoterapia com polens, com testes cutâneos positivos para *Phl p* e *Ole e*. Todos realizaram um painel de testes em picada a aeroalergênicos e determinação de IgE séricas específicas para *Phl p*, *Ole e*, rPhl p1, rPhl p5, rPhl p7, rPhl p 12, rOle e 1, nOle e 7, rBet v2. **Resultados:** Foram incluídos 40 adultos. Em relação à sIgE para *Phl p* e *Ole e*, 83% e 65% dos pacientes apresentaram positividade para ambos, usando o *cut-off* de 0,35 kUA/L e 0,70 kUA/L, respectivamente. A positividade para Phl p1 e/ou Phl p 5 foi encontrada em 42,5%, para Ole e 1 apenas em 2,5%, enquanto 47,5% apresentaram sIgE positivo para ambos (*cut-off* corte de 0,35 kUA/L). Aumentando o *cut-off* para 0,7 kUA/L, 55% foram sensibilizados para Phl p1 e/ou Phl p5, nenhum paciente foi sensibilizado apenas para Ole e 1. Após a determinação dos alergênicos para os componentes moleculares, a escolha de imunoterapia foi alterada em 15 (37,5%) pacientes, com uma diminuição no número de vacinas para *Phleum* + *Olea e* apenas para *Olea e* e um aumento na prescrição de vacinas para *Phleum*. **Conclusão:** A sensibilização genuína do *Olea europaea* foi reduzida e os padrões de sensibilização foram heterogêneos. O conhecimento da sensibilização aos componentes moleculares dos alergênicos mudou a prescrição de imunoterapia em mais de um terço dos pacientes.

Descritores: Rinite alérgica, diagnóstico por componentes moleculares, pólen de gramíneas, imunoterapia, pólen de oliveira.

1. Centro Hospitalar Universitário de Lisboa Norte - Hospital de Santa Maria, Serviço de Imunoalergologia - Lisboa, Lisboa, Portugal.

2. Faculdade de Medicina de Lisboa, Clínica Universitária de Imunoalergologia - Lisboa, Lisboa, Portugal.

3. Unidade de Imunologia Clínica, Faculdade de Medicina, Instituto de Medicina Molecular, Universidade de Lisboa - Lisboa, Portugal.

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Introduction

Grasses, such as timothy grass (*Phleum pratense* [*Phl p*]), and olive tree (*Olea europaea* [*Ole e*]) are common sources of allergenic pollen in Mediterranean countries¹⁻⁴ that share cross-reactivity panallergens.^{3,5} In Portugal, these pollens are highly allergenic and have overlapping pollination periods from May to June. Therefore, in patients with allergic rhinitis (AR), a careful history, despite being important, is not enough to a precise identification of the clinically relevant sensitizers, especially when we consider prescribing immunotherapy.^{6,7} Component resolved diagnosis (CRD) is, for this reason, essential to allow the identification of the allergenic molecules involved in the etiological process of allergic diseases, to establish the individual reactivity profile of each patient, and to identify the relevant sensitizers (genuine or primary sensitization) and cross-reactivity patterns.^{3,8-10}

Molecular and biochemical characterization of *Phl p* and *Ole e* has revealed several relevant allergen components. For *Phl p*, Phl p1 (beta-expansin) and Phl p5 (a probable ribonuclease) are the most common genuine sensitization allergens, whereas *Ole e*1 (trypsin inhibitor) is considered the most common for *Ole e*.^{3,5} Cross-reactive panallergens found across many species of plants are also relevant: profilin (Phl p12) and polcalcin (Phl p7) for *Phl p*, and nonspecific lipid transfer protein (LTP) *Ole e*7 for olive tree pollen.^{3,5,9}

In Spain, some authors^{6,8-10} have already characterized the sensitization patterns to the major grass and olive pollen allergens using CRD in adults and children with seasonal AR. These authors also determined how this knowledge affected physicians' selection of allergen immunotherapy (AIT).

In Portugal, no study has been developed yet in order to identify our national *Phl p* and *Ole e* molecular sensitization patterns. Therefore, the aim of this study was to describe the frequency and the molecular sensitization patterns of a sample of Portuguese AR patients sensitized to pollens and to compare AIT selection based on two methodologies: patients' specific IgE (sIgE) to *Phl p* and *Ole e* whole extracts and sIgE to *Phl p* and *Ole e* molecular allergens (Phl p1, Phl p5, Phl p7, Phl p12, *Ole e*1, *Ole e*7). Additionally, we also aimed to investigate the presence of cross-reactivity by determining the levels of sIgE positivity to Bet v2 (profilin). This profilin is a minor allergen from birch pollen and is also involved in pollen-pollen and pollen-food cross-reactivity,¹¹ but

few data have been published regarding its frequency in the Mediterranean countries.

We believe that these data contribute to improving our knowledge of sensitization patterns in Portuguese AR patients and allows us to compare our results with those from our neighboring country, Spain.

Materials and methods

Patients and study design

Patients were recruited from an outpatient clinic, in Lisbon, Portugal, during the first 3 months of 2017. All patients had a medical consistent diagnosis of seasonal moderate-to-severe AR according to the Allergic Rhinitis and its Impact on Asthma (ARIA) classification,¹² with or without associated asthma or conjunctivitis, and clinically related to grass and olive pollinosis. Asthma was classified according to 2018 Global Initiative for Asthma (GINA) criteria.¹³ All patients had positive skin-prick test (SPT) results to both *Phl p* and *Ole e* pollens and had indication for AIT according to their attending allergy specialist. This indication was also revised by two of the authors according to patients' clinical history and laboratory results (sIgE to *Phl p* and *Ole e* extracts and molecular allergens) and following the recommendations for AIT established by the European Academy of Allergy and Clinical Immunology (EAACI).¹⁴ Exclusion criteria were pregnancy, age less than 18 years, previous AIT, or any contraindications for AIT. Patients' demographic, clinical and laboratory data were collected during normal practice. Serum samples were obtained after written informed consent. The study was approved by the Ethical Boards of Centro Hospitalar Universitário de Lisboa Norte/Faculdade de Medicina da Universidade de Lisboa.

Skin prick tests

SPTs were performed as described by the EAACI guidelines.¹⁵ All patients underwent SPT with a panel of allergens: dust mite (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Euroglyphus maynei*, *Lepidoglyphus destructor*, *Blomia tropicalis*, *Glycyphagus domesticus*, *Acarus siro*, *Tyrophagus putrescentiae*), pollens (wild grass mixture, grown grass mixture, *Phleum pratense*, *Parietaria judaica*, *Olea europaea*, *Artemisia vulgaris*, *Plantago lanceolata*, plane tree), molds (*Alternaria alternata*, *Aspergillus fumigatus*), cat and dog epithelia, Pho d2 (palm tree profiling extract), and

Pru p3 (peach LTP extract), all from Bial-Aristegui®. Histamine (10 mg/mL) and a 0.9% saline solution were used as positive and negative controls, respectively. Wheals with a mean diameter > 3 mm compared to the negative control were considered positive, but as this study was related to choices of AIT with grass and/or olive pollen, we chose to include only patients that had SPT wheals for these pollens at least 6 mm greater than the negative control, to ensure a stronger sensitization criterion.

Determination of sIgE levels

We evaluated serum levels of sIgE to *Phl p* and *Ole e* whole extracts and to molecular allergens for *Phl p* (*Phl p*1, *Phl p*5, *Phl p*7, *Phl p*12), *Ole e* (*Ole e*1 and *Ole e*7) and *Betula verrucosa* (*Bet v*2). These assessments were performed using the ImmunoCAP® system according to manufacturer's instructions (ThermoFisher Scientific, Uppsala, Sweden). For further analysis, we considered two cut-off values for sIgE (≥ 0.35 kU/L and ≥ 0.70 kU/L).

AIT selection

Considering clinical data, SPT results, and the different laboratory results, two of the authors reached a consensus on the indication and composition of AIT for each patient. To make this choice, the authors considered both the clinical data and the SPT results of each patient. Besides this, on a first stage the results of the sIgE to *Phl p* and *Ole e* whole extracts were considered; while, on a second stage, the authors looked at the results of the sIgE to *Phl p* and *Ole e* molecular allergens. Based on this, one of four AIT options was chosen for each patient: AIT with grass pollen, AIT with olive pollen, AIT with both allergens, or no AIT in patients with sIgE < 0.70 kU/L.

Statistical analysis

A descriptive analysis of the demographic and clinical characteristics of the study population was performed. For SPT diameters and sIgE values, median and interquartile range were presented. The number and percentage of patients were used to describe categorical variables. The Spearman's rho correlation index was used to find correlations between in vivo and in vitro tests. A p-value of less than 0.05 was considered significant. Data was compared using GraphPad Prism v5.01 (GraphPad Software Inc.).

Results

Clinical and demographical data

Demographical and clinical data of the studied population are shown in Table 1. Forty patients (65% female, mean±SD age 42.8±16.3 years) were included. All patients had moderate-to-severe rhinitis, according to the ARIA classification¹², and 10 (25%) had asthma. Of these, 40% had mild persistent asthma, according to 2018 GINA guidelines¹³. The median diameter of the SPT was 11 [9-14.3] mm for *Phl p* extract and 9 [8-12] mm for *Ole e* pollen extract. Conjunctivitis was the most common comorbidity (n=25; 62.5%), and 10 (25%) patients had concomitant asthma. House dust mites were the most frequent co-sensitizations, found in 29 (72.5%) patients.

In vitro sensitization to *Phl p* and *Ole e* whole extracts and molecular allergens

Table 2 shows the results of the different studied sIgE specificities (sIgE to *Phl p* and *Ole e* whole extracts, sIgE to *Phl p* and *Ole e* molecular allergens, and sIgE to *Bet v* 2), according to the two different cut-offs used.

Considering the 0.35 kUA/L cut-off, 33 (82.5%) patients tested positive to *Phl p* and *Ole e*, while six (15%) tested positive only to *Phl p*, and one (2.5%) only to *Ole e*. Despite being SPT-positive to both *Phl p* and *Ole e* extracts (≥ 6 mm), one patient was sIgE-positive to neither *Phl p* nor *Ole e*. With the 0.70 kUA/L cut-off, the number of patients who tested positive to both *Phl p* and *Ole e* decreased to 26 (65%), and the number of those positive only to *Phl p* increased to 12 (30%). One (2.5%) patient was sIgE-positive only to *Ole e*, and one (2.5%) was positive to neither *Phl p* nor *Ole e*.

Some differences between the two cut-off values were also observed for the genuine sensitization allergens *Phl p*1, *Phl p*5, and *Ole e*1 (Table 3). Using the 0.35 kUA/L cut-off value, 17 (42.5%) patients were positive only to *Phl p*1 and/or *Phl p*5, one (2.5%) patient only to *Ole e*1, and 19 (47.5%) to the genuine sensitization allergens of both species. By increasing the cut-off value to 0.70 kUA/L, 22 (55%) patients were sensitized only to *Phl p*1 and/or *Phl p*5, 13 (32.5%) were sensitized to genuine sensitization allergens of both species, and no patient was sensitized only to *Ole e*1. For the 0.35 kUA/L cut-off, all patients were positive both to *Phl p*5 and *Phl p*1. For the 0.70 kUA/L cut-off, one patient had sensitivity to *Phl p*5 but not to *Phl p*1.

Regarding the cross-reactivity allergens, 8-12.5% of patients were sensitized to profilins, 10% to polcalcin, and 5-7.5% to LTP (depending on the two cut-offs of positivity used), as shown in Table 2.

Five (12.5%) patients had positive sIgE values for Bet v 2, all of them with sIgE values above 0.7 kUA/L.

Four of these patients were also sensitized to Phl p12 (Table 2).

There was a very strong correlation between sIgE values to *Phl p* and to Phl p1 (Spearman rho coefficient 0.89; $p < 0.05$) and between sIgE levels to *Phl p* and to Phl p5 (Spearman rho coefficient 0.84;

Table 1

Characteristics of the studied population

Demographic and clinical characteristics	
Characteristics	N° patients (%)
Number of patients	40 (100)
Age (years), mean±SD	42.8 ±16.3
Female	26 (65)
Sensitization (according to SPT)	
<i>Phleum pratense</i>	
Wheal diameter 6-10 mm	17 (43)
Wheal diameter ≥ 11 mm	23 (58)
<i>Olea europaea</i>	
Wheal diameter 6-10 mm	28 (70)
Wheal diameter ≥ 11 mm	12 (30)
Other pollens	
Wild grass mixture	39 (98)
Grown grass mixture	37 (93)
<i>Parietaria judaica</i>	11 (28)
<i>Artemisia vulgaris</i>	9 (23)
<i>Plantago lanceolata</i>	16 (40)
Plane tree	15 (38)
Just grass and Olea pollens	10 (25)
Molds	5 (13)
Cat/dog epithelia	18 (45) / 8 (20)
Dust mites	29 (73)
Palm profilin (Pho d 2)	7 (18)
Peach LTP (Pru p 3)	4 (10)
Allergic symptoms	
Rhinitis (persistent moderate-to-severe)	40 (100)
Asthma (2018 GINA criteria ¹³)	10 (25)
Intermittent	2 (5)
Mild persistent	4 (10)
Moderate persistent	3 (7.5)
Severe persistent	1 (2.5)
Conjunctivitis	25 (63)
Eczema	3 (8)
Food allergy symptoms	6 (15)

$p < 0.05$), as show in Figure 1a and 1b. The correlation between sIgE values to *Ole e* and to *Ole e1* was moderate (Spearman rho coefficient 0.45; $p < 0.05$) (Figure 1c).

Sensitization patterns

For molecular allergens sIgE results, several patterns of sensitization were found (Table 4). We found 14 different sensitization patterns with the cut-off point of 0.35 kUA/L, and 13 with the higher cut-off point (> 0.70 kUA/L).

AIT prescription

All the 40 patients in our study were considered by their allergy specialists, after reviewing clinical history and SPT results, to have indication to receive pollen AIT. The selection of the precise AIT changed after knowing the different laboratory results (Figure 2).

When considering only the results of sIgE to *Phl p* and *Ole e* whole extracts, the predominant immunotherapy choice was AIT with both grass and olive pollens ($n=26$; 65%). In 12 (30%) patients the choice was only grass AIT and in 1 (2.5%) patient only

Table 2

Frequency and median levels of sIgE to *Phl p*, *Ole e*, and molecular allergens

Parameters		Determination of sIgE								
		<i>Phl p</i>	<i>Ole e</i>	<i>Phl p1</i>	<i>Phl p5</i>	<i>Phl p7</i>	<i>Phl p12</i>	<i>Ole e1</i>	<i>Ole e7</i>	<i>Bet v 2</i>
sIgE ≥ 0.35	Patients, n (%)	39 (97.5)	34 (85)	36 (90)	17 (42.5)	4 (10)	4 (10)	20 (50)	3 (7.5)	5 (12.5)
	Median [Q1-Q3]	4.6	1.5	3.9	5.9	2.5	0.9	2.1	9.6	1.6
	levels, kU/L	[2.1-12.7]	[0.9-3.3]	[1.9-7.3]	[3-8.9]	[1.9-7.8]	[0.7-1.3]	[0.7-4.5]	[5.1-22]	[1.3-4.5]
sIgE ≥ 0.70	Patients, n (%)	38 (95)	27 (68)	34 (85)	18 (45)	4 (10)	3 (8)	13 (32.5)	2 (5)	5 (12.5)
	Median [Q1-Q3]	4.7	2.6	4.1	5.9	2.5	0.9	3.1	22.6	1.6
	levels, kU/L	[2.6-13]	[1.2-4.8]	[2.1-7.3]	[3-8.9]	[1.9-7.8]	[0.8-1.7]	[1.6-5.2]	[16.1-29.1]	[1.3-4.5]

sIgE = specific IgE; *Phl p* = *Phleum pratense*; *Ole e* = *Olea europaea*.

Table 3

Frequency of patients with genuine sensitization to molecular allergens of *Phleum pratense* and *Olea europaea*

		<i>Phl p1</i> + <i>Phl p5</i>	<i>Phl p1</i>	<i>Phl p1</i> +	Only	Only	Only	None
		+ <i>Ole e1</i>	+ <i>Ole e1</i>	<i>Phl p5</i>	<i>Phl p1</i>	<i>Phl p5</i>	<i>Ole e1</i>	
sIgE ≥ 0.35	n (%)	7 (17.5)	12 (30)	10 (25)	7 (17.5)	0 (0)	1 (2.5)	3 (7.5)
sIgE ≥ 0.70	n (%)	4 (10)	9 (22.5)	13 (32.5)	8 (20)	1 (2.5)	0 (0)	5 (12.5)

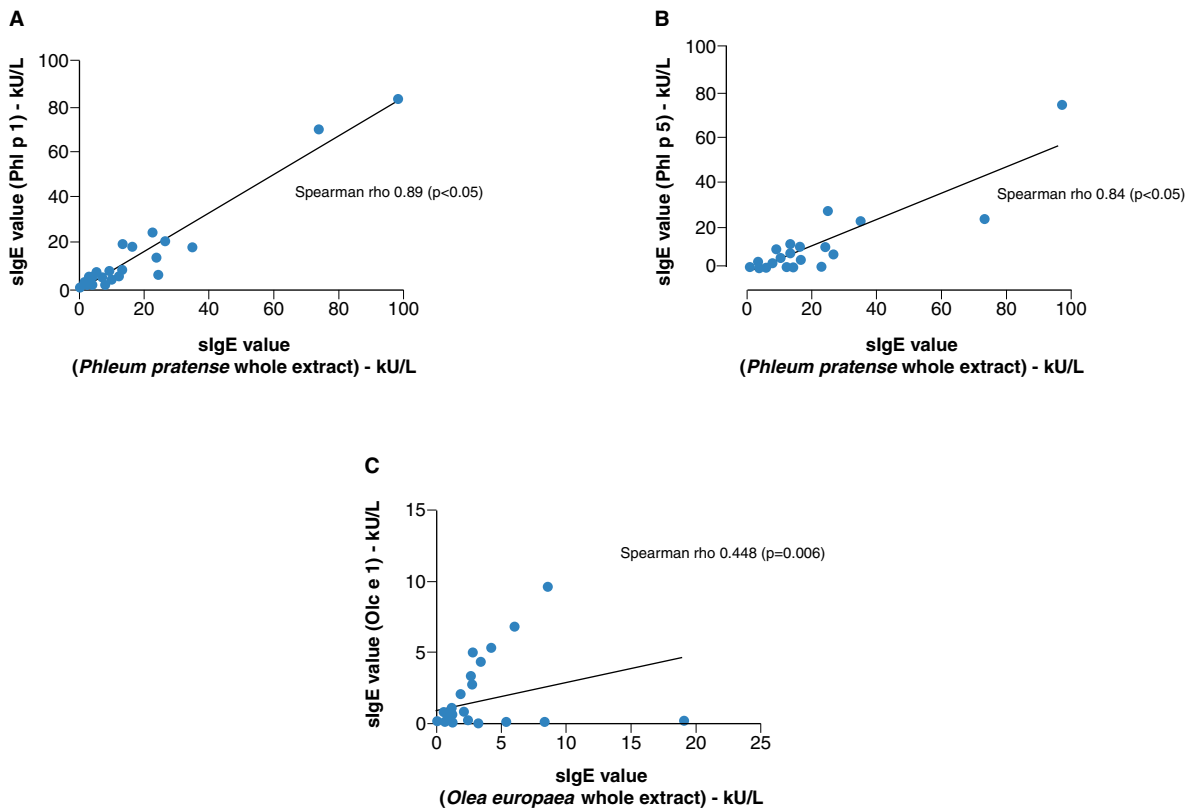


Figure 1
 Correlations - a) *Phleum pratense* whole extract sIgE and Phl p 1; b) *Phleum pratense* whole extract sIgE and Phl p 5; c) Between *Olea europaea* whole extract sIgE and Ole e 1

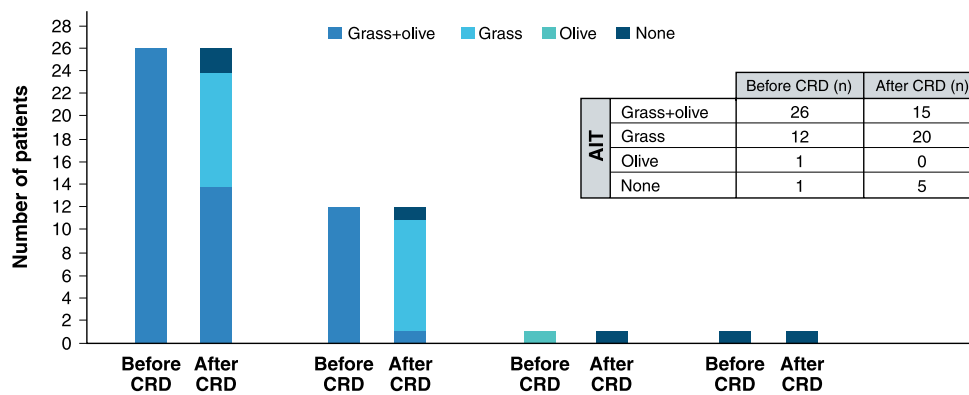


Figure 2
 Allergen immunotherapy (AIT) selection before and after component resolved diagnosis (CRD)

olive AIT. No AIT was selected in one other patient (2.5%).

After knowing the results of sIgE to molecular allergens, the choice of AIT changed: AIT with both grass and olive tree pollens was selected in only 15 patients (37.5%), and AIT only with grass pollen

was the choice in 20 patients (50%). In five patients (12.5%), AIT was not recommended (Table 4).

Overall, these outcomes confirmed a change in AIT selection in 15 (37.5%) patients, with a decrease in the number of patients receiving olive immunotherapy (olive + grass or only olive AIT) while the number

Table 4

Cross table of sensitization patterns according to different sIgE cut-off values

Sensitization patterns		sIgE ≥ 0.35 cut-off				Total
		<i>Phl p</i>	<i>Ole e</i>	<i>Phl p + Ole e</i>	None	
sIgE ≥ 0.35 cut-off	Phl p1 + Ole e1	0	0	10	0	10
	Phl p1 + Phl p5 + Ole e1	0	0	5	0	5
	Phl p1 + Phl p5 + Ole e1 + Ole e7	0	0	1	0	1
	Phl p1 + Phl p5 + Phl p7 + Ole e1	0	0	1	0	1
	Phl p1 + Ole e1 + Ole e7	0	0	1	0	1
	Phl p1 + Phl p12 + Ole e1	0	0	1	0	1
	Phl p1 + Phl p5	2	0	6	0	8
	Phl p1	3	0	2	0	5
	Phl p1 + Phl p5 + Phl p12	0	0	2	0	2
	Phl p1 + Phl p7	0	0	1	0	1
	Phl p1 + Ole e7	0	0	1	0	1
	Ole e1 + Phl p12	0	1	0	0	1
	Phl p7	0	0	2	0	2
	None	1	0	0	0	1
	Total	6	1	33	0	40

Sensitization patterns		sIgE ≥ 0.70 cut-off				Total
		<i>Phl p</i>	<i>Ole e</i>	<i>Phl p + Ole e</i>	None	
sIgE ≥ 0.70 cut-off	Phl p1 + Ole e1	0	0	8	0	8
	Phl p1 + Phl p5 + Ole e1	0	0	3	0	3
	Phl p1 + Phl p5 + Ole e1 + Ole e7	0	0	1	0	1
	Phl p1 + Phl p12 + Ole e1	0	0	1	0	1
	Phl p1 + Phl p5	6	0	4	0	10
	Phl p1	4	0	2	0	6
	Phl p1 + Phl p5 + Phl p12	0	0	2	0	2
	Phl p1 + Phl p7	0	0	1	0	1
	Phl p1 + Ole e7	0	0	1	0	1
	Phl p1 + Phl p5 + Phl p7	1	0	0	0	1
	Phl p5	0	0	1	0	1
	Phl p7	0	0	2	0	2
	None	1	1	0	1	3
	Total	12	1	26	1	40

of patients receiving only grass immunotherapy increased. The number of patients for which no vaccine was recommended also increased.

Discussion

Our study revealed a substantial degree of heterogeneity of molecular sensitization profiles and a reduced number of positive sIgE to cross-reactive molecules. Moreover, there were changes in AIT prescriptions in more than one fourth of the patients after CRD determination.

The 40 patients included in this study were AIT candidates with pollen AR and positive SPT to *Phl p* and *Ole e* with a wheal diameter ≥ 6 mm. Despite these SPT results, only 33 patients (82.5%) had sIgE values ≥ 0.35 kUA for both pollens. Increasing the cut-off point to ≥ 0.70 kUA/L reduced this number to 65%.

It is known that allergens extract for SPT and for sIgE determination with whole extracts exhibit a considerable heterogeneity regarding the presence of individual allergens, which may contribute to a variability in its results.^{16,17} Hence, a single molecular allergen or a combination of a few major molecular allergens can be used for a more precise diagnosis. In this study, we determined seven molecular allergens (Phl p1, Phl p5, Phl p7, Phl p12, Ole e1, Ole e7, and Bet v 2). Considering the *Phl p* species-specific allergens, the percentage of sensitization to Phl p1 (90%) was higher than to Phl p5 (42.5%). These results are in line with the those previously published by Rossi et al.¹⁸ that, in a population of 77 grass allergic adults identified a frequency of 93.5% of sensitization to Phl p1 and of 72.7% to Phl p5 and by Darsow et al.¹⁹ that documented, 101 adults with pollen allergy, a frequency of sensitization to Phl p1 and Phl p5 of 92% and 81%, respectively. These differences between sensitization to Phl p1 and Phl p5 are also found in pediatric patients, since studies have also shown a greater prevalence of sensitization to Phl p1 (90-99%) than to Phl p5 (50-67%).^{20,21}

Regarding sensitization to Ole e1, which indicates primary sensitization to Oleaceae pollens,⁸ only 50% of our patients were sensitized to this allergen, considering the 0.35 kUA/L cut-off value, and only one patient was sensitized to Ole e1 without positivity to any of the *Phl p* genuine sensitization allergen. These results are different to those published in previous studies.^{9,10,22} In Spain, another Mediterranean country, sensitization to Ole e1 varies between 75.3%²¹ and

83%⁹ in adults. Also, in Spanish pediatric population, Martínez-Cañavate Burgos et al.¹⁰ found a frequency of sensitization to Ole e1 (89%) higher than ours.

In our study, when the 0.35 kUA/L cut-off was considered, there was only one patient sensitized to Ole e1 who did not test positive to any other genuine sensitization molecular allergen. Using the 0.70 kUA/L cut-off, we did not find any patient sensitized only to Ole e1. This finding is different from that of the study by Moreno et al.,⁹ which showed that, in a subset of adults polysensitized to olive and grass, the higher cut-off point for sIgE, the higher the percentage of patients sensitized only to Ole e1. We may argue that our results are due to the peculiar characteristics of exposure to this allergen in the Lisbon metropolitan area (Atlantic littoral zone).

Our percentage of sensitization to panallergens was relatively small (10% sensitized to Phl p7; 10% to Phl p12, and 7.5% to Ole e7). Regarding Phl p7 and Phl p12, the frequency of sensitization to these allergens in other European studies is also small (7-32% for Phl p7 and 5-35% to Phl p1.^{29,23} However, our percentage of sensitization to Ole e7 was significantly lower than that of those published in previous studies. Scala et al.,²⁴ for example, reported a 21.5% sensitization to this allergen in a subset of olive tree-pollen allergic while Barber et al.²² demonstrated a frequency of sensitization of approximately 50%. We believe that this discrepancy between frequencies of sensitization is probably related to a geographical variation in allergen exposure and to the different populations included.

In order to confirm whether there was any difference using other pollen species cross-reactivity allergen, we also determined the levels of sIgE to Bet v2. However, in our population, these levels were similar values to those of sIgE to Phl p12 and were positive in almost the same patients. For this reason, levels of sIgE to Bet v2 did not show any additional value in our study.

Our study revealed differences between AIT choices before and after CRD. Overall, in 15 (37.5%) patients AIT was changed after CRD determination. These changes were mainly due to a decrease in the number of candidates to AIT with both grass and olive pollens and to an increase in the number of candidates to AIT with grass pollen.

In other European countries, similar changes in AIT composition have already been reported. Sastre et al.²⁵ and Letran et al.⁶, for example, documented

a difference in AIT composition before and after molecular diagnosis in, respectively, 54% and 50% of Spanish patients with pollen sensitization. More recently, Moreno et al.⁹ also reported a change in AIT composition after CRD in 56.8% patients allergic to pollen. Similar results were reported by Martínez-Cañavate Burgos et al.¹⁰ in a population of 281 children with grass and olive sensitization: AIT composition was changed in 52.87% of cases after CRD.

Apparently, our results showed a slightly smaller percentage of changes in AIT than results previously published^{6,8,9,10,25} in Spanish studies. Although one should note that, in all those studies,^{6,9,10,25} changes were considered comparing decisions based on clinical and skin tests with decisions after CRD, while in our study we compared AIT choice based on clinical data, SPT, and sIgE to *Phl p* and *Ole e* whole extracts with AIT choice after CRD.

To our best knowledge, this is the first Portuguese study performed with the purpose of portraying the molecular sensitization profile of polysensitized AIT candidates with sensitization to both grasses and olive tree, in order to achieve a better knowledge of Portuguese molecular sensitization profiles and a more appropriate AIT choice. Nevertheless, some limitations should be considered: patient sensitization was based only on SPT results, and no confirmation was made using provocation tests; no information was considered regarding pollen counts in the area of residence of patients; only patients living in Lisbon area were included; the only *Ole e* major allergen determined was *Ole e*1; and the impact of cross-reactive carbohydrate determinants was not assessed.

Conclusion

In conclusion, in our study, the frequency of genuine sensitization to *Ole e* and to cross-reactivity allergens was reduced, and the sensitization patterns found were highly heterogeneous. Cross-reactive allergen molecules were present only in a minority of our patients. After CRD, AIT was changed in 15 (37.5%) of the patients, stressing the relevance of CRD in AIT selection.

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Corresponding author:
Joana Cosme
joanamcosme@gmail.com