[17]Immunoallergology Service, Castelo Branco Local Health Centre, Castelo Branco, Portugal
[18]Immunoallergology Service, Lisbon Lusiadas Hospital, Lisboa, Portugal.
[19]Immunoallergology Unit, HPA Saúde Hospital, Alvor, Portugal
[20]Faculty of Medicine and Biomedical Sciences, University of Algarve, Gambelas Campus, Faro, Portugal
[21]Immunoallergology Unit, Terra Quente Hospital, Mirandela, Portugal
[22]Immunoallergology Service, Luz Hospital, Lisboa, Portugal
[23]Comprehensive Health Research Centre, NOVA Medical School, NOVA University of Lisbon, Lisboa, Portugal
[24]Macro Array Diagnostic, Vienna, Austria
[25]Diater Laboratory, Laboratory of Diagnostic and Therapeutical Applications, Madrid, Spain

Abstract

Background. Grass and olive pollens have overlapping pollination periods and are common allergens in the Iberian Peninsula. Objective: To determine the sensitization pattern to major *Phleum pratense* and *Olea europaea* pollens in the Portuguese population with pollen allergic rhinitis (AR) using molecular allergen diagnosis (MAD).

Methods. Seasonal AR patients (≥ 12 years), with positive skin prick tests (SPT) to Phleum and Olea were recruited from 16 centers. Using ALEX^2, specific IgE to Phl p1, Phl p2, Phl p5, Phl p6, Phl p7, Phl p 12, Ole e1, Ole e7 and Ole e9 were determined. Immunoblotting of Olea allergic patients was performed.

Results. Included 175 patients (55.4% female; mean age 31.6 ± 13.3 years; 85.7% adults; 40% asthmatic, Coast 28%/Inland 72% and North 29.1%/Centre 20.6%/South 50.3%). Considering Phleum MAD, 85.7% were sensitized to Phl p1, 45.7% to Phl p2, 50.3% to Phl p5, 45.7%, to Phl p6, 10.9% to Phl p7 and 22.9% to Phl p12. Sensitization to Ole e1 was found in 56.6%, to Ole e7 in 1.7% and Ole e9 in 3.4%

patients. Sensitization to Phl p7 was more frequent in asthmatics (17.4% vs 6.6%; p = 0.044). Sensitization to Phl p5, Phl p6, Phl p12 and Ole e1 was more frequent in inland. Regarding sensitization patterns: 53.1% patients were sensitized to both species genuine´ sIgE, 38.3% to Phleum and 3.4% only to Olea species' sIgE. Immunoblotting of Olea allergic patients showed a high intensity band that may correspond to Ole e12.

Conclusions. MAD showed "genuine" Grass and Olea sensitization in approximately 50% of our patients.

Key words: Allergic rhinitis, molecular allergen diagnosis, *olea europaea*, *Phleum pratense*, sensitization.

Impact statement: The use of molecular allergen diagnosis allowed us to make a more detailed characterization of the sensitization profile from pollinic (grass and olive pollens) rhinitis patients. This was the first multicentric national study regarding this topic.

Introduction

Allergic rhinitis (AR) is the most common chronic disease worldwide (1). In Europe, AR frequency ranges from 16.9% to 28.5% (1,2). Portuguese epidemiological studies estimated a rhinitis prevalence of 26.1% (3). Within the different age groups, the Portuguese rhinitis prevalence is 21.5%-24% in children, 27% in adolescents and 29.8% in the elderly (4–6).

Pollen allergy has had a remarkable clinical impact in Europe (7). Portugal, due to its climate, geographical characteristics, and the influence of Mediterranean pollens, has a distinctive vegetation with various types of allergenic pollen. Some of those are different from the central and northern Europe pollens (8).

In Portugal, grasses such as timothy grass (*Phleum pratense*), and olive tree (*Olea europaea*) are highly allergenic, and both have overlapping pollination periods from May to June. In grass and olive AR patients, a comprehensive evaluation of the patient's medical history together with *in vivo* and *in vitro* diagnostic tests, such as skin prick test (SPT) and specific IgE (sIgE) for the whole allergen extracts, may not allow a precise identification of the clinically relevant sensitizers, especially when we are considering allergen immunotherapy (AIT) prescription (9, 10, 11). Thus, molecular allergen diagnosis (MAD) is essential to establish the individual reactivity profile and to identify the relevant sensitizers (genuine or primary sensitization) and cross-reactivity patterns (10,11).

The aim of this study was to determine the sensitization pattern to *Phleum pratense* and *Olea europaea* pollens in the Portuguese population who presented a concise history of pollinic AR with positive SPT to both Phleum and olive pollen. To better characterize our population sensitization profile to olive pollen, we performed an Immunoblotting with Olive pollen allergic patients' sera.

Materials and Methods

Study design, data collection and selection of the patients

This was a multicenter, cross-sectional, observational, national study conducted under routine clinical practice, during 2021 (first 8 months) in 16 allergy centers/units of mainland Portugal. There were included patients older than 11 years

old with a consistent diagnosis of seasonal allergic rhinitis, according to Allergic Rhinitis and its Impact on Asthma (ARIA) classification, clinically related to grass and olive pollinosis. In patients with concomitant asthma, its diagnosis was made according to 2020 Global Initiative for Asthma (GINA) criteria.

All patients had double sensitization to grass and olive pollens on SPT. These tests were performed as described by the EAACI guidelines (12,13). All patients underwent SPT with the same panel of allergens: dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Lepidoglyphus destructor*) moulds (*Alternaria alternata, Aspergillus fumigatus*), cat and dog epithelia and *Phleum pratense* (Diater®) and *Olea europaea* (Diater ®) pollens. Other grass (Multiple grass mix), tree (*Platanus acerifolia*, *Cupressus sempervirens*) or weed (*Parietaria judaica, Artemisia vulgaris*) pollens were added to the SPT. Histamine (10 mg/mL) and a negative control (0.2% phenolic and 50% glycerinated physiology saline solution) (Diater®) were used as positive and negative controls (13), respectively. Wheals with a mean diameter > 3 mm compared to the negative control were considered positive.

To investigate possible geographical differences, it was performed a country division in 2 regions: coast (the region close to Atlantic Ocean) and inland (the interior region of Portugal, next to Spain). With this division, we intended to evaluate the influence of the proximity of the Ocean in the sensitization rate.

We also considered another division in 3 other zones: the northern, the center, and the southern (including Lisbon area, Alentejo and Algarve) zones. This late division followed the current administrative division of the mainland area of Portugal.

Using a standardized questionnaire we collected sociodemographic data (age, gender, region), clinical and laboratory data. To all patients that accepted to participate in the study, we collected 10 mL of blood for the laboratory determinations.

The study was approved by all Hospitals Ethics Committee and all patients received written information and provided their consent. A specific informed consent form for pediatric patients was used which was signed by a legal representative.

Exclusion criteria were pregnancy, previous pollen AIT or any contraindications for SPT or for AIT.

***Study variables: sIgE, in vitro* sensitization patterns and Olea Europa immunoblotting**

Considering the laboratory study we determined 4 Phleum pratense species' specific IgE (Phl p1, Phl p2, Phl p5 and Phl p6) and 2 Olea europaea species' specific IgE (Ole e1 and Ole e9). We also determined cross reactivity sIgE: Phl p7 and Phl p12 for Phleum and Ole e7 for olive tree pollen.

Specific IgE to *Phleum pratense* and *Olea europaea* whole extracts and sIgE to Phl p 1, Phl p 2, Phl p 5, Phl p 6, Phl p 7, Phl p 12, Ole e1, Ole e7 and Ole e 9 were measured in Macro Array Diagnostic Laboratory in Vienna (Austria), using ALEX system (MacroArrayDX®)(14). ALEX is a multiplex array containing 282 reagents (157 extractive allergens and 125 molecular components). The different allergens and components are coupled onto polystyrene nano-beads, and then the allergen beads are deposited on a nitrocellulose membrane the sIgE levels were expressed in kUA/L and values ≥0.30 kUA/L were considered positive.

In this study we intended to determine molecular *in vitro* sensitization patterns to Phleum pratense and Olea europaea specific IgE (sIgE) in the included patients. We defined the following sensitization patterns: 1) Double sensitization to Phleum and Olea species´ specific, 2) Phleum species 'specific IgE; 3) Sensitization to Olea species 'specific IgE and 4) Sensitization only cross reactivity IgE. For the definition of patterns 1 to 3, we considered for each patients the presence of at least one IgE to the species 'specific allergens. These patients could also be sensitized to cross reactivity allergens. Pattern 4 included the patients that were only sensitized to cross reactivity allergens.

In addition, we analyzed the sera of our olive pollen sensitized patients by immunoblotting. In immunoblotting, proteins from Olea europaea extracts were analyzed in a procedure performed in three steps: separation of proteins by electrophoresis, transfer to a polyvinylidene difluoride membrane and identification of specific proteins by their binding to sIgE. With this additional procedure we intended to characterize Olive sensitization and investigate whether there are other allergens involved in the Portuguese population.

Statistical analysis

A descriptive analysis of the demographic data and clinical characteristics of the study population was performed. For sIgE values, median and interquartile range were presented. The number and percentage of patients were used to describe categorical variables. Hypothesis tests according to their nature were conducted for comparison between variables. According to variables, there were used a chi-squared test, Fisher's exact test and McNemar test were conducted, as applicable. A level of significance of 0.05 was used for all statistical tests. Results were analyzed globally

and stratified by geographic area (inland/coast and north/center/south). Data analysis was performed using the SPSS version 28 (SPSS, Chicago, IL, US).

Results

Patients' demographic and clinical characteristics

A total of 175 patients with seasonal allergic rhinitis were included from 16 Allergy Units/Centers of the mainland regions of Portugal. Table I summarizes patients´ demographic data and the clinical characteristics. Within the included patients, the majority were adults, females and lived in inland areas of Portugal. Conjunctivitis was the main comorbidity (72%) and 40% of the patients had asthma. The most common co sensitization was to house dust mites (69.7%).

Specific IgE determinations

Regarding *Phleum pratense* sIgE, the most frequent molecular sIgE identified was Phl p1 (150; 85.7%) followed by Phl p5 (88; 50.3%). The number of patients sensitized to Phl p2 and Phl p6 was the same (80; 45.5%). The sensitization to cross-reactivity allergens was reduced in our populations, with 19 (10.9%) patients sensitized to Phl p7 and 40 (22.9%) to Phl p12. Figure 1A shows the frequency of sensitization and the median [Q1-Q3] values of all sIgE determined for *Phleum pratense*.

For Olea pollen, the main molecular allergen found was Ole e1 (99; 56.6%). Figure 1B indicates the frequency of sensitization and the median values [Q1-Q3] to all Olea molecular allergens determined.

In relation to Portuguese geographical areas, Figure 2 exhibits the frequency of sensitization to Phleum and Olea molecular allergens in the coats and inland. Apart from Phl p7, sensitization to all *Phleum pratense* molecular sIgE was more frequent in the Inland with significant differences for Phl p5 (p=0.004), Phl p6 (p=0.003) and Phl p12 (p=0.003).

Regarding Olea europaea, sensitization to Ole e1, Ole e7 and Ole e9 was more frequent in Inland than in Coast regions with significant differences for Ole e1 sIgE (p=0.041).

We did not find any differences within the sensitizations between North, Centre, and South of Portugal.

We also did not find any difference regarding sensitization to all the sIgE tested according to the patients age group or according to the rhinitis severity. In our population, sensitization to Phl p7 was higher in the asthmatic patients (17,4% vs 6,6%; p=0,044).

In-vitro molecular sensitization patterns

Four sensitization patterns were found, and 7 (4%) patients did not show any positivity to the sIgE tests. The molecular sensitization patterns are indicated on table II.

Regarding the sensitization patterns: 93 (53.1%) were sensitized to both Phleum and Olea species' specific IgE, 67 (38.3%) were sensitized to Phleum species' specific IgE exclusively, 6 (3.4%) were sensitized to Olea species' specific IgE exclusively and 2 (1.1%) were sensitized exclusively to Phl p7. Sensitization to Ole e 9 occurred exclusively in the context of double sensitization to Phleum and Olea species.

We did not find any significant difference between these patterns within the country regions (inland/coast) nor between children and adults. All the 6 patients sensitized exclusively to Olea species' specific IgE were adults.

Olea europaea Immunoblotting

Immunoblotting of selected individuals sensitized to Olea europaea showed a repertoire of IgE response with molecular weights ranging from ≈30 to 37 kD, that may correspond to Ole e 12 allergen (Figure 3).

Discussion

In our study, from the 176 patients with AR with symptoms in the spring and positive SPT to Phleum and Olea, only 53.1% were sensitized to both species specific Phleum and Olea allergens and 4% had no positivity for the studied allergens. MAD showed that Phl p1, Phl p5 and Ole e1 were the most frequent allergens. Apart from Phl p7, sensitization to the other molecular allergens was more frequent in Inland, with significant differences for Phl p5, Phl p6, Phl p12 and Ole e 1. We found 4 sensitization profiles.

Regarding Phleum sensitization, our study showed similar frequencies of sensitization to the previously published international (83-99% to Phl p1; 50-73%, to Phl p5; 7-32% to Phl p7 and to 5-35% Phl p12) (14-17) and national studies (18, 19).

Cipriani F et al showed that Phl p7 is a biomarker of asthma and of a more severe AR form in a cohort of grass allergic patients. In our study, sensitization to Phl p7 was higher in the asthmatic patients; however, we found no differences regarding AR severity (20).

Olive pollen allergy is common in the Peninsula Iberian countries, although this sensitization is widely variable within Peninsula Iberian countries. This difference can be explained by the intensity of olive tree cultivation in each country. For instance, in Spain, in the regions with intensive olive pollination, the frequency of sensitization to Ole e1 varies between 75.3% e 80% (21), while on areas of low exposure to olive pollens as Navarra, the frequency of sensitization to Ole e1 is of 28.2% (22). In our study, Ole e 1 was the main olive allergen identified (56.6%) being less prevalent in coast regions (51.6%) than in inland (69.4%) where olive cultivation is more intense.

In our study, sensitization to Ole e7 and Ole e9 was residual. Regarding Ole e 9, our sensitization (3.4%) is quite inferior to the Spanish sensitization rate both in areas with high pollen exposure (over 35%), and with low olive pollen counts (10.7%) (21). Our frequency of sensitization to Ole e 7 was also lower to our neighbor country (1.7% vs 14.4-47%) (23).

Our data showed an interesting result as we have identified in Immunoblotting a broad IgE reaction binding band in the 37kDa range, corresponding to Ole e12 molecular weight. According to Castro et Al (24), Ole e12 is a isoflavone reductase that is found in patients with olive pollinosis and peach allergy. Being Portugal, a country with high frequency of peach allergy and olive allergy, our data highlights the importance of studying this allergen in our population.

Molecular sensitization profile characterization is important for etiological management of allergic disease and may help improving AIT selection in polysensitized patients (25,26). Identification of species-specific allergens and cross reactivity allergens is important before considering AIT prescription. In our

population, about half of the included patients had IgE sensitization to the species-specific allergens of both pollens. Considering this, only these patients were candidates for AIT with both pollens, about 38% were candidates for grass pollen immunotherapy, 3.4% to olive pollen AIT and 5.1% had no criteria for grass and olive AIT prescription. In conclusion, after the MAD study, in 46.9% of the patients there was an alteration in the AIT composition. This percentage are distinctive from the data previously published in two Spanish studies (56.8% in Moreno C et al and 52.87% in Martínez-Cañavate Burgos A et al) (27,28). Even though we cannot comment about statistically significant differences, we believe that our results highlight the particularities of the Portuguese population.

Our study has some limitations. The main one is the reduced number of included patients. The exclusion of patients younger than 11 years old is also a limitation. The inclusion of a larger sample with younger patients would allow to identify evolution of Phleum and Olive molecular allergens according to age. Despite these limitations, it is important to state that this is the first multicenter Portuguese study that characterizes Phleum and Olive sensitization patterns.

Our data regarding Phleum and Olive sensitization patterns slightly differed from the data previously published in Spanish studies (our neighbor country) and added information regarding the Iberian Peninsula sensitization profile.

In conclusion, in our study, MAD showed that about half of the included patients were sensitized to species specific Phleum and Olive pollen allergens. Using MAD, sensitization to Phleum was more prevalent in the Inland and Phl p7 was more frequent in asthmatic patients. Regarding Olive, Ole e1 was the main allergen, with a

predominance in the Inland, that may be probably related to the intensive cultivation of Olive trees in this area.

After MAD performance, in about half of the included patients, we maintained the indication for AIT with both pollens. In the remaining patients we changed either the AIT composition or the AIT indication.

Recognizing sensitization profiles is useful to select the allergens that should be included in AIT. With the advent of molecular AIT, we consider that establishing national and regional sensitization profiles will be essential for choosing a tailored maid AIT composition.

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Author Contributions: JC: Conceptualization, Investigation, Methodology, Project administration, Resources, Software, Writing - original draft, Writing - review & editing. EP, MCPC, AL, JC, MP, ET, SF, EM, AP, HJ, MASB, FR, MJP, NS, SRC, RDF, ATB, FR, PBA, AM, HF, LC, IF, EN, JLP, DS, MJV, RS, CA, AFH, SM, CLI, LPV, PMS, PMS, CN, JCV, LMB, CC and SP: Data curation. FP and MA: Investigation (laboratory). JA: Funding acquisition, Writing - review & editing. ASS:

conceptualization, Investigation, Methodology, Project administration, Writing - original draft, Writing - review & editing.

Informed Consent Statement and Ethics: Informed consent was obtained from all subjects involved in the study. Ethical approval was obtained by the ethical board of all medical centers involved in the study.

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References

1.Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. Eur Respir J. 2004;24(5):758-64. doi: 10.1183/09031936.04.00013904

2.Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy. 2008;63 Suppl 86:8-160. doi: 10.1111/j.1398-9995.2007.01620.x

3.Todo-Bom A, Loureiro C, Almeida MM, Nunes C, Delgado L, Castel-Branco G, et al. Epidemiology of rhinitis in Portugal: evaluation of the intermittent and the persistent types. Allergy. 2007;62(9):1038-43. doi: 10.1111/j.1398-9995.2007.01448.x

4.Morais-Almeida M, Nunes C, Gaspar, Angela, Falcão H, Branco Ferreira, M, Todo-Bom A, et al. Rinite em idade pré-escolar: Prevalência e caracterização. Estudo ARPA Kids. Rev Port Imunoalergologia. 2007;15(5):387-410.

5.Morais-Almeida M, Pite H, Pereira AM, Todo-Bom A, Nunes C, Bousquet J, et al. Prevalence and classification of rhinitis in the elderly: a nationwide survey in Portugal. Allergy. 2013;68(9):1150-7. doi: 10.1111/all.12207

6.Morais-Almeida M, Loureiro C, Todo-Bom A, Nunes C, Pereira C, Delgado L, et al. Rhinitis prevalence and characterization survey in primary care centres of mainland Portugal – ARPA study. Rev Port Imunoalergologia. 2005; 13 (1): 69-80

7.D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, et al. Allergenic pollen and pollen allergy in Europe. Allergy. 2007;62(9):976-90. doi: 10.1111/j.1398-9995.2007.01393.x

8.D'Amato G, Liccardi G. Pollen-related allergy in the European Mediterranean area. Clin Exp Allergy. 1994;24(3):210-9. doi: 10.1111/j.1365-2222.1994.tb00222.x

9.Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Grönlund H. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). Clin Exp Allergy. 1999;29(7):896-904. doi: 10.1046/j.1365-2222.1999.00653.x

10.Luengo O, Labrador-Horrillo M. Molecular Allergy Diagnosis in Clinical Practice: Frequently Asked Questions. J Investig Allergol Clin Immunol. 2022;32(1):1-12. doi: 10.18176/jiaci.0769

11.Barber D, Diaz-Perales A, Escribese MM, Kleine-Tebbe J, Matricardi PM, Ollert M, et al. Molecular allergology and its impact in specific allergy diagnosis and therapy. Allergy. 2021;76(12):3642-58. doi: 10.1111/all.14969

12.Van Kampen V, De Blay F, Folletti I, Kobierski P, Moscato G, Olivieri M, et al. EAACI position paper: skin prick testing in the diagnosis of occupational type I allergies. Allergy. 2013;68(5):580-4. doi: 10.1111/all.12120

13.Heinzerling L, Mari A, Bergmann KC, Bresciani M, Burbach G, Darsow U, et al. The skin prick test – European standards. Clin Transl Allergy. 2013;3:3. doi: 10.1186/2045-7022-3-3

14.Letrán A, Espinazo M, Moreno F. Measurement of IgE to pollen allergen components is helpful in selecting patients for immunotherapy. Ann Allergy Asthma Immunol. 2013 Oct;111(4):295-7. doi: 10.1016/j.anai.2013.07.005. Epub 2013 Aug 2. DOI: 10.1016/j.anai.2013.07.005

15.Movérare R, Larsson H, Carlsson R, Holmquist I. Mugwort-Sensitized Individuals from North Europe, South Europe and North America Show Different IgE Reactivity Patterns. Int Arch Allergy Immunol. 2011;154:164–172. doi: 10.1159/000320231

16.Sastre J, Landivar ME, Ruiz-García M, Andregnette-Rosigno MV, Mahillo I. How Molecular Diagnosis Can Change Allergen-Specific Immunotherapy Prescription in a Complex Pollen Area. Allergy. 2012;67:709–711. doi:10.1111/j.1398-9995.2012.02808.x

17.Darsow U, Brockow K, Pfab F, Jakob T, Petersson CJ, Borres MP, Ring J, Behrendt H, Huss-Marp J. Heterogeneity of Molecular Sensitization Profiles in Grass

Pollen Allergy—Implications for Immunotherapy? Clin Exp Allergy. 2014;44:778–786. doi: 10.1111/cea.12303

18.Gonçalves Nunes I, Loureiro G, Tavares B, Todo-Bom A, Cunha R. Sensitization to genuine markers of timothy grass pollen (Phleum pratense) in the North-Central region of Portugal. Eur Ann Allergy Clin Immunol 2022. doi: 10.23822/EurAnnACI.1764-1489.269.

19.Almeida E, Caeiro E, Todo-Bom A, Duarte A, Gazarini L. Sensitization to grass allergens: Phl p1, Phl p5 and Phl p7 Phl p12 in adult and children patients in Beja (Southern Portugal). Allergol Immunopathol (Madr). 2019;47(6):579-584. doi: 10.1016/j.aller.2019.04.006

20.Cipriani F, Mastrorilli C, Tripodi S, Ricci G, Perna S, Panetta V, et al; Italian Pediatric Allergy Network (I-PAN). Diagnostic relevance of IgE sensitization profiles to eight recombinant Phleum pratense molecules. Allergy. 2018;73(3):673-682. doi: 10.1111/all.13338

21.Barber D, de la Torre F, Feo F, Florido F, Guardia P, Moreno C, et al. Understanding patient sensitization profiles in complex pollen areas: a molecular epidemiological study. Allergy. 2008;63(11):1550-8. doi: 10.1111/j.1398-9995.2008.01807.x

22.García BE, Lizaso MT, Moreno C, Rodríguez R, Villalba MT, Ledesma A, et al. Oleaceae-induced pollinosis in an area with exposure to olive and ash trees. J Investig Allergol Clin Immunol. 2011;21(1):34-7. PMID: 21370721

23.Tejera ML, Villalba M, Batanero E, Rodríguez R. Identification, isolation, and characterization of Ole e 7, a new allergen of olive tree pollen. J Allergy Clin Immunol. 1999 Oct;104(4 Pt 1):797-802. doi: 10.1016/s0091-6749(99)70290-3

24.Castro L, Crespo JF, Rodríguez J, Rodríguez R, Villalba M. Immunoproteomic tools are used to identify masked allergens: Ole e 12, an allergenic isoflavone reductase from olive (Olea europaea) pollen. Biochim Biophys Acta. 2015;1854(12):1871-1880. doi: 10.1016/j.bbapap.2015.09.004

25.Sastre J, Sastre-Ibañez M. Molecular diagnosis and immunotherapy. Curr Opin Allergy Clin Immunol. 2016 Dec;16(6):565-570. doi: 10.1097/ACI.0000000000000318

26.Izmailovich M, Semenova Y, Abdushukurova G, Mukhamejanova A, Dyussupova A, Faizova R, et al. Molecular Aspects of Allergen-Specific Immunotherapy in Patients with Seasonal Allergic Rhinitis. Cells. 2023;12(3):383. doi: 10.3390/cells12030383

27.Moreno C, Justicia JL, Quiralte J, Moreno-Ancillo A, Iglesias-Cadarso A, Torrecillas M, et al. Olive, grass or both? Molecular diagnosis for the allergen immunotherapy selection in polysensitized pollinic patients. Allergy. 2014; 69(10):1357-63. doi: 10.1111/all.12474

28.Martínez-Cañavate Burgos A, Torres-Borrego J, Molina Terán AB, Corzo JL, García BE, Rodríguez Pacheco R, ET AL. Molecular sensitization patterns and influence of molecular diagnosis in immunotherapy prescription in children sensitized to both grass and olive pollen. Pediatr Allergy Immunol. 2018;29(4):369-374. doi: 10.1111/pai.12866

Table I - Demographic and clinical characteristics

Demographic and clinical characteristics	
Number of included patients – n(%)	175 (100)
Demographic data	
Female, n (%)	97 (55.4)
Adults, n (%)	150 (85.7)
Age – mean ± SD (min – max)	31.6±13.3 (12-75)
Age groups, n (%)	
12-17 y	25 (14.3)
18-30 y	70 (40)
31-50 y	63 (36)
51-75 y	17 (9.7)
Residence area in mainland Portugal, n (%)	
Coast/Inland	49 (28) / 126 (72)
North/central/south	51 (29.1)/ 36 (20.6)/ 88 (50.3)
Rhinitis – ARIA classification, n (%)	
Intermittent mild	18 (10.3)
Intermittent moderate/severe	25 (14.3)
Persistent mild	27 (15.4)

Persistent moderate/severe	105 (60)
Concomitant allergic diseases, n (%)	
Asthma	70 (40)
Conjunctivitis	126 (72)
Eczema	40 (22.9)
Co-sensitizations, n (%)*	
House dust mites	122 (69.7)
Weed pollens	98 (56)
Other tree pollens	63 (36)
Cat/Dog epithelia	78 (44.6)
Moulds	35 (20)

* House dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Lepidoglyphus destructor*), Weed pollens (*Parietaria judaica, Artemisia vulgaris*), Tree pollens (*Platanus acerifolia*, *Cupressus sempervirens*), Moulds (*Alternaria alternata, Aspergillus fumigatus*).

Table II - Molecular sensitization patterns

Sensitization patterns	**n (%)**
Double sensitization to *Phleum* and *Olea* species' specific IgE*	**93 (53.1%)**
Phl p1 and/or Phl p2 and/or Phl p5 and/or Phl p6 + Ole e1	60
Phl p1 and/or Phl p2 and/or Phl p5 and/or Phl p6 + Ole e1 + Ole e9	3
Phl p1 and/or Phl p2 and/or Phl p5 and/or Phl p6 + Ole e1 + Phl p7	6

Phl p1 and/or Phl p2 and/or Phl p5 and/or Phl p6 + Ole e1 + Phl p12	20
Phl p1 and/or Phl p2 and/or Phl p5 and/or Phl p6 + Ole e1 + Ole e9 + Phl p12	2
Phl p1 and/or Phl p2 and/or Phl p5 and/or Phl p6 + Ole e1 + Ole e7	1
Phl p1 and/or Phl p2 and/or Phl p5 and/or Phl p6 + Ole e1 + Ole e7 + Ole e9	1
Sensitization to *Phleum* species' specific IgE*	**67 (38.3%)**
Phl p1 and/or Phl p2 and/or Phl p5 and/or Phl p6	40
Phl p1 and/or Phl p2 and/or Phl p5 and/or Phl p6 + Phl p7	8
Phl p1 and/or Phl p2 and/or Phl p5 and/or Phl p6 + Phl p12	16
Phl p1 and/or Phl p2 and/or Phl p5 and/or Phl p6 + Phl p7 + Phl p12	2
Phl p1 and/or Phl p2 and/or Phl p5 and/or Phl p6 + Ole e7	1
Sensitization to Olea species' specific IgE*	**6 (3.4%)**
Ole e1	5
Ole e1 + Phl p7	1
Ole e9	0
Sensitization only to cross reactivity IgE	**2 (1.1%)**
Phl p 7	2
No CRD (None of the studied sIgE)	**7 (4%)**

* with or without associated cross-reactivity allergens (Phl p7, Phl p12, Ole e7)

Figure 1: Frequency of sensitization to Phleum pratense and Olea europaea sIgE (**Fig. 1a** - Frequency of sensitization to Pheum pratense sIgE; **Fig. 1b** - Frequency of sensitization to Olea europaea sIgE)

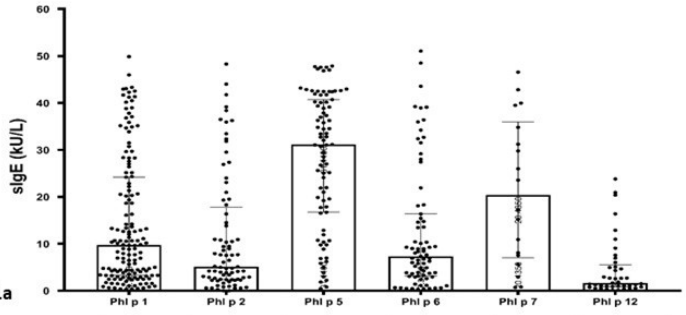

Fig. 1a

	Phl p 1	Phl p 2	Phl p 5	Phl p 6	Phl p 7	Phl p 12
Sensitization, n (%)	150 (85.7)	80 (45.7)	88 (50.3)	80 (45.7)	19 (10.9)	40 (22.9)
sIgE median values– kU/L	9.34	5.65	31.05	6.94	16.27	1.77
[Q1-Q3]– kU/L	3.3-24.3	2.4-18.3	17.5-40.7	3.2-16.4	7.05-35.9	0.87-5.6

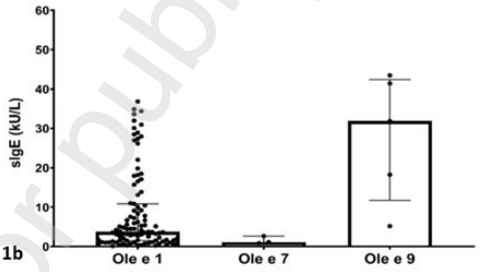

Fig. 1b

	Ole e 1	Ole e 7	Ole e 9
Sensitization, n (%)	99 (56.6)	3 (1.7)	6 (3.4)
sIgE median values – kU/L	3.70	1.15	25.06
[Q1-Q3]– kU/L	1.5– 10.6	0.86 – 2.7	11.7 – 42.5

Figure 2: Sensitization to Pheum pratense and Olea europaea sIgE according to Inland and Coast regions

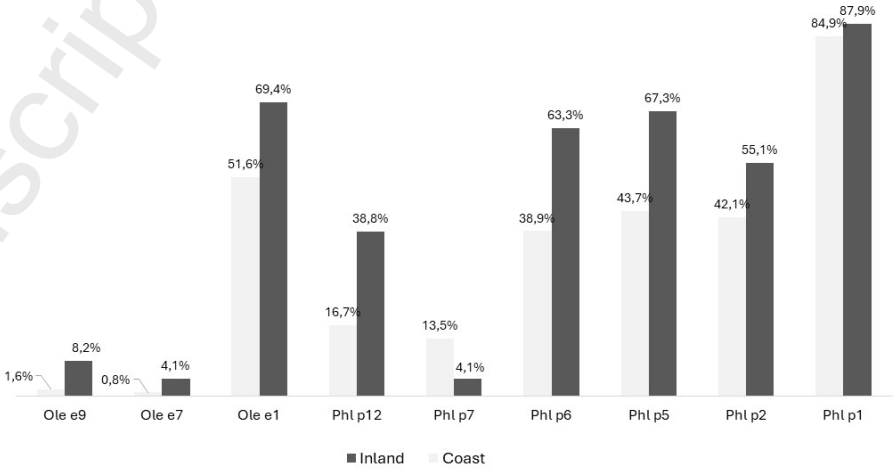

Figure 3: IgE-Immunoblotting of selected patients (each lane corresponds to a hospital center) for the allergenic extracts of Olea europaea.