ORIGINAL ARTICLE

Seasonal Local Allergic Rhinitis in Areas With High Concentrations of Grass Pollen

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Abstract

Background: Local allergic rhinitis (LAR) is a phenotype of allergic rhinitis characterized by the presence of a localized immune response in the nasal mucosa of patients with negative skin prick test (SPT) results and undetectable serum specific IgE (sIgE). It unknown whether LAR is limited to areas with low or moderate aeroallergen exposure.
Objective: To explore the presence of LAR and the clinical and immunological characteristics of this entity in geographic areas with high grass pollen loads.
Methods: A cross-sectional observational study was carried out in 2 hospitals in central Spain (Madrid and Ciudad Real). Sixty-one patients with seasonal rhinitis and negative SPT results and undetectable serum sIgE were evaluated using a clinical questionnaire, determination of serum total IgE, and a nasal allergen provocation test (NAPT) with *Phleum* species. The response to NAPT was monitored using assessment of nasal symptoms, acoustic rhinometry, and determination of sIgE, tryptase, and eosinophil cationic protein in the nasal cavity.
Results: Seasonal LAR was detected in 37 patients (61%) using the techniques described above. Eleven percent of patients with LAR were adolescents or children, and 14% reported onset of rhinitis in childhood. Most patients reported persistent-moderate seasonal nasal symptoms, and 41% reported worsening of the disease during the last 2 years. Conjunctivitis was the most common comorbidity, affecting 95% of cases.
Conclusions: LAR to grass pollen is relevant in patients with seasonal symptoms indicative of allergic rhinitis but with a negative skin test result who live in areas with high allergenic pollen loads. This entity should be included the differential diagnosis of rhinitis.

Key words: Grass pollen. Local allergic rhinitis. Local specific IgE. Nasal allergen provocation test.

Resumen

Introducción: La rinitis alérgica local (RAL) es un fenotipo de rinitis alérgica (RA) caracterizado por la presencia de una respuesta inmunológica localizada en la mucosa nasal de pacientes con pruebas cutáneas (PC) negativas e IgE específica (sIgE) sérica no detectable. Se desconoce si la RAL es una entidad limitada a áreas con baja o moderada exposición a aeroalérgenos.
Objetivos: Explorar la presencia y características clínico-inmunológicas de la RAL en áreas geográficas con alta concentración atmosférica de polen de gramíneas.
Métodos: Estudio observacional-transversal realizado en dos hospitales de la zona centro de España (Madrid y Ciudad Real). Sesenta y un pacientes con rinitis estacional, PC negativas y sIgE sérica no detectable fueron evaluados mediante cuestionario clínico, IgE total sérica, y test de provocación nasal con *Phleum* (TPN-*Phleum*). La respuesta al TPN se monitorizó mediante síntomas, rinometría acústica, y determinación de sIgE, triptasa y proteína catiónica de eosinófilos en secreciones nasales.
Resultados: Se detectó RAL estacional en 37 pacientes (61%) mediante TPN-*Phleum*. El 11% de los pacientes eran adolescentes o niños, y el 14% habían comenzado con los síntomas en la infancia. La mayoría presentaban rinitis estacional persistente-moderada, y el 41% refirió empeoramiento en los 2 últimos años. La conjuntivitis fue la enfermedad asociada más frecuente, afectando al 95% de los sujetos con RAL.
Conclusiones: La RAL por polen de gramíneas es una enfermedad frecuente en pacientes con síntomas indicativos de RA estacional y PC negativas que viven en áreas con alta concentración atmosférica de pólenes, y debe ser incluida en el diagnostico diferencial de la rinitis.

Palabras clave: Polen de gramíneas. Rinitis alérgica local. IgE específica local. Test de provocación nasal.

Introduction

In 1975, Huggins and Brostoff [1] first detected local specific IgE (sIgE) to *Dermatophagoides pteronyssinus* in the nasal secretions of individuals with negative skin prick test (SPT) results and undetectable sIgE in serum [1]. Since then, various studies have confirmed the existence of an in situ allergic response in patients with nonatopic rhinitis [2-10]. This clinical entity, known as local allergic rhinitis (LAR) [8] or entopy [3], is considered a new phenotype of allergic rhinitis (AR) that must be differentiated from nonallergic rhinitis [11-14].

The immunological characteristics of LAR include a T_H2 pattern of nasal mucosal inflammation [3-6,15] with local production of sIgE [1,5,6] and a positive response to a nasal allergen provocation test (NAPT) [2,5-7,10]. LAR is a common respiratory disease in young people [8-10,16], can affect up to 25.7% of persons in some populations, and requires an allergy workup for confirmation [16]. It can affect individuals from different countries and ethnic groups [2,5,7,10,17-20]. A preliminary observational study showed that specific immunotherapy with grass pollen is a safe and effective treatment in LAR, with significant improvement in nasal tolerance to NAPTs and a clinical response to natural exposure to the allergen [21]. However, the influence of environmental factors in the development of LAR is not well known, and it remains unclear whether LAR is limited to geographic areas with low or moderate atmospheric concentrations of aeroallergens or can also occur in areas of higher concentrations.

LAR to grass pollen has been detected in 62.5% of patients with nonatopic seasonal rhinitis evaluated in Málaga, Spain [6]. This city, which is located on the Mediterranean coast, has one of the lowest pollen records in the Autonomous Community of Andalusia. A general increase in the pollen index, peak value, and severity has been detected in recent years. The pollen index ranged from 887 in 2005 to 4570 in 2007, and the peak value from 54 grains/m^3 in 1992 to 815 grains/m^3 in 2003. The severity of the season was seen in its increased duration, from 3 days (25 grains/m^3) in 1992 to 38 days in 2007 [22].

In order to explore the clinical and immunological characteristics of LAR in geographic areas with a high allergenic load, we undertook collaborative studies in several centers in Spain and other countries and compared the results with those of previous studies performed in areas with a low-moderate atmospheric allergen concentration. The methodology used in these studies was the same as previously reported [6,8,16].

We present our findings from the first of these studies, which were carried out in 2 hospitals in central Spain (Madrid and Ciudad Real), a large region with a high atmospheric concentration of grass pollen.

Methods

Patients

We performed a cross-sectional observational study in 70 adults and children diagnosed with seasonal nonallergic rhinitis from 2011 to 2013 in Hospital Infanta Leonor, Madrid, Spain and Hospital General, Ciudad Real, Spain.

These centers cover an area in central Spain with similar climates and an intense grass pollen season, reaching peaks of 585 grains/m^3 and annual totals of 4500 grains/m^3 [23]. The local ethics committees approved the study, and all participants and parents gave written their informed consent.

The inclusion criteria were age 6-70 years, clinical history of seasonal rhinitis during grass pollen season over a period of at least 2 years, negative SPT results to a wide panel of aeroallergens including pollens, mites, molds, and animal epithelium (LETI, SL), and undetectable serum sIgE. The exclusion criteria were pregnancy/breastfeeding, immunological diseases, chronic rhinosinusitis with/without nasal polyposis (nasal endoscopy and/or computed tomography scan), respiratory infections in the previous 4 weeks, and treatment with corticosteroids or antihistamines in the previous 3 weeks.

All patients completed a questionnaire to provide demographic and clinical data and underwent intradermal skin tests (IDST) with *Phleum* species and NAPT with *Phleum* pollen extract (LETI, SL).

Study Groups

The patients included in the study were categorized into 2 groups:

- LAR: seasonal nasal symptoms with positive NAPT, and negative SPT, IDST, and serum sIgE.
- Negative NAPT: seasonal nasal symptoms with negative NAPT, SPT, IDST, and serum sIgE.

In order to evaluate the diagnostic value of NAPT with *Phleum*, we compared the responses to the nasal challenge with the results of SPT and serum sIgE to *Phleum* in 2 control groups:

- Positive controls: 20 patients with seasonal AR and a positive SPT and serum sIgE to *Phleum*.
- Negative controls: 20 patients with perennial AR to *D pteronyssinus* with positive SPT results and serum sIgE to *D pteronyssinus* and negative SPT and serum sIgE to *Phleum* species.

Demographic and Clinical Data

Demographic and clinical data on the persistence and severity of nasal symptoms, disease course, and the presence of comorbidities were obtained from the clinical history and a detailed questionnaire, as reported elsewhere [16].

Chronology of Nasal Symptoms

The presence of rhinitis symptoms (nasal itching, sneezing, rhinorrhea, or nasal obstruction) from January to December was recorded by the patients over the previous 3 years according to the following scale: 0, no symptoms; and 1, presence of ≥1 rhinitis symptoms.

Persistence of Nasal Symptoms

According to the duration of nasal symptoms and the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines, rhinitis was defined as *intermittent* (symptoms present for

<4 days/week or for <4 consecutive weeks) or *persistent* (symptoms present for >4 days/week or for >4 consecutive weeks) [24].

Severity of Rhinitis

Depending on the number of impaired items (sleep, daily activities/sport/leisure, work/school performance, and troublesome symptoms), severity of symptoms was categorized as *mild* (no affected item), *moderate* (1-3 affected items), or *severe* (4 affected items), following the modified ARIA severity classification [25].

Comorbidities

The presence of conjunctivitis, asthma, atopic dermatitis, and allergic reactions to drugs and/or foods was also explored in the clinical history.

Pollen Counts

Pollen grains were measured using a Burkard spore trap (Burkard Manufacturing Co Ltd) in Madrid and Ciudad Real during the sampling period, which lasted from January 1 to December 31. The sampling airflow rate was 10 L/min, and the size of the orifice of the Hirst-type sampler was 14×2 mm. Grains were caught on 24-mm wide transparent tape coated with a thin film of petroleum jelly. This tape was mounted on a cylinder rotating at a speed of 2 mm/hour. To study the pollens caught over a 24-hour period, a 48-mm sweep (2 mm/h × 24 h = 48 mm) was performed with the oil immersion objective (×10 eyepiece, ×100 objective, with a field diameter of 0.18 mm).

Nasal Allergen Provocation Test

The NAPT was performed as described elsewhere [6] outside the grass pollen season when patients were free of symptoms. A bilateral saline challenge was performed to exclude nasal hyperactivity. If the result was negative, three 100-µL 10-fold increasing concentrations (1/100, 1/10, and 1/1) of freshly reconstituted *Phleum* extract (30 HEP/mL, LETI, SL) were administered in each nostril every 15 minutes.

To minimize the variability of external conditions, all the tests were performed simultaneously in both hospitals for a week. Depending on the availability of patients and centers, the response to NAPT was evaluated at baseline and 15 minutes and 1 hour after provocation based on subjective parameters (nasal and ocular symptoms score) and objective parameters (the volume of nasal cavity from 2-6 cm [VOL 2-6cm] assessed using acoustic rhinometry [6]; SRE 2000, RhinoMetrics). A 0-15 nasal-ocular symptoms score including 4 individual nasal symptoms (obstruction, rhinorrhea, itching, and sneezing) and 1 ocular symptom (itching, redness, or watery eyes) was used to categorize symptoms, as follows: 0, no symptoms; 1, mild; 2, moderate; and 3, severe. A positive NAPT was considered to be an increase >30% in the symptoms score and a decrease >30% in the %VOL 2-6cm in acoustic rhinometry compared with baseline.

Nasal Inflammatory Mediators and Phleum sIgE

Bilateral nasal lavage was performed using the Naclerio method [26] with 8 mL of physiologic saline administered with the participants seated with their necks extended approximately 30º backwards. After 10 seconds, the participants expelled the sample of mucus and saline into a container. The procedure was then repeated for the other nostril. Samples were centrifuged at 2000 rpm (1069*g*) for 7 minutes at 48ºC, and the supernatant was stored at –20ºC for the measurement of tryptase, eosinophil cationic protein (ECP), and sIgE to *Phleum* pollen by immunoassay (UniCAP, Phadia). The cutoff values for the immunoassays were 1 ng/mL for tryptase, 2 ng/mL for ECP, and 0.35 kU_A/L for sIgE.

Statistical Analysis

The statistical analysis was performed with SPSS 15.0 (SPSS Inc). Continuous data were expressed as the mean (SD) and categorical data as percentages. The concordance between NAPT and SPT was analyzed using the κ index. The clinical and demographic data were compared between groups using the chi-square test and ANOVA. *P* values <.05 (2-sided) were considered significant.

Results

Of the 70 patients evaluated, 9 presented nasal hyperreactivity to saline challenge and were excluded (13%). Demographic data are shown in Table 1. No significant differences were observed between the patients included and excluded. The study group consisted of 61 patients (42 women [69%]) with a mean age of 36.15 (15.89) years (range, 7-67 years). Thirty-six patients (59%) were from Hospital Infanta Leonor in Madrid, and 25 (41%) were from Hospital General in Ciudad Real.

Seasonal LAR to *Phleum* pollen was confirmed in 37 patients (61%) by a positive response to NAPT. The result of NAPT was negative in 24 participants (39%). There were no significant differences between patients from both hospitals.

Table 1. Baseline Demographic Data of Patients Evaluated

	Patients Included (Study Group)	Patients Excluded (NHR)	*P* Value
Participants, No.	61	9	
Mean (SD) age, y	36.15 (15.89)	29.56 (11.25)	.235
Gender, No. (%)			
Female	42 (67)	6 (67)	.895
FHA, No. (%)	39 (62)	5 (56)	.627
Smoking habit, No. (%)			.853
Yes	21 (33)	3 (33)	
No	29 (46)	5 (56)	
Ex-smoker	11 (18)	1 (11)	
Positive Phleum-NAPT, %	37 (61)	NA	NA
Dwelling			
Urban	44 (72)	6 (67)	.735
Rural	17 (28)	3 (33)	

Abbreviations: FHA, family history of atopy; NA, not applicable; NAPT, nasal allergen provocation test with *Phleum*; NHR, nasal hyperreactivity.

Total concordance was obtained between NAPT and SPT results in positive and negative control groups of patients with AR caused by *Phleum* species and *D pteronyssinus* (κ index, 1). No false-negative or false-positive responses to the NAPT were recorded.

LAR patients had a mean age of 37 years, 8-11% were children or adolescents, and 14% reported onset of the disease in childhood (age <14 years). Most of the patients were nonsmoking women with a family history of atopy. No significant differences were found between LAR and NAPT-negative patients (Table 2).

The comparison between patients with LAR and patients with seasonal nonallergic rhinitis aged <18 years and ≥18 years did not reveal statistically significant clinical or demographic differences. The chronology of symptoms and atmospheric grass pollen concentration showed that most LAR patients (70%-100%) had symptoms of persistent and moderate rhinitis (Table 2) from April to June (Figure 1), when the grass pollen concentration in the air was higher (Figure 2). A similar percentage of patients (75%-100%) also reported symptoms form April to June in the NAPT-negative group, and 20% reported symptoms from December to February despite the absence of grass pollen in the air. Conjunctivitis was the main comorbidity for 95% of LAR patients. The main clinical diagnosis was rhinitis-conjunctivitis for 59% of patients, followed by rhinitis-conjunctivitis-asthma for 35% (Table 2).

Table 2. Clinical Characteristics of LAR and Negative NAPT Patients

	LAR	Negative NAPT	*P* Value
Participants, No.	37	24	
Mean (SD) age, y	36.59 (15.06)	35.46 (17.39)	.787
0-18 y, No. (%)	4 (11)	5 (21)	.298
0-14 y, No. (%)	3 (8)	3 (13)	.850
Mean age at onset, y	29.25 (15.07)	30.70 (16.96)	.744
0-18 y, No. (%)	11 (30)	6 (26)	.761
0-14 y, No. (%)	(14)	5 (22)	.406
Persistence of symptoms, No. (%)			
Intermittent	10 (27)	4 (17)	.219
Persistent	27 (73)	20 (83)	
Severity of symptoms, No. (%)			.333
Mild	8 (22)	8 (33)	
Moderate	21 (57)	14 (58)	
Severe	8 (22)	2 (8)	
Comorbidities, No. (%)			
Conjunctivitis	35 (95)	23 (96)	.827
Asthma	13 (35)	14 (58)	.075
Dermatitis	2 (5)	-	ND
Drug allergy	2 (5)	-	ND
Food allergy	1 (3)	1 (4)	ND
High blood pressure	3 (8)	2 (8)	ND
Hypothyroidism	2 (5)	1 (4)	ND
Fibromyalgia	1 (3)	1 (4)	ND
Clinical diagnosis, No. (%)			
Rhinitis	2 (5)	1 (4)	.965
Rhinitis-conjunctivitis	22 (59)	14 (58)	
Rhinitis-conjunctivitis-asthma	13 (35)	9 (38)	
Mean (SD) duration, y			
Rhinitis	6.97 (7.60)	4.61 (3.91)	.147
Conjunctivitis	5.86 (7.63)	4.43 (4.14)	.569
Asthma	1.73 (3.84)	1.09 (2.26)	.978
Disease course, No. (%)			.622
Improvement	4 (10.81)	3 (12.50)	
No changes	18 (48.65)	14 (58.33)	
Worsening	15 (40.54)	7 (29.17)	

Abbreviations. LAR, local allergic rhinitis; NAPT, nasal allergen provocation test; ND: not done.

LAR patients reported a mean 7-year history of rhinitis. Most reported no changes in the disease (48.65%) or worsening (40.54%) in the previous 2 years, and only 10.81% reported an improvement in their symptoms. Similar data were reported by NAPT-negative patients (Table 2).

Analysis of the time course of the comorbidities showed that ocular symptoms had been present for longer than bronchial symptoms in both groups (Table 2).

Analysis of the nasal allergic response to NAPT in the LAR group showed that all patients had an immediate allergic response after the challenge (15 minutes to 1 hour). Most had a positive response at 15 minutes (86%) with low concentrations of *Phleum* extract (0.3 μg/mL [32%] and 3 μg/mL [47%]). Only 8 patients (22%) had a positive response to NAPT with the highest concentration of *Phleum* (30 μg/mL) (Figure 3).

Monitoring of the local inflammatory response to NAPT revealed significant increases in nasal secretions of ECP (64.7%) and tryptase (44.1%) in LAR patients at 15 minutes (ECP, *P*=.002; tryptase, *P*=.001) and 1 hour (ECP, *P*=.001; tryptase, *P*=.005). The kinetics of release was different for tryptase and ECP, with a peak at 15 minutes for tryptase and a peak at 1 hour for ECP. No significant increases in nasal secretion of tryptase or ECP were detected in NAPT-negative patients (Figure 4).

Nasal sIgE antibodies against *Phleum* were found in 3 LAR patients (8.10%) with the maximum production at 1 hour after NAPT (Figure 4). Detectable levels at baseline and 15 minutes after challenge were observed in 1 patient. No NAPT-negative patients presented detectable levels of *Phleum* sIgE in the nasal cavity.

Discussion

The demonstration of LAR in patients with nonatopic rhinitis has generated major clinical and epidemiological concerns with respect to its prevalence, natural history, and response to specific immunotherapy, as well as to the influence of environmental factors [11-13, 21]. Although prevalence data are lacking for the general population, several authors have reported that LAR is a commonly under/misdiagnosed respiratory disease that affects patients from different countries, ethnic groups, and age groups [2,5-7,10,16,18,19,27].

We evaluated the presence of LAR and the clinical and immunological characteristics of this entity in an area with a much higher atmospheric concentration of grass pollen than the rest of Spain [22,23,28]. In Ciudad Real and Madrid, both of which are in central Spain, grasses are the source of the most relevant pollen allergens [23]. Pollination in this area occurs

between May and June (about 6 weeks). The threshold for producing symptoms is set at 35 grains/m^3, which is frequently surpassed during the pollen season, reaching 585 grains/m^3 on peak days. As a result, grass allergy affects 81% of pollen-allergic patients from Madrid and Ciudad Real [23,28].

The NAPT combined with assessment of nasal-ocular symptoms and acoustic rhinometry was the diagnostic test used to identify LAR to *Phleum* pollen. NAPT is a very specific and sensitive diagnostic test for LAR [5,6,8-10,17]. In addition, we confirmed the accuracy of NAPT with *Phleum*

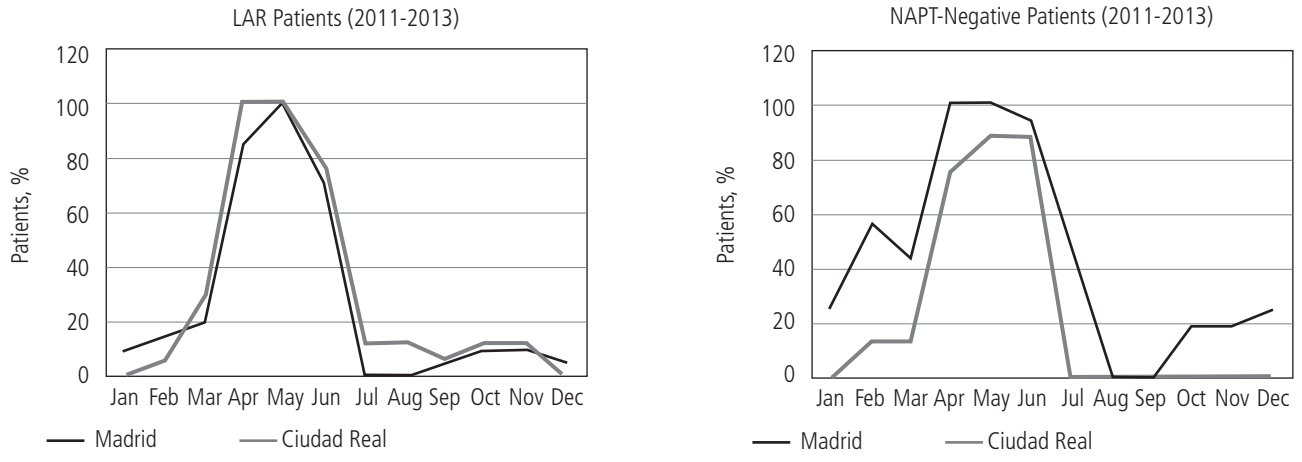

Figure 1. Chronology of nasal symptoms in patients with LAR and negative NAPT results. NAPT indicates nasal allergen provocation test; LAR, local allergic rhinitis.

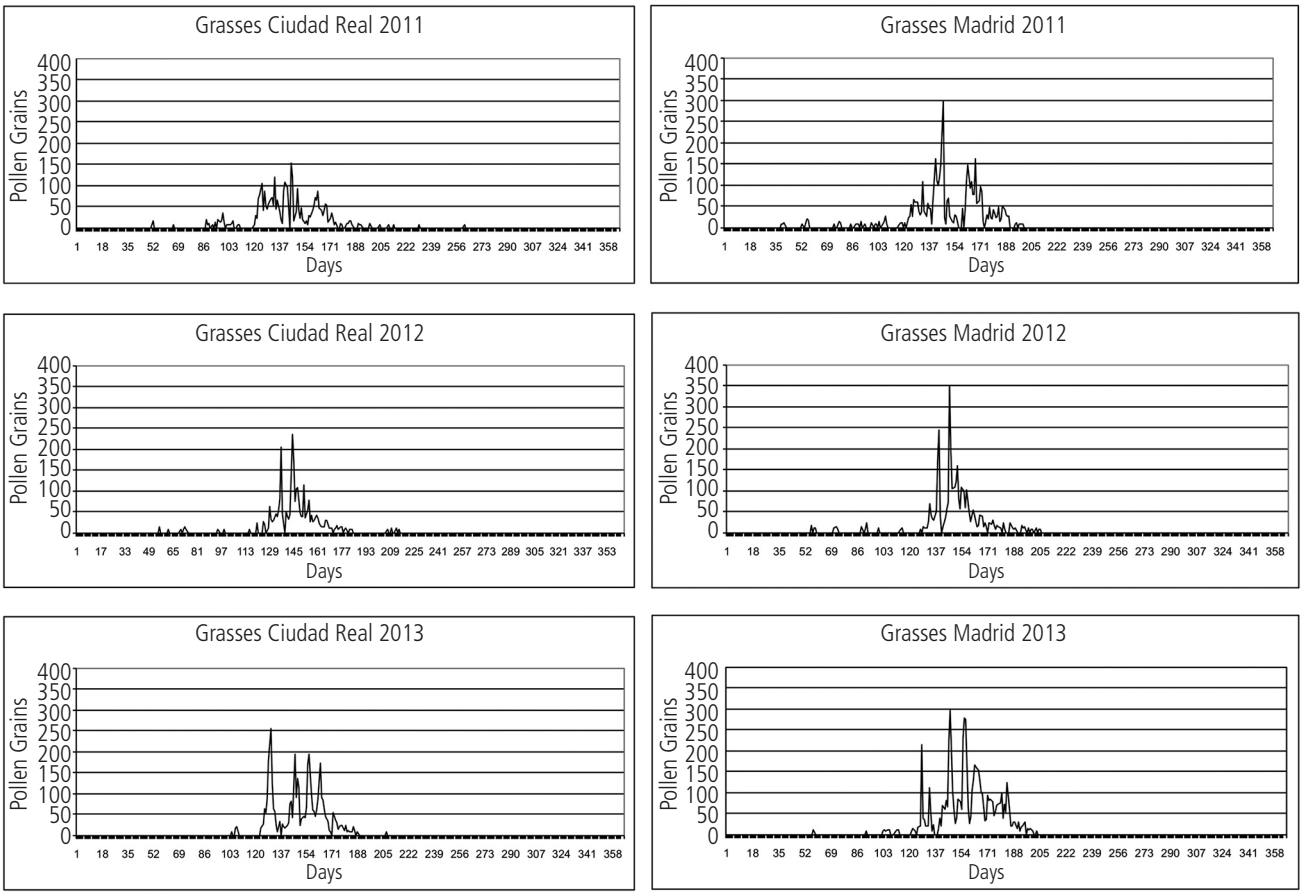

Figure 2. Atmospheric concentration of grass pollen in Ciudad Real and Madrid.

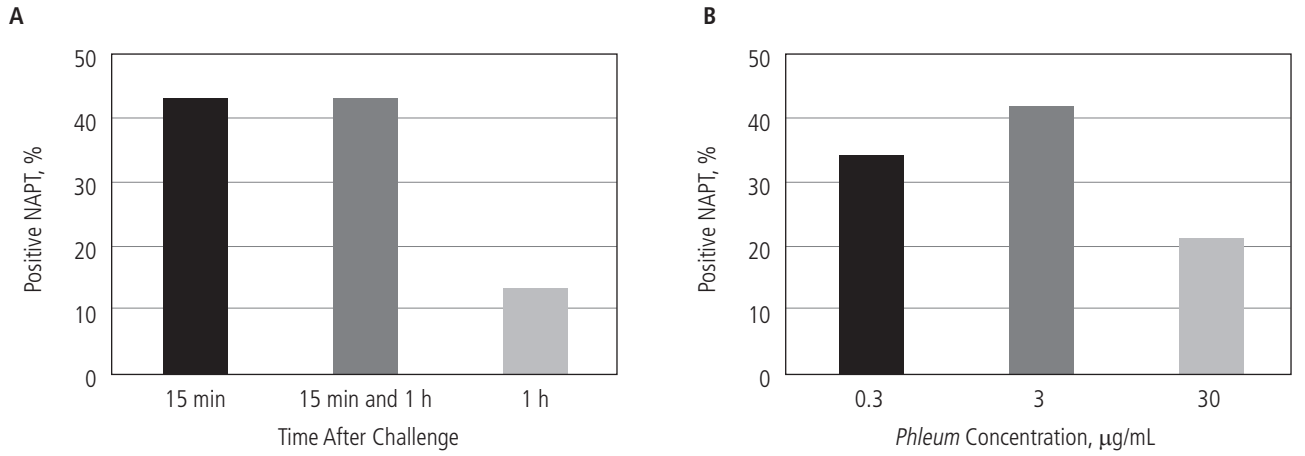

Figure 3. Time of response (A) and threshold *Phleum* concentration (B) for the nasal allergen provocation test (NAPT) in patients with seasonal local allergic rhinitis. Data are expressed as percentage of patients with a positive response to nasal challenge. NAPT indicates nasal allergen provocation test.

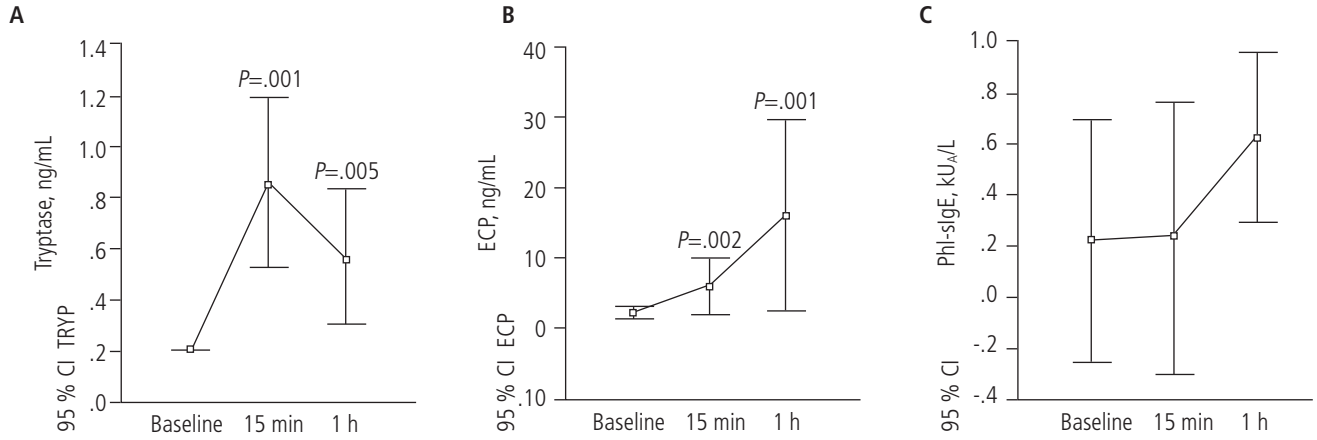

Figure 4. Kinetics of nasal secretion of tryptase (A), ECP (B), and Phl-sIgE in LAR patients after NAPT. Data are expressed as mean (SD). Comparisons between groups were made using the Mann-Whitney test (*P* values). ECP indicates eosinophil cationic protein; LAR, local allergic rhinitis; NAPT, nasal allergen provocation test.

pollen extract by obtaining total concordance with SPT in a control group comprising patients with AR caused by *Phleum* and a negative control group consisting of patients with AR to *D pteronyssinus*.

We demonstrated the existence of seasonal LAR to *Phleum* pollen in 61% of patients from Madrid and Ciudad Real. In most cases, the allergic response was triggered by low concentrations of allergen. Similar data were previously reported in a study performed using the same NAPT methodology in Málaga [6], which has a low-moderate atmospheric load of grass pollen. These results raise questions about whether the development of LAR to grass pollen might be influenced by the concentration of pollen in the air, and whether a mean daily atmospheric concentration of grass pollen ≥35 grains/m^3 in spring (the threshold grass pollen concentration for symptoms in AR patients [23]) is sufficient to induce nasal allergic sensitization. However, more studies need to be carried out in this respect. Preliminary studies indicate that LAR induced by house dust mite is more commonly detected in areas with high mite concentrations in the environment [20].

A study carried out in 2005 in Sweden identified LAR to birch and timothy pollen using NAPT in 47% of patients with seasonal nonatopic rhinitis [7]. Although the concentration of allergen used for NAPT was similar to that used in our study, the volume of allergen was lower than currently recommended [29]. We wonder whether the use of the recommended volume (100 μL) would have revealed more positive cases.

In the present study, patients with LAR to *Phleum* and NAPT-negative patients presented similar clinical characteristics, namely, most participants had symptoms of persistent and moderate rhinitis associated with conjunctivitis and asthma during the grass pollen season (April-June) and worsening of the disease in the last 2 years. A possible explanation for this worsening could be the increase in the amount of grass pollen in the atmosphere during recent

years [22]. It is therefore impossible to distinguish between them using the clinical history alone, thus supporting the need for an allergy workup for the target organ (NAPT, determination of nasal sIgE, or both) in patients with clinical signs of LAR, as previously recommended [13,14].

Twenty percent of NAPT-negative patients had AR symptoms both in spring and December-February, suggesting the involvement of other pollens, such as olive and *Chenopodiacea* during spring-summer [30,31] and *Cupressaceae* in December-February [22].

The prevalence of an association with asthma was similar to data previously reported in LAR [6,16] and classic AR [32]. However, the association with conjunctivitis was more frequent, affecting up to 95% of LAR patients [6,16], and symptoms started earlier than asthma symptoms during the course of the disease. A possible explanation for this finding may be the strong inflammatory response in the ocular conjunctiva caused by a high atmospheric concentration of grass pollen. In order to investigate this possibility, conjunctival provocation tests are now in progress to detect LAR by studying patients in different environments.

An interesting finding of our study was the detection of LAR in children and adolescents. Consistent with data from previous studies, 11% of the patients were aged <14 years at clinical evaluation, and 14% first reported symptoms in childhood [10,16]. These data need to be further evaluated in epidemiological studies to detect the true prevalence and clinical relevance of this entity in children and adults.

Although nasal mediators were only evaluated during the hour after challenge, comparison with previous studies showed that the kinetics of production of inflammatory mediators and sIgE in the nasal cavity was the same as previously reported in LAR patients sensitized to grass pollen [8,9] and house dust mites, with a peak production of tryptase during the first 15 minutes after challenge, followed by secretion of ECP and sIgE (last evaluation). Given restrictions on patients' availability and time, no further determinations were made in nasal lavage.

When we compared the number of cases with elevated ECP and tryptase during NAPT and the amount of ECP produced with previous data from a similar group of patients in Malaga [8], values tended to be higher in the present study. A possible explanation for these differences is the stronger nasal inflammatory response in patients living in areas of high atmospheric concentrations of *Phleum* pollen. Comparative studies in LAR and AR patients during natural exposure to *Phleum* pollen in geographic areas with different allergenic loads are currently being carried out to investigate this possible correlation.

In our study, the concentration of nasal sIgE at 15 minutes and 1 hour after NAPT (last evaluation) was similar to that recorded in previous studies using the Naclerio method for nasal lavage [8]. The low number of positive cases detected in our study could be explained by the concentration of sIgE reported in nasal lavage samples [6-9], the dilution effect of the technique [6-9], and the kinetics of nasal production of sIgE, with progressive increases reaching the maximum level at 24 hours after challenge [8,9].

We conclude that LAR to *Phleum* species is a relevant entity that can be found in up to 60% of individuals living in areas of high exposure to grass pollen. The remaining patients evaluated with suspicion of LAR during this period did not show significant clinical differences in the persistence/severity of symptoms and comorbidities, suggesting that other environmental allergens, most likely pollens, could be responsible for this finding. Our findings show that LAR may exist in regions of high allergen load, similar to areas of low-moderate loads, indicating that in any case of suspicion of LAR, diagnosis should be based on an appropriate nasal allergy workup.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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