



# Persistent allergic rhinitis with high sensitization to grass pollen: personalized medicine can identify immunotherapy patients who are truly allergic to pollen

*Rinite alérgica persistente com elevada sensibilização a pólen de gramíneas: a medicina personalizada pode identificar os verdadeiros alérgicos a polens na imunoterapia*

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## ABSTRACT

This study aimed to evaluate patients with persistent allergic rhinitis who are sensitized to house mites and have high sensitization to grass pollen without seasonal symptoms. Molecular diagnosis was used to determine patients truly allergic to grass pollen. This retrospective study analyzed the medical records of patients from areas of Caxias do Sul and nearby municipalities (all with the same climatic characteristics) in the state of Rio Grande do Sul, Brazil between 2016 and 2017. Fifty patients allergic to dust mites were selected through a prick test (papule  $\geq 5$  mm) and grass pollen (papule  $\geq 7$  mm), but were asymptomatic in the spring. A total of 52% were female, and their ages ranged from 4 to 56 (mean 26.6) years. Specific serum IgE levels for grass pollen antigens, such as Phl p1, Phl p5, and Cyn d1, were investigated in all patients. Thirteen patients (26%) were diagnosed with at least one studied molecular antigen. The restricted sample included 5 (10%) patients with Phl p5 > Phl p1, ie, truly allergic to the *Pooideae* subfamily, while 2 (4%) had Cyn d1 (*Chloridoideae* subfamily) > Phl p1. The results indicate that among patients with persistent allergic rhinitis polysensitized to mites and grass pollen but without characteristic seasonal symptoms, molecular tests can diagnose those who are truly allergic to pollen.

**Keywords:** Mites, pollen, allergic rhinitis, diagnosis, immunotherapy.

## RESUMO

Este trabalho teve como objetivo avaliar pacientes com rinite alérgica persistente, sensibilizados a ácaros domésticos, associado à elevada sensibilização por pólen de gramíneas, sem sintomatologia estacional. Usou-se como método o diagnóstico molecular por componentes para selecionar os verdadeiramente alérgicos ao pólen de gramíneas. Foi realizado um estudo retrospectivo com análise de prontuários de pacientes em áreas de Caxias do Sul e municípios próximos no estado do RS, nos anos de 2016 e 2017, com as mesmas características climáticas. Foram selecionados 50 pacientes com alergia a ácaros, através de teste de punctura (pápula  $\geq 5$  mm) associado ao pólen de gramíneas (pápula de  $\geq 7$  mm) sem sintomatologia na primavera. Um total de 52% era do sexo feminino, a idade variou entre 4 e 56 anos, com uma média de 26,6 anos. Pesquisou-se a dosagem de IgE específica no soro para antígenos moleculares de pólen de gramíneas como estes: Phl p1, Phl p5, Cyn d1, em todos os pacientes. Houve 13 pacientes (26%) com diagnóstico, pelo menos, a um dos antígenos moleculares estudados. A amostra restringida apresentou 5 (10%) deles que possuíam Phl p5 > Phl p1, ou seja, eram verdadeiramente alérgicos à subfamília *Poideae*, enquanto 2 (4%) apresentaram Cyn d1 (subfamília *Chloridoideae*) > Phl p1. O estudo mostra que, em pacientes com rinite alérgica persistente, polissensibilizados a ácaros associados a pólen de gramíneas, sem sintomas estacionais característicos, os testes moleculares podem diagnosticar os verdadeiros alérgicos ao pólen.

**Descritores:** Ácaros, pólen, rinite alérgica, diagnóstico, imunoterapia.

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The occurrence of symptoms of allergic rhinitis can be persistent or seasonal, the latter being particularly related to exposure to allergenic pollens during the pollen season. In southern Brazil, grass pollen triggers symptoms in previously sensitized individuals during spring (September to December).<sup>1,2</sup> The presence of rhinitis and/or bronchial asthma for two or more consecutive years is relatively easy to diagnose, especially when accompanied by conjunctivitis and a positive skin prick test.

House dust mites are the main etiological agents of rhinitis, especially *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Blomia tropicalis*.<sup>3</sup> When associated with grass pollen, it can be difficult to diagnose a true pollen allergy. Patients may not be able to explain or identify the reflex symptoms that occur in their environment during the pollen season (spring). This would be a bias for indicating pollen-specific immunotherapy.

Skin prick tests to aeroallergens are the main resources for the diagnosis of respiratory allergy and can identify IgE-mediated allergic reactions. However, the diagnosis of allergy should not be based solely on skin prick test responses but also on the correlation between symptoms, clinical history, and test results.<sup>3,4</sup>

Component-resolved molecular diagnosis, with a large number of recombinant or purified antigens, is a novel tool that uses biomarkers to achieve a clinical diagnosis of excellence in allergic diseases and to guide specific immunotherapy.<sup>5</sup>

The ImmunoCAP-Solid phase Allergen Chip (ISAC) is an *in vitro* molecular allergy test used to detect IgE antibodies specific for 103 to 112 different recombinant or purified natural allergens from serum or plasma samples. Results are expressed in a range of 0.3 to 100 ISAC standardized units (ISU). The method includes the grass *Phleum pratense* (subfamily: *Pooideae*), which is not found in Brazil but shows extensive cross-reactivity with *Lolium multiflorum* (ryegrass), the main pollen antigen in southern Brazil.<sup>5,6</sup>

IgE antibodies to Phl p1, Phl p2, Phl p5, and Phl p6 recombinant antigens are biomarkers of true sensitization to the *Poaceae* family.<sup>7</sup> Group 5 allergens are restricted to the *Pooideae* subfamily, such as *Lolium multiflorum*, with limited cross-reactivity with components of the *Chloridoideae* and *Panicoideae* subfamilies, which mainly contain group 1 allergens.<sup>5-7</sup>

Combined positivity for Phl p1 and Phl p5 characterizes a true pollen allergy to the *Pooideae* subfamily. This suggests that recombinant *Phleum pratense* allergens could also be used for diagnosis and specific immunotherapy in the population living in southern Brazil.<sup>5</sup>

Molecular allergy diagnosis represents a major contribution to personalized medicine by assisting in the assessment of risk prediction, disease severity, and genuine sensitization/cross-reactivity and in the application of treatment strategies.<sup>8,9</sup>

Component-resolved molecular diagnosis is used to guide the prescription of grass pollen immunotherapy in regions of the world where grass pollen seasons overlap with other types of pollen.<sup>8</sup> However, differently, there are no associated studies in polysensitized individuals that have included house dust mites. The following databases were searched: PubMed and Elsevier, using keywords such as grass pollen, sensitization, perennial symptoms, house dust mites, allergy, molecular diagnosis, and immunotherapy.

The main objective was to evaluate patients with persistent allergic rhinitis caused by house dust mites associated with high sensitization to grass pollen, without characteristic seasonal symptoms or with symptoms difficult to characterize, in a region of pollinosis. Component-resolved molecular diagnosis was used to determine patients with true allergy and screen them for possible pollen-specific immunotherapy.

## Methods

A retrospective study was conducted with a review of the medical records of patients seen at an allergy and immunology clinic in Caxias do Sul, southern Brazil, between 2016 and 2017, who lived in the municipality or nearby regions (all with similar climate and vegetation characteristics).

Fifty patients with persistent allergic rhinitis for two or more consecutive years were included in the study, and the characteristic symptoms (sneezing, itching, rhinorrhea, nasal obstruction) occurred for consecutive days, for more than one hour, in most participants.

Patients had immediate skin prick test results with papules  $\geq 5$  mm in diameter for the following house dust mites: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Blomia tropicalis*.

Papules were  $\geq 7$  mm for mixed grass pollen and *Lolium multiflorum*. Saline solution and histamine (10 mg/mL) served as controls. Papule size was determined by the average of their perpendicular diameters, in relation to the negative control (extracts provided by FDA Allergenic/Immunotech, RJ, Brazil).

We adopted the random cutoff of papule size for the mites ( $\geq 5$  mm), confirming the clinical diagnosis of persistent allergic rhinitis, associated with symptoms. However, we adopted papule  $\geq 7$  mm for grass pollen, which characterizes high sensitization (without seasonal symptoms) capable of causing confusion, requiring the diagnosis of a true allergy.

All patients (or their legal representatives) were informed of the procedures and provided written consent, including laboratory tests, which would define a true pollen allergy and accurate treatment indication.

None of the participants had or reported seasonal symptoms during the grass pollen season (September to December), were taking medication that could affect the test results, or had previously undergone specific immunotherapy with the allergens under study.

We performed a component-resolved analysis of sera from 100% of patients for the presence of IgE antibodies specific for Phl p1, Phl p5, and Cyn d1 antigens using the ImmunoCAP-ISAC (ThermoFischer Scientific®).

## Results

Of the 50 patients included in the study, 52% were female, with a mean age of 26.6 years (median, 25 years; range, 4 to 56 years).

The duration of symptoms was greater than or equal to two consecutive years, with or without associated conjunctivitis.

Thirteen patients (26%) had a positive diagnosis for at least one of the antigens under investigation (Phl p1, Phl p5, and Cyn d1). However, the restricted sample showed a significant change: only 5 patients (10%) were truly allergic to the *Pooideae* subfamily with Phl p5 > Phl p1, whereas 2 (4%) were allergic to the *Chloridoideae* subfamily with Cyn d1 > Phl p1 (Table 1).

## Discussion

Aerobiology studies have identified a grass pollen season in southern Brazil, during spring.<sup>1,10,11</sup> This

coincides with the characteristic symptom complex of pollinosis reported in previously sensitized patients, which repeats annually.

In Brazil, there is no succession of other important allergenic pollens in nature, as observed in other countries, mainly in the Northern Hemisphere. However, there are potent indoor allergens such as dust mites, which, in real life, can act as a diagnostic confounder when associated with grass pollens not only in southern Brazil but also in some regions of the Brazilian tropics.<sup>12</sup> Here, it is possible to identify potential candidates for personalized medicine, also known as precision medicine.

In our environment, a continuous air monitoring system has been used for more than a decade with a Burkard volumetric pollen and spore trap, located on the main campus of the University of Caxias do Sul, in southern Brazil. Grass pollens are the main allergenic pollens and can reach high concentrations in November (spring), ranging from 512 to 949 grains/m<sup>3</sup> of air in that specific area.<sup>10</sup>

It is estimated that most grass pollen-allergic patients show symptoms with daily levels between 30 and 50 grains/m<sup>3</sup> of air. This estimate may be lower in the presence of a preexisting mite-induced inflammatory process, similarly to what occurs in successive pollen seasons.

Patients sensitized to grass pollen, when exposed to the external environment, will have symptoms of rhinitis associated with a high frequency of conjunctivitis characterized mainly by severe ocular itching.<sup>13</sup>

Immediate skin prick testing allows us to confirm, or not, sensitization and atopy when associated with clinical history and physical examination, which altogether enable a diagnosis to be made.

The presence of a mean papule diameter  $\geq 3$  mm, compared with the negative control, associated with a well-circumscribed erythematous plaque > 10 mm, characterizes sensitized patients.<sup>4</sup> Patients selected for inclusion in this study had papules  $\geq 7$  mm in mean diameter for grass pollen, associated with papules  $\geq 5$  mm for dust mites. We acknowledge that high sensitization to grass pollen can make the diagnosis difficult, even for the most experienced physicians, since there is associated persistent allergic rhinitis.

In real life, high pollen sensitization may be included in the pollens associated with perennial antigens in a potential immunotherapy, without a true diagnosis.

The reflex symptoms obtained via patient symptoms could be altered by the use of masks during the period of the COVID-19 pandemic, a fact that did not occur at the time of the study.

Nasal or conjunctival provocation tests with grass allergens would be indicated if there was doubt about the diagnosis.<sup>14</sup> In polysensitized patients, it can be complemented with the measurement of specific IgE levels (ImmunoCAP-ISAC), using components such as Phl p1, Phl p5, and Cyn d1.<sup>5,12</sup>

Cyn d1 (*Cynodon dactylon* - Bermuda grass) is the main pollen allergen of the *Chloridoideae* subfamily, widely distributed in Brazil and with high allergenic potential.<sup>12</sup>

Monosensitization to Phl p1 is related to the detection of low IgE levels for *Lolium multiflorum*, when tests are performed with pollen extracts. However, specific IgE anti-Phl p5 antibody would be a true

allergy biomarker for the *Pooideae* subfamily, rarely found as the only sensitizer.<sup>5,6</sup> In summary, when there is IgE positivity for the association of Phl p5 > Phl p1, defined by their potencies and frequencies, the *Pooideae* subfamily can be considered the cause of pollen allergy.<sup>5</sup> The same is true for the *Chloridoideae* subfamily when Cyn d1 > Phl p1.

Component-resolved molecular diagnosis has been suggested to facilitate the identification of true disease-causing allergens and the prescription of allergen-specific immunotherapy.<sup>9</sup> This information could be extended to the patient.

The discrepancies between the results obtained from the extracts used in the skin prick tests and those from the molecular diagnosis are possibly due to cross-reactivity between allergens from unrelated plant species, such as profilins and other cross-reactive allergens.<sup>9</sup>

**Table 1**

Sample restricted to patients with true allergy: potential candidates for specific immunotherapy, within a group of 50 patients

Age	Sex	Phl p5	Phl p1	Cyn d1	Eosinophils	Total IgE*
26	M	3.37	4.53	26.8	2.5/129	393
41	F	27.3	21.1	3.46	0.5/46	198
37	F	0.1	0.1	2.16	1.4/155	1020
12	F	9.18	3.28	0.14	ND	582.7
26	F	33.8	19.8	3.74	7.4/438.82	108
4	M	45.1	34.8	10.6	10.4/1280	534
32	F	11.4	4.77	2.23	ND	144

- Group of patients with grass pollen allergy: n = 7 (14% of the total).
- Monosensitization to *Cynodon* (Cyn d1).

ISAC Standardized Units (ISU)	Class		
< 0.3			Not detectable
> 0.3 to ≤ 1			Low
> 1 to ≤ 15			Moderado
> 15			High

\* Degree of sensitization (kU/L) - ImmunoCAP.

## Conclusion

Molecular tests for grass pollen, restricted to Phl p1, Phl p5, and Cyn d1, can be included in cases of doubtful diagnosis. This would make the indication for immunotherapy more accurate and reduce costs in polysensitized patients with a difficult diagnosis, not only in the southern region but also in other specific regions of Brazil.

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