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Interference of *Dermatophagoides pteronyssinus* sensitization in grass pollen allergy

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Summary
Background. *Climate conditions in the northwest of Spain are from the rest of the country, and the pollen sensitisation rates and allergens involved are different. The present study aimed to investigate the sensitisation profile of patients with grass pollen allergy and the interference of other sensitisations in respiratory symptoms.* ***Methods.*** *A total of 959 Spanish patients with seasonal respiratory symptoms and a positive skin prick test (SPT) to Phleum pratense pollen were studied. Patients were classified as having rhinitis and/or bronchial asthma. A battery of SPTs, including common weeds and tree pollens, profilin, polcalcin, moulds, Dermatophagoides pteronyssinus, Lepidoglyphus destructor, and cat and dog dander were performed. Serum specific IgE (sIgE) to Phl p 1 and Phl p 5, adding sIgE to Phl p 7, Phl p 12 and house dust mites (HDMs) or other pollens in selected cases were measured.* ***Results.*** *The majority (89.8%) of the patients were polysensitised according to SPT. HDM co-sensitisation was the most prevalent (62.3%). Profilin and polcalcin rendered a positive result in 25.9% and 18.7% of the patients, respectively. A higher proportion of patients recognized sIgE to Phl p 1 (88.7%) with respect to Phl p 5 (59%). Phl p 1-sIgE levels were higher than Phl p 5-sIgE levels, and no differences were found in patients with rhinitis and/or asthma. However, total serum IgE was higher in patients with asthma. Multivariate regression analyses revealed that only sIgE to Dermatophagoides pteronyssinus (after adjusting by sIgE to Phl p 1, Phl p 5 and Lepidoglyphus destructor) was associated with a greater risk of asthma.* ***Conclusions.*** *Phl p 1 is the most relevant allergen in patients with grass pollen allergy in the northwest of Spain. Sensitisation rates against panallergens are low. Even in patients with grass pollen allergy, HDM sensitisation plays a relevant role in asthma.*

Introduction

The northwest of Spain is a wet region with mild temperatures similar to Northern and Central Europe in terms of pollination (1-3). In our health care area, *gramineae* (*Poaceae*) is by far the most relevant pollen from an allergological point of view (1, 4). Other relevant pollens are those from the *Betula* and *Parietaria* genus. However, house dust mites (HDMs) constitute the major source of aeroallergens and are the leading cause of allergic rhinitis and allergic asthma in this region (5-7). In a previous prospective study, we have shown that the majority of patients with HDM allergy without any other sensitisation recall seasonal changes in symptoms (8), making it difficult to differentiate seasonal pollen symptoms from HDM allergies in polysensitised patients.

Over the last 3 decades, the emergence of molecular diagnosis has been considered an essential tool for the diagnosis and treatment of allergic diseases (9-14). However, molecular diagnoses must be complemented with a clinical approach and a profound knowledge of the main allergens in a specific area. Regarding grass pollen allergy, the grass species commonly used as a model is *Phleum pratense*, in which more than 13 different allergens have been identified (15). Although sensitisation rates for every

allergen component vary from region to region, it has been accepted that Phl p 1 and Phl p 5 are major allergens from *Phleum pratense*, found in 95% and 65%-85% of patients, respectively (14, 16). The presence of specific immunoglobulin E (sIgE) against Phl p 1 and Phl p 5 is considered to indicate genuine sensitisation to grass pollen. In contrast, profilin (Phl p 7) and polcalcin (Phl p 12), widely distributed among pollens and vegetables, are known as panallergens and could be considered as markers of disease progression, polysensitisation, and/or provoking type II food allergy symptoms (4, 17). Their sensitisation rate varies from 10% to 20% (4) in Spanish populations but reaches 30% when including patients from other European countries (18, 19). The present study aimed to investigate the sensitisation profile of our patients with grass allergy and the interference of other sensitisation in the interpretation of the clinical pattern, the type of respiratory symptoms and/or food allergy.

Patients and methods

Study population and design

This was a cross-sectional study that enrolled 959 adults with seasonal respiratory symptoms and/or symptoms compatible with oral allergy syndrome (OAS) and a positive skin prick test (SPT) to *Phleum pratense* pollen who first attended our reference University Hospital Allergy Department in northwest Spain from January 2007 to December 2011. Patients were referred either from Primary Care or from the Ear, Nose and Throat Department. The hospital covers an area of approximately 500,000 people; nearly 90,000 live in the city of Santiago de Compostela, and the remainder live in primarily rural areas. The median age was 26 years (interquartile range (IQR), 17-35 years) and 513 (53.5%) were female. The local weather is warm and humid, and as previously stated, grass pollen is the most relevant pollen in the area (4) even though HDM sensitisation is higher than pollen sensitisation (5). Patients were classified according to their clinical histories and spirometry values (FEV1 increase greater than 12% and 200 mL after bronchodilator test) as having rhinitis or rhinoconjunctivitis (n = 553, 57.7%), asthma (n = 17, 1.8%) or both (n = 377, 39.3%). The remaining 11 (1.1%) patients did not meet the clinical criteria for respiratory allergy despite having a positive SPT. A physician-administered questionnaire was completed for every patient, including data on symptoms after eating vegetables and fruits.

Complementary studies

SPTs were performed on the volar surface of the forearm using a battery of common allergens in the region, including *Phleum pratense*, *Plantago lanceolata, Parietaria judaica, Artemisia vulgaris, Betula alba, Alnus glutinosa, Olea europaea, Fraxinus excelsior, Quercus robur, Platanus acerifolia,* purified natural date palm profilin (Pho d 2 50 µg/mL), date palm polcalcin, *Dermatophagoides pteronyssinus, Lepidoglyphus destructor, Alternaria alternata, Aspergillus fumigatus* and cat and dog dander (ALK-Abelló, Madrid, Spain).
Total serum IgE was measured by latex-enhanced nephelometry in a BN-II analyser (Siemens, Germany). Allergen-sIgE was measured by the Immuno-CAP-250™ system (Thermo-Fisher Scientific, Sweden) and included sIgE against Phl p 1 and Phl p 5 as molecular markers of grass pollen sensitisation and sIgE against house dust mites or other pollens if a positive SPT was found with presumably clinical relevance. In patients with a positive SPT against profilin and/or polcalcin and patients with symptoms of OAS, food allergy or positive SPT against pollen allergens different from grasses, sIgE to Phl p 7 and/or Phl p 12 were also measured. Following the manufacturer's instructions, sIgE levels ≥ 0.1 kU_A/L were deemed positive; for analyses, however, the classic 0.35 kU_A/L threshold level was used.

Statistical analyses

Continuous variables were expressed as median and IQR. Categorical variables were expressed as absolute numbers and percentages. Spearman´s rank tests were employed for evaluation of correlation of continuous variables. Logistic regression analysis was employed to estimate the association between serum specific IgEs and asthma. Statistical significance was accepted at $p < 0.05$. All analyses were developed using the Statistical Package for Social Sciences (IBM SPSS, v. 19).

Ethics

The study was approved by the Institutional Review Board of Complejo Hospitalario Universitario de Santiago de Compostela and complied with the recommendations of the Declaration of Helsinki.

Results

According to our inclusion criteria, all patients presented a positive SPT to *Phleum pratense*, and the majority of respiratory symptoms could be classified as upper and/or lower airway disease (see Methods). Only 121 (12.6%) patients reported OAS symptoms when eating vegetables or fruits. More than 50% of the patients reported mild respiratory symptoms at home related to dust exposure.
The majority (844, 89.8%) of our patients presented a positive SPT to at least one allergen different from grass pollen. HDM sensitisation was detected in more than 50% of this population (a higher sensitisation rate against *Dermatophagoides pteronyssinus* than against *Lepidoglyphus destructor*, 585 (62.3%) *vs* 408 (43.5%), respectively). The highest sensitisation rates against other pollens were 30.8%, 25.2% and 23.5% against *Betula alba, Olea europaea* and *Parietaria judaica*, respectively. SPT

against profilin and polcalcin rendered a positive result in 249 (25.9%) and 180 (18.7%), respectively.

Specific IgE to Phl p 1 and Phl p 5 was available for 904 and 913 patients, respectively. Regarding grass pollen sensitisation, a higher proportion of patients had sIgE to Phl p 1 (88.7%) with respect to Phl p 5 (59%). Concentrations of Phl p 1-sIgE were higher than those of Phl p 1-sIgE (median, 7.24 kU_A/L (IQR 1.26-22.87 kU_A/L) *vs* median 2.09 kU_A/L (IQR 0.01-17.6 kU_A/L), respectively). A significant correlation was found between Phl p1-sIgE and Phl p 5-sIgE ($R = 0.7$, $P < 0.001$) (**figure 1**).

Serum sIgE against profilin (Phl p 12) was measured in 471 patients (443 because of more than one pollen sensitisation and 249 because of a positive SPT against profilin). Also, sIgE against polcalcin (Phl p 7) was measured in 400 patients (180 with a positive SPT). In both cases, sIgE levels were low (Phl p 7-sIgE median, 0.01 kU_A/L (IQR 0.01-0.07 kU_A/L) and Phl p 12-sIgE median 0.19 kU_A/L (IQR 0.02-1.23 kU_A/L). The concordance of the SPT extracts of profilin and polcalcin and sIgE to the corresponding panallergens was evaluated. A higher diagnostic value was observed for profilin SPT (positive concordance 85.5%; negative concordance 77.98%) than for polcalcin SPT (positive concordance 42.8%; negative concordance 93.6%) (**table I**). Only 60.1% of patients reporting OAS presented a positive result to Phl p 12 in serum.

Concentrations of sIgE to Phl p 1 and sIgE to Phl p 5 did not differ according to the patients' clinical diagnoses, rhinitis and/or asthma (**table II**). However, total serum IgE was significantly higher in patients with asthma with or without concomitant rhinoconjunctivitis (**table II** and **figure 2**). The comparison of total IgE among patients with other pollen sensitisations yielded no differences (data not shown). Nevertheless, patients with HDM sensitisation, especially those sensitised to *Dermatophagoides pteronyssinus*, presented higher levels of both total IgE and also sIgE to *Dermatophagoides pteronyssinus* and *Lepidoglyphus destructor* in patients with asthma (**table II** and **figure 3**). In fact, multivariate regression analyses revealed that only sIgE to *Dermatophagoides pteronyssinus* (after adjusting by sIgE to Phl p 1, Phl p 5, and *Lepidoglyphus destructor*) was significantly associated with a greater risk of asthma (**table II**); the same result was obtained even after adding sIgE to Phl p 7 and sIgE to Phl p 12 as variables into the equation. The risk of asthma significantly increased as the level of sIgE to *Dermatophagoides pteronyssinus* increased (**figure 4**).

Discussion

The results of our study suggest that even in patients with grass pollen allergy, the weight of HDM sensitisation in the clinical expression is high in the northwest of Spain.

Regarding SPT results, almost 90% of our patients with grass pollen allergy were polysensitised. However, the most frequent sensitisation found was to HDM, given 62% of patients showed

Figure 1 *- Scatterplots representing the relationship of serum specific IgE to Phl p 1 with specific IgE to Phl p 5.*

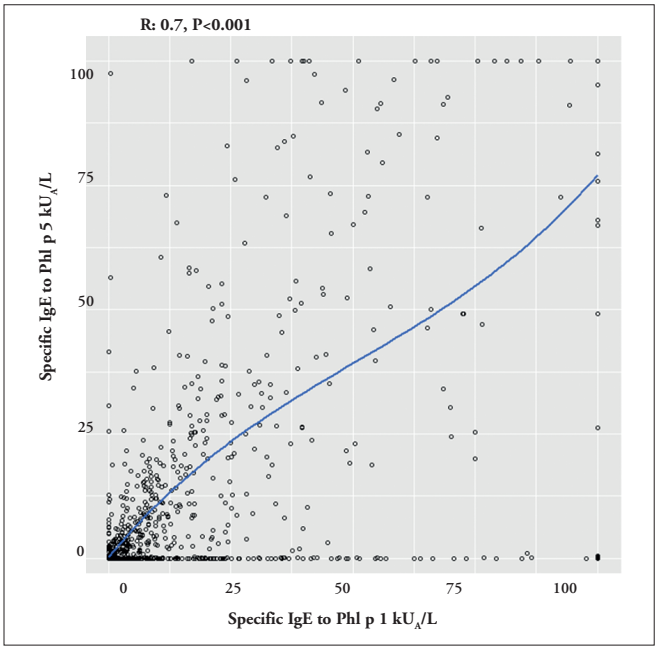


Figure 2 *- Total serum IgE levels (IU/mL) in patients with grass pollen allergy according to the clinical diagnosis of rhinitis and/or bronchial asthma.*

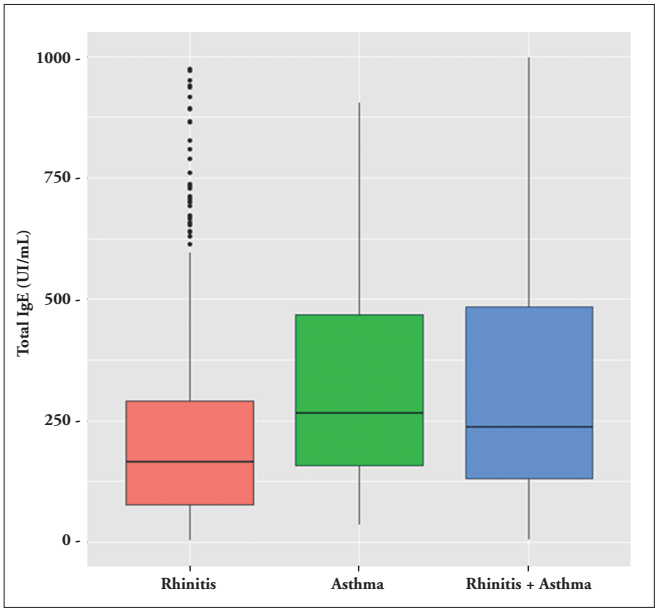


Figure 3 *- Serum specific IgE levels to Dermatophagoides pteronyssinus and Lepidglyphus destructor in patients with grass pollen allergy according to the clinical diagnosis of rhinitis with and/or bronchial asthma.*

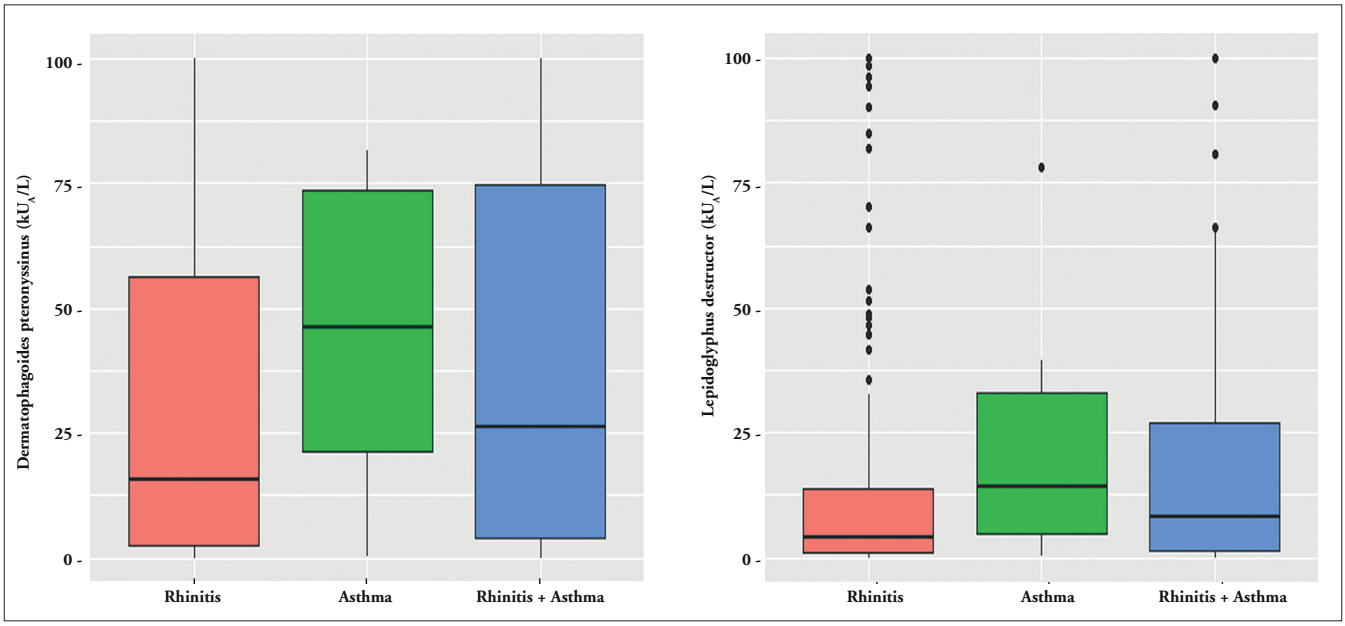


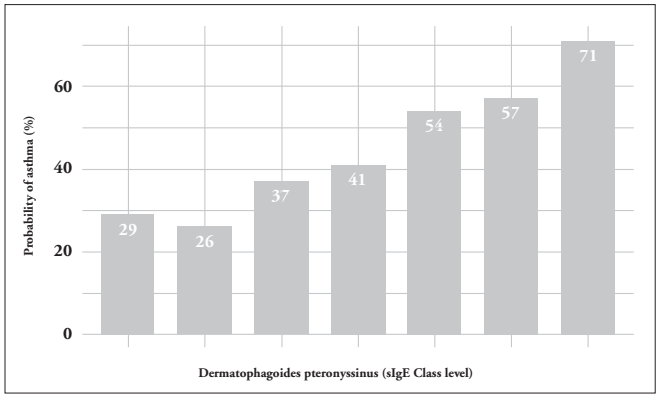
Table I *- sIgE to Phl p 1, Phl p 5, Dermatophagoides pteronyssinus, and Lepidoglyphus destructor and total serum IgE in patients with grass pollen allergy patients with or without bronchial asthma.*

Allergen	Rhinitis (n = 553)	Asthma (n = 17)	Rhinitis + Asthma (n = 377)
Phl p 1	6.6 (1.0-21.7) kU$_A$/L (n = 525)	8.3 (1.6-13.3) kU$_A$/L (n = 14)	7.8 (1.7-23.9) kU$_A$/L (n = 355)
Phl p 5	1.5 (0.0-14.7) kU$_A$/L (n = 527)	5.7 (0.0-17.3) kU$_A$/L (n = 15)	2.4 (0.0-22.5) kU$_A$/L (n = 362)
Dermatophagoides pteronyssinus	30.1 (6.8-67.3) kU$_A$/L (n = 292)	42.7 (19.6-78.9) kU$_A$/L (n = 11)	44.2 (12.7-99.9) kU$_A$/L (n = 214)
Lepidoglyphus destructor	4.3 (1.1-14.1) kU$_A$/L (n = 179)	14.1 (3.4-37.2) kU$_A$/L (n = 8)	8.5 (1.6-27.2) kU$_A$/L (n = 146)
Total IgE	183 (90-399) UI/mL*	282 (157-533) UI/mL	289 (141-725) UI/mL

sIgE: specific IgE. Figures are shown as median (IQR). Cases with sIgE above the analytical limit (100 kUA/L) were deemed to have 100 kUA/L. (*) Patients with rhinitis presented lower levels of total serum IgE than patients with asthma (with or without concomitant rhinitis) ($p < 0.001$).

a positive response to *Dermatophagoides pteronyssinus*, an allergen commonly found in this region (5-7, 20). Only 47.1% of patients presented positive SPTs to pollens different from grass pollen, a lower prevalence than the 70% reported in other regions in Spain (21). The prevalence of sensitisation to profilin and polcalcin was similar to that reported by other authors in Spain (22-25); however, the prevalence of OAS in our population was at least 3-fold lower than that reported by the same authors. A possible explanation for the low rate of OAS in our patients could be a lower sIgE level against Phl p 7 and Phl p 12 in our patients, perhaps indicating a lower environmental exposure (17). Taking into account the low rate of OAS in our population, the concordance between sIgE determination and a positive SPT to profilin and that only 60% of patients with OAS presented positive sIgE to Phl p 12, we suggest that SPT is enough for the diagnoses (4).

Figure 4 *- Prevalence of asthma in grass pollen allergic patients with co-sensitization against HDM (Dermatophagoides pteronyssinus) in relation to the level of specific IgE to Dermatophagoides pteronyssinus scored in classes: Class 0: no detection of sIgE < 0.35kUA/L; Class 1: 0.36-0.70 kUA/L; Class 2: 0.71-3.50 kUA/L; Class 3: 3.51-17.5 kUA/L. Class 4: 17.51-50 kUA/L; Class 5: 50-100 kUA/L; Class 6: > 100 kUA/L.*



With respect to the sensitisation profile against grass pollen, Phl p 1 is both more frequently detected and has higher sIgE levels, as has been established in previous series in Europe (4, 11, 26). A few studies have noted the relationship between sensitisation profile and the severity of the respiratory disease or its clinical expression. Savi et al had studied 140 patients with grass pollen allergy and found that patients without asthma presented low values of sIgE to Phl p 5, suggesting that high levels of sIgE to Phl p 5 could be used as markers for potential risk of future asthma (27). We did not find the same association. In fact, neither sIgE-Phl p 1 nor sIgE-Phl p 5 were related to asthma. However, our patients with asthma presented not only higher levels of total IgE but also higher levels of sIgE against HDM, supporting the hypothesis that a more severe allergic respiratory disease is associated with higher levels of both total IgE (28, 29) and sIgE (30). However, and even though the study was performed in a population of patients with grass pollen allergy, our results led us to suspect that the weight of HDM sensitisation is what really mattered in our patients. This idea is supported by the result of the multivariate analysis, which demonstrated that the only variable relevant in asthmatic patients was HDM sensitisation, specifically sensitisation against *Dermatophagoides pteronyssinus*, the main allergen in our region (5-8, 20). Furthermore, the higher level of sIgE to *Dermatophagoides pteronyssinus,* the higher the risk of asthma. From a clinical standpoint, these results might be of importance, given dual sensitisation against grass pollen and *Dermatophagoides pteronyssinus* could help identify persons at risk of asthma in regions with similar levels of exposure to HDM. Nevertheless, a clinical problem arises when both sensitisations, grass pollen and HDM, coexist in the same patient. In an observational, Internet- and telephone-based prospective survey performed in Italy, France, and Spain of 313 patients with HDM allergy, 67% of the Spanish patients with HDM allergy reported co-sensitisation to grass pollen (8). The problem is that even 74% of patients allergic to HDM alone reported moderate seasonal variations in symptom scores, with peaks in spring and autumn, and these recalled seasonal changes could not be explained by concomitant, intermittent grass pollen allergy, but by the HDM sensitisation itself (8, 31, 32).

Table II *- Analysis of variables associated with bronchial asthma.*

	Coefficient (B)	SE (B)	P-value
sIgE to Phl p 1	0.002	0.008	0.753
sIgE to Phl p 5	0.007	0.008	0.346
sIgE to *Dermatophagoides pteronyssinus*	0.017	0.004	< 0.001
sIgE to *Lepidoglyphus destructor*	0.004	0.007	0.588

Multiple regression analysis. All selected variables entered the equation. B: slope of the regression model. SE: standard error.

Conclusions

Taken together, these results suggest Phl p 1 is the most relevant allergen in patients with grass pollen allergy in the northwest of Spain, where profilin and polcalcin appear to be of low relevance. However, putting aside the sensitisation profile of patients with grass pollen allergy, HDM sensitisation is the most important variable that influences the clinical expression of respiratory symptoms.

Conflict of interests

The authors declare that they have no conflict of interests.

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