ORIGINAL ARTICLE | Eur Ann Allergy Clin Immunol | Vol 56, N.2, 65-70, 2024

Inês Nunes[1], Graça Loureiro[1], Beatriz Tavares[1], Ana Todo-Bom[1], Rosário Cunha[2]

Sensitization to genuine markers of timothy grass pollen (*Phleum pratense*) in the North-Central region of Portugal

[1]Department of Allergy and Clinical Immunology, Hospital and University Center of Coimbra, Coimbra, Portugal
[2]Department of Clinical Pathology, Hospital and University Center of Coimbra, Coimbra, Portugal

Key words

Grass pollen allergy; Phleum pratense; Phl p1; Phl p5b; timothy grass.

Corresponding author
Inês Nunes
Department of Allergy and Clinical Immunology
Hospital and University Center of Coimbra
Praceta Professor Mota Pinto
3004-561, Coimbra, Portugal
ORCID: 0000-0003-3889-2748
E-mail: inesnunes0929@gmail.com

Doi
10.23822/EurAnnACI.1764-1489.269

Impact statement

Phl p1 sensitization, marking genuine grass pollen sensitization, is the most frequently found in the North-Central region of Portugal.

Summary

Background. *Pollen is the main cause of respiratory allergy and its prevalence is increasing. Timothy grass (Phleum pratense) is one of the most common pollen sources, and one of the best characterized allergenic grasses. The major allergens Phl p1, Phl p2, Phl p5 and Phl p6 are considered markers of genuine grass pollen sensitization.* ***Methods.*** *It is a retrospective study. IgE levels of Phl p1 and Phl p5 in patients living in the North-Central region of Portugal were analyzed, considering age and area of residence (inland or coastal).* ***Results.*** *Among the 188 patients with IgE results for Phl p1 and Phl p5, sensitization to Phl p1 and Phl p5b was observed in 97.87% and 63.83%, respectively. The majority demonstrated co-sensitization to both Phl p1 and Phl p5b, while 68 patients were monosensitized to Phl p1 and 4 patients were monosensitized to Phl p5b. Either patients living in coastal or inland areas showed IgE levels of Phl p1 higher than Phl p5b.* ***Conclusions.*** *Regarding genuine grass pollen sensitization, Phl p1 sensitization is more prevalent than Phl p5b in the North-Central region of Portugal.*

Introduction

In the last decades, the prevalence of respiratory allergies induced by pollens has been increasing. Atmospheric pollen in the outdoor environment is the main cause of rhinitis, bronchial asthma, rhinoconjunctivitis and eczema in individuals with pollinosis (1). In Europe, especially in the Mediterranean countries, Poaceae family pollen (grasses) is one of the most important causes of pollinosis (2). In the Pooideae subfamily, timothy grass (*Phleum pratense*), orchard grass (*Dactylis glomerata*), perennial ryegrass (*Lolium perenne*) and bluegrass (*Poa pratensis*) are the most common pollen sources (3). Poaceae pollen is the most prevalent pollen and the main cause of pollinosis in Portugal (4). In pollen allergy diagnosis, it is important to distinguish co-sensitization to several allergen sources and sensitization to cross-reactive components. In pollen allergy treatment, the content of commercial pollen allergen immunotherapy (AIT) extracts is not always known, but the presence of major allergens is often stated (5, 6). Molecular diagnosis can improve allergy diagnosis (7), selecting patients eligible for AIT and allowing an accurate individual prescription, which will avoid inappropriate or ineffective therapies (8). Timothy grass pollen (*Phleum pratense*) is one of

***Table I** - Timothy allergenic molecules listed by the IUIS Allergen Nomenclature Sub-Committee (http://www.allergen.org/index.php).*

Allergen name	Biochemical name	Allergenicity
Phl p 1	CCD-bearing protein/beta-expansin	95%
Phl p 2	Grass group II/III	65%
Phl p 3	Pollen allergen 1	60%
Phl p 4	CCD-bearing protein/ berberine bridge enzyme	75%
Phl p 5	Grass group V	95%
Phl p 6	Grass group VI	75%
Phl p 7	Polcalcin	10%
Phl p 11	Ole e 1-related protein	32%
Phl p 12	Profilin	15%
Phl p 13	Grass group XIII/ polygalacturonase	50%

the best characterized allergenic grasses (9), with ten allergenic molecules already described and officially listed by the IUIS Allergen Nomenclature Sub-Committee, at date (**table I**) (3, 10). ImmunoCAP singleplex IgE tests available for the Phl p allergenic molecules are Phl p1, Phl p2, Phl p4, Phl p5b, Phl p6, Phl p7, Phl p11 and Phl p12.

The major allergens Phl p1, Phl p2, Phl p5 and Phl p6 are considered markers of genuine sensitization (3). The present study aims at characterizing molecular allergens of timothy grass pollen genuine sensitization in patients living in the North-Central region of Portugal.

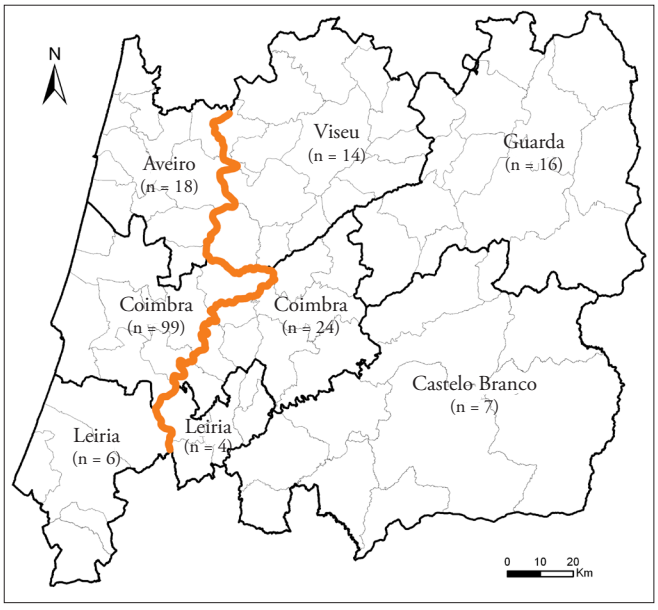
Materials and methods

Retrospective study performed in the Clinical Pathology Department of Coimbra University Hospital, a central hospital that receives patients from all over the North-Central region of Portugal. A parameterized search in Clinidata XXI 5.3.12 SP1 software was made. Patients who were asked for specific IgE antibodies to Phl p1, Phl p5 and Phl p6 were included, from 01/06/2014 until 31/07/2020. Since our laboratory does not have Phl p2 analysis available, it was not considered. For allergen 5, the isoform available is Phl p5b. IgE assay was made by fluorescence enzyme immunoassay (ImmunoCAP, Thermo Fisher Scientific) with a detection sensitivity between 0.01 and 100 kilounits per liter (kU_A/L). Results were classified as positive or negative according to the cutoff point of 0.35 kU_A/L. Exclusion criteria were previous AIT, repeated determinations from the same patient (the highest levels or those prior to AIT treatment were considered) and patients living outside North-Central region of Portugal (included districts of Coimbra, Guarda, Leiria, Castelo Branco, Viseu and Aveiro). As only one result of Phl p6 was found, genuine molecular sensitization pattern of *Phleum pratense* allergens focused only in the allergens Phl p1 and/or Phl p5b specific IgE assays. To characterize and compare the genuine molecular sensitization pattern of *Phleum pratense*, only patients who were asked for the two allergens and that were positive to at least one Phl p1 and/or Phl p5b specific IgE were considered. These patients were divided according to sensitization profile: monosensitization to Phl p1 (group I), monosensitization to Phl p5b (group II) and sensitization to both Phl p1 and Phl p5b allergens (group III). In each group the sensitization profile was analyzed according to age (children if age < 18 years and adult if age ≥ 18 years) and area of residence (coastal *versus* inland areas) (**figure 1**).

Statistical analysis

Data analysis was carried out using SPSS version 25 for Windows. Results were presented as median (interquartile range (IQR)) and mean (standard deviation). Statistical analysis was performed with nonparametric tests (Wilcoxon signed-rank and Mann Whitney U tests). A level of significance of 0.05 was considered (P-value ≤ 0.05).

***Figure 1** - Map of the North-Central region of mainland Portugal.*



The orange line limits the coast (on the left) and the inland areas (on the right side). The number of patients included in each district is shown on the map. Adapted from https://www.ccdrc.pt/.

Results

A total of 508 results were found, corresponding to 319 patients who were asked for at least one specific IgE to Phl p1 and/or Phl p5b (168 female, mean age 25.21 ± 1.67 years). The prevalence of Phl p1 and Phl p5b sensitization was 296/302 and 134/206, respectively, as shown in **table II**.

To characterize and compare the genuine molecular sensitization pattern of *Phleum pratense*, from the initial 319 patients, we considered the 188 patients with available results to both molecular allergens. None of the patients had both negative results. All the included patients had a positive result to Phl p1 and/or Phl p5b. This total group included 95 female and 93 male aged 4 to 65 years, of which 59 were children (mean age 11.44 ± 3.65 years, 66.1% male) and 159 were adults (mean age 31.43 ± 11.40 years, 41.86% male). In **table III** the characteristics of these patients divided in the three groups are described: monosensitized to Phl p1 (group I), monosensitized to Phl p5b (group II) and sensitized to both Phl p1 and

Table II - *Prevalence of Phl p1 and Phl p5b sensitization.*

	Total	Phl p1	Phl p5b
Number of results	508	302	206
Number of positive results (> 0.35 kU_A/L)	430	296	134

Table III - *Characteristics of sensitization to Phl p1 and/or Phl p5b.*

	Total Group		Group I	Group II	Group III	
	Phl p1	Phl p5b	Phl p1	Phl p5b	Phl p1	Phl p5b
Total (n)	188	188	68	4	116	116
Gender (f/m)	95/93	95/93	40/28	03-gen	52/64	52/64
Age (years)	25.15 ± 13.40	25.15 ± 13.40	25.97 ± 14.11	35 ± 16.67	24.34 ± 12.82	24.34 ± 12.82
IgE (kU_A/L)						
Mean ± SD	27.59 ± 31.34	20.89 ± 29.40	12.70 ± 21.26	22.43 ± 20.10	37.26 ± 32.90	33.07 ± 31.43
Median	14.75	6.85	4.09	15.50	25.75	21.9
Minimum	0	0	0.48	6.91	0.37	0.6
Maximum	100	100	98.90	51.80	100	100
Q_1	4.15	0.01	1.68	8.43	10.3	8.15
Q_3	39.15	32.70	14.23	43.35	56.92	47.95
Children (n)	59	59	21	1	37	37
Gender (f/m)	20/39	20/39	lug-14	0/1	13/24	13/24
Age (years)	11.44 ± 3.65	11.44 ± 3.65	11.38 ± 3.53	10	11.51 ± 3.81	11.51 ± 3.81
IgE (kU_A/L)						
Mean ± SD	43.25 ± 37.39	31.71 ± 37.11	20.62 ± 30.63	18	57.25 ± 34.34	50.07 ± 35.80
Median	29.90	15.30	7.92	18	52.4	41.6
Minimum	0.20	0	0.48	18	4.59	0.6
Maximum	100	100	98.90	18	100	100
Q_1	8.00	0.01	2.98	18	24.4	18.75
Q_3	91.70	49.00	18	18	100	100
Adults (n)	129	129	47	3	79	79
Gender (f/m)	75/54	75/54	33/14	3/0	61/68	61/68
Age (years)	31.43 ± 11.40	31.43 ± 11.40	32.49 ± 12.01	43.33 ± 0.58	30.34 ± 11.0	30.34 ± 11.0
IgE (kU_A/L)						
Mean ± SD	20.42 ± 25.24	15.94 ± 23.67	9.16 ± 14.46	23.90 ± 24.35	27.90 ± 27.81	25.11 ± 25.77
Median	10.60	5.64	3.28	13	18.1	15
Minimum	0	0	0.49	6.91	0.37	0.65
Maximum	100	100	84.7	51.8	100	100
Q_1	2.92	0.00	1.59	n	7.4	6.52
Q_3	26.75	21.40	11.3	n	33.9	35.4

Group I: monosensitization to Phl p1; group II: monosensitization to Phl p5b; group III: sensitization to both Phl p1 and Phl p5b; f: female; m: male; SD: standard deviation. Descriptive statistic: mean, standard deviation, median, minimum, maximum, Q_1: first quartile and Q_3: third quartile.

Figure 2 - *Graphical representation of IgE levels (kU_A/L) for allergens Phl p1 and Phl p5b (y-axis) in the total group and children and adults' subgroups (x-axis).*

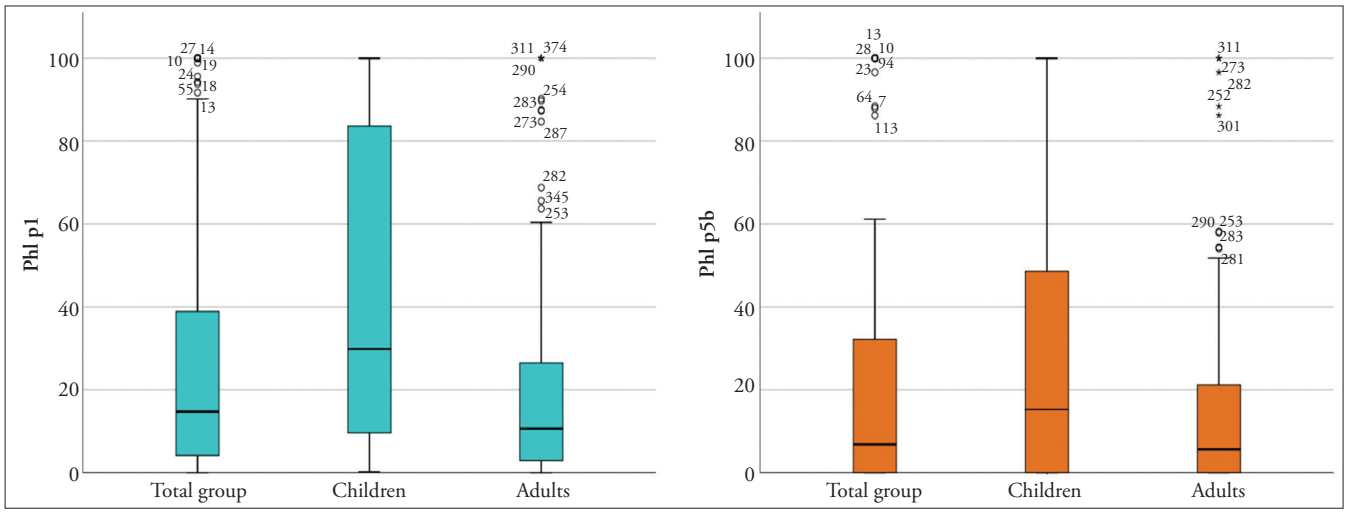


Table IV - *Characteristics of sensitization to Phl p1 and/or Phl p5b according to the area of residence.*

	Total Group		Group I	Group I	Group III	
	Phl p1	**Phl p5b**	**Phl p1**	**Phl p5b**	**Phl p1**	**Phl p5b**
Coastal areas						
Total (n)	123	123	48	3	72	72
Gender (f/m)	66/57	66/57	28/20	02-gen	36/36	36/36
Age (years)	25.45 ± 13.36	25.45 ± 13.36	25.90 ± 13.32	32 ± 19.05	24.88 ± 13.29	24.88 ± 13.29
IgE (kU_A/L)						
Mean ± SD	25.91 ± 31.99	18.76 ± 29.05	12.34 ± 21.35	27.6 ± 21.11	36.04 ± 34.64	30.89 ± 32.36
Median	11.3	5.3	3.42	18	22.7	15
Minimum	0	0	0.49	13	0.37	0.6
Maximum	100	100	98.9	51.8	100	100
Q_1	3.07	0	1.58	N	8.14	6.45
Q_3	32	26.5	13.55	N	57.08	44.88
Inland areas						
Total (n)	65	65	20		44	44
Gender (f/m)	29/36	29/36	12-ago	1/0	16/28	16/28
Age (years)	24.6 ± 13.57	24.6 ± 13.57	26.15 ± 16.24	44	23.45 ± 12.10	23.45 ± 12.10
IgE (kU_A/L)						
Mean ± SD	30.75 ± 30.07	24.92 ± 29.88	13.58 ± 21.57	6.91	39.26 ± 30.12	36.64 ± 29.87
Median	20.1	15.0	4.51	N	29.4	26.7
Minimum	0.01	0.00	0.48	N	1.87	1.03
Maximum	100	100	93.80	N	100	100
Q_1	7.03	0.01	2.04	N	14.95	14.18
Q_3	43.45	41.1	15.43	N	56.83	52.68

Group I: monosensitization to Phl p1; group II: monosensitization to Phl p5b; group III: sensitization to both Phl p1 and Phl p5b; f: female; m: male. Descriptive statistic: mean, standard deviation, median, minimum, maximum, Q_1: first quartile and Q_3: third quartile.

Phl p5b molecular allergens (group III). IgE levels of the total group are also represented in **figure 2**. In the total group of 188 patients, 72 (38.3%) patients were sensitized to only one molecular allergen, while the majority, 116 patients (61.7%), were sensitized for both molecular allergens. Concerning age subgroups, the same pattern was observed (62.7% of the children and 61.2% of the adults were sensitized to both allergens). Levels of IgE to Phl p1 were higher than IgE to Phl p5b, in total group and in both subgroups, children and adults ($p < 0.001$, $p = 0.002$ and $p < 0.001$ respectively). Higher levels of both molecular allergens were found in children when compared to adults (Phl p1 $p < 0.001$ and Phl p5b $p = 0.016$).
Concerning patients of group III (sensitized to both allergens), Phl p1 levels were higher than Phl p5b, besides no statistical difference was found ($p = 0.074$). In children and adult groups, Phl p1 levels were also higher than Phl p5b ($p = 0.162$ and $p = 0.208$, respectively). Comparing children to adult groups, children have higher IgE levels than adults of both Phl p1 and Phl p5b ($p < 0.001$).
Considering the 72 patients with only one sensitization, 68 (94.4%) were only sensitized to Phl p1 (Phl p5b negative, representing 36.2% of the total group) and only four patients (5.6%) were sensitized to Phl p5b (Phl p1 negative, representing 2.1% of the total group). In group I, children also showed higher levels of IgE comparing to adults. Although the sample of group II is small, the 4 patients exclusively sensitized to Phl p5b showed higher IgE levels comparing to IgE levels of group I patients.
Levels of IgE to Phl p1 in group III were significantly higher than in group I ($p < 0.001$).
Considering the area of residence, the 188 patients' group was organized into two groups, according on whether they lived inland or coastal (**table IV**). Either patients living in coastal or inland areas showed IgE levels of Phl p1 higher than IgE to Phl p5b ($p < 0.001$ and $p = 0.009$, respectively). This could be mainly because the number of monosensitized patients to Phl p1. If we focus on group III, IgE to Phl p1 is also higher, but the difference is not significative ($p = 0.106$ for coastal and $p = 0.414$ for inland). Considering group II, only three patients from coastal areas (2.4% of coastal residents) and one patient from inland (1.5% of inland residents) were monosensitized to Phl p5b. No significant differences were found between IgE coastal levels and IgE inland levels.

Discussion and conclusions

Phl p1 and Phl p5 are considered major allergens – allergens recognized by IgE antibodies in over 50% of allergic patients to an allergen source (3). Both are also considered markers of genuine sensitization to timothy grass pollen. Sensitization to Phl p1 is the most prevalent component sensitization, with a sensitization prevalence of > 90% and Phl p5 has a lower sensitization prevalence with specific IgE present in 65-90% of grass pollen allergic patients (9). Besides less prevalent, Phl p5 often has higher IgE levels (3). In our analysis group we found sensitization to Phl p1 in 97.87% of the patients and sensitization to Phl p5b in 63.83%. Similar frequency percentages were found between children and adults for both allergens (respectively 98.3% of children and 97.67% of adults for Phl p1, and 64.40% of children and 63.57% of adults for Phl p5b).
Besides Phl p1 is not the only marker of genuine sensitization, IgE to other markers (Phl p2, Phl p5 and Phl p6) are rarely observed in the absence of IgE to Phl p1 (3). According to our data, the majority of the patients (61.7%) showed sensitization to both genuine markers or monosensitization to Phl p1 (36.2%). We only found four patients (2.1%) with exclusive sensitization to Phl p5b (Phl p1 negative) and these four patients showed, in fact, higher IgE levels to Phl p5b comparing to monosensitization levels to Phl p1. Other authors also showed that monosensitization to Phl p5 is rare (10).
Sensitization to *Phleum pratense* molecules usually start in early childhood with an IgE response to Phl p1 that later grows by involving other grass molecules (10). Hatzler *et al*. studied the progression of IgE sensitization responses against grass pollen showing that in > 75% of cases, Phl p1 sensitization precedes other grass pollen sensitizations (11). In our population, only one child had exclusive sensitization to Phl p5b without any IgE response to Phl p1. Comparing children and adults, except in group II, children showed higher IgE levels.
Our data showed also higher levels of IgE in polysensitized patients comparing to IgE levels of monosensitized patients. According to geographic area we found similar frequencies. Sensitization to Phl p1 was found in 97.56% and 98.46% in cosatal and inland areas respectively. The frequency of Phl p5b sensitization was slightly higher in inland comparing to coastal, with 69.2% *versus* 60.98% of the patients. On the other hand, monosensitization to Phl p1 was lower in inland comparing to coastal, 30.77% *versus* 39.0% of the patients.
There are no other studies about molecular sensitization to pollen in the North-Central region of Portugal. Tavares *et al*. analyzed sensitization to pollens based on skin prick tests (12). Sensitization to grass pollen was detected in 72.0% of the patients with pollen sensitization, of which 27.6% were also sensitized to the pan-allergen profilin (Pho d2). Monosensitization to grass pollen was not associated with sensitization to profilin. Considering the large extent of cross-reactivity between plant profilins, sensitivity to Phl p12 will probably have a similar frequency (*Phleum pratense* profilin, minor allergen).
In the Southern region of Portugal, a characterization of molecular profiles of *Phleum pratense* pollen was made. Almeida *et al*. also found that the majority of patients were sensitized to Phl p1; patients sensitized to both allergens had higher IgE levels; monosensitization frequency to Phl p1 was similar (40.0% *versus* 36.2%), and children also showed higher mean IgE levels than adults (13). On

the other hand, in the North-Central region, mean IgE levels were higher comparing to the South, both for children (43.25 kU/L *versus* 22.49 kU/L and 31.71 kU/L *versus* 20.23 kU/L, respectively, for Phl p1 and Phl p5b) and adults (20.42 kU/L *versus* 10.46 kU/L and 15.96 kU/L *versus* 8.43 kU/L, respectively for Phl p1 and Phl 5b). Sensitization to Phl p5b was more frequent in our data (63.83% *versus* 44.4%) while sensitization to Phl p1 was > 90% in both studies. IgE to Phl p1 seems to be the most important molecular allergen of *Phleum pratense* in Portugal.

Barber *et al*. measured IgE to the different allergens in different regions of Spain (14), including Phl p1 and Phl p5. Spain borders Portugal and, in particular, the provinces of Salamanca and Cáceres border the central region of Portugal (districts of Guarda and Castelo Branco, respectively). The authors found a very high prevalence of Phl p1 (> 76%) with IgE median of 10.83 kU/L in both provinces. In Portuguese inland areas, prevalence of Phl p1 was also very high (97.56%) but the median IgE seems to be higher (20.1 kU/L). Considering sensitization to Phl p5, Spanish border regions showed a prevalence between 26-50% *versus* 69.2% in Portuguese inland areas, with median IgE to Phl p5 of 14.52 kU/L (Salamanca) and 9.31 kU/L (Cárceres) *versus* 15.0 kU/L in Portuguese inland areas.

We acknowledge study limitations. As a retrospective study, it was only possible to analyze the previous available results facing the limitation that not all patients were tested for all allergens. Furthermore, no correlation was made with clinical severity of allergic disease.

In conclusion, our characterization of timothy grass pollen molecular pattern showed that, in the North-Central region of Portugal, Phl p1 sensitization is more common than Phl p5b. Besides both being major and also markers of genuine sensitization, when considering AIT, Phl p1 would be more sensible. IgE to Phl p5b should not be considered alone, not least because monosensitization to Phl p5b is rare. As Phl p5b sensitization can also indicate long-term disease, in children and/or patients with recent onset disease it may still not be detected, which can lead to wrong conclusions.

Fundings

None.

Contributions

IN: conceptualization, methodology, investigation, data curation, formal analysis, writing – original draft, writing – review & editing. GL: conceptualization, methodology, writing – review & editing. BT: conceptualization, formal analysis, writing – review & editing. ATB: writing – review & editing. RC: resources, writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

References

1. 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.
2. Camacho I, Caeiro E, Nunes C, Morais-Almeida M. Airborne pollen calendar of Portugal: a 15-year survey (2002-2017). Allergol Immunopathol (Madr). 2020;48(2):194-201. doi: 10.1016/j.aller.2019.06.012.
3. Matricardi PM, Kleine-tebbe J, Hoffmann J, Valenta R, Ollert M, Ree R Van, et al. EAACI Molecular Allergology User's Guide. Pediatr Allergy Immunol. 2016;27 Suppl 23:1-250. doi: 10.1111/pai.12563.
4. Caeiro E, Camacho IC, Lopes L, Gaspar Â, Todo-Bom A, Ferraz De Oliveira J, et al. Análise das concentrações de pólen de gramíneas na atmosfera de Portugal Continental. Rev Port Imunoalergologia. 2014;22(2):125-38. Available at: http://hdl.handle.net/10400.13/3069.
5. Asero R, Mistrello G, Amato S. Detection of pan-allergens in commercial pollen extracts for allergen immunotherapy. Ann Allergy Asthma Immunol. 2016;117(2):180-5. doi: 10.1016/j.anai.2016.05.010.
6. Tripodi S, Frediani T, Lucarelli S, MacR F, Pingitore G, Di Rienzo Businco A, et al. Molecular profiles of IgE to Phleum pratense in children with grass pollen allergy: Implications for specific immunotherapy. J Allergy Clin Immunol. 2012;129(3):834-9.e8. doi: 10.1016/j.jaci.2011.10.045.
7. Sastre J, Sastre-Ibañez M. Molecular diagnosis and immunotherapy. Curr Opin Allergy Clin Immunol. 2016;16(6):565-570. doi: 10.1097/ACI.0000000000000318.
8. Douladiris N, Savvatianos S, Roumpedaki I, Skevaki C, Mitsias D, Papadopoulos NG. A molecular diagnostic algorithm to guide pollen immunotherapy in southern Europe: towards component-resolved management of allergic diseases. Int Arch Allergy Immunol. 2013;162(2):163-72. doi: 10.1159/000353113.
9. Pablos I, Wildner S, Asam C, Wallner M, Gadermaier G. Pollen Allergens for Molecular Diagnosis. Curr Allergy Asthma Rep. 2016;16(4):31. doi: 10.1007/s11882-016-0603-z.
10. Cipriani F, Mastrorilli C, Tripodi S, Ricci G, Perna S, Panetta V, et al. Diagnostic relevance of IgE sensitization profiles to eight recombinant Phleum pratense molecules. Allergy. 2018;73(3):673-82. doi: 10.1111/all.13338.
11. Hatzler L, Panetta V, Lau S, Wagner P, Bergmann RL, Illi S, et al. Molecular spreading and predictive value of preclinical IgE response to Phleum pratense in children with hay fever. J Allergy Clin Immunol. 2012;130(4):894-901.e5. doi: 10.1016/j.jaci.2012.05.053.
12. Tavares B, Machado D, Loureiro G, Cemlyn-Jones J, Pereira C. Sensitization to profilin in the Central region of Portugal. Sci Total Environ. 2008;407(1):273-8. doi: 10.1016/j.scitotenv.2008.08.013.
13. 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):579-84. doi: 10.1016/j.aller.2019.04.006.
14. Barber D, De La Torre F, Lombardero M, Antépara I, Colas C, Dávila I, et al. Component-resolved diagnosis of pollen allergy based on skin testing with profilin, polcalcin and lipid transfer protein pan-allergens. Clin Exp Allergy. 2009;39(11):1764-73. doi: 10.1111/j.1365-2222.2009.03351.x.