Local allergic rhinitis in children: clinical characteristics and role of basophil activation test as a diagnostic tool.

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Abstract

Background Local allergic rhinitis (LAR) is a condition involving a localized nasal allergic response in absence of systemic atopy. We aimed to describe clinical characteristics of LAR and non-allergic rhinitis (NAR) pediatric patients, their clinical evolution over a 7-year follow-up period and to study the role of basophil activation test (BAT), for the diagnosis of LAR. Methods Forty-four children with non-allergic rhinitis (NAR) were included (24 males, 20 females, aged under 15 years). Nasal allergen provocation test (NAPT) and BAT were performed with *Dermatophagoides pteronyssinus* and *Phleum pratense*. Results Seven patients (16%) were diagnosed of LAR. Seven reacted to *D pteronyssinus* and one also to *P pratense*. All LAR and 86% of NAR patients presented perennial symptoms. Fifty-seven percent of NAR and LAR patients referred persistent symptoms. Three LAR patients associated conjunctival symptoms. BAT was positive after stimulation with *D pteronyssinus* only in one LAR patient. On follow-up, 3 LAR patients and 10 of the 25 NAR patients who agreed to be retested, presented systemic sensitization. Conclusions LAR should be considered in children with NAR. Almost half of children with LAR and one fourth of NAR children will develop systemic sensitization over time. BAT shows low sensitivity for the diagnosis of LAR in children. Key message: Since sixteen percent of initially diagnosed as non-allergic rhinitis children present local allergic rhinitis, we suggest performance of nasal provocation test in those cases to achieve a correct diagnosis. Basophil activation test seems to be less sensitive for the diagnosis of local allergic rhinitis in children than in adults. Follow up over would be interesting since a significant number local allergic rhinitis children and non-allergic rhinitis children will eventually develop systemic sensitization to aeroallergens.

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iii. Abstract, Key message and keywords:

Background

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Methods

Forty-four children with non-allergic rhinitis (NAR) were included (24 males, 20 females, aged under 15 years). Nasal allergen provocation test (NAPT) and BAT were performed with *Dermatophagoides pteronyssinus*and *Phleum pratense* .

Results

Seven patients (16%) were diagnosed of LAR. Seven reacted to *D pteronyssinus* and one also to *P pratense* . All LAR and 86% of NAR patients presented perennial symptoms. Fifty-seven percent of NAR and LAR patients referred persistent symptoms.

Three LAR patients associated conjunctival symptoms. BAT was positive after stimulation with *D pteronyssinus* only in one LAR patient.

On follow-up, 3 LAR patients and 10 of the 25 NAR patients who agreed to be retested, presented systemic sensitization.

Conclusions

LAR should be considered in children with NAR. Almost half of children with LAR and one fourth of NAR children will develop systemic sensitization over time.

BAT shows low sensitivity for the diagnosis of LAR in children.

Key message: Since sixteen percent of initially diagnosed as non-allergic rhinitis children present local allergic rhinitis, we suggest performance of nasal provocation test in those cases to achieve a correct diagnosis. Basophil activation test seems to be less sensitive for the diagnosis of local allergic rhinitis in children than in adults.

Follow up over would be interesting since a significant number local allergic rhinitis children and non-allergic rhinitis children will eventually develop systemic sensitization to aeroallergens.

Key words: basophil activation test, BAT, local allergic rhinitis, LAR, nonallergic rhinitis, NAR, nasal allergen provocation test.

iv. Main text:

INTRODUCTION

Local allergic rhinitis (LAR) is a clinical rhinitis phenotype defined by the presence of nasal symptoms of allergic rhinitis (AR) in patients with negative skin prick test (SPT) and undetectable serum specific-IgE (sIgE) against inhalant allergens, but with positive nasal provocation test (NAPT) with suspected allergens and good response to allergen specific immunotherapy (1).

LAR is characterized by the development of a mucosal Th2 immune response in sensitized patients during natural exposure to aeroallergens with local increased levels of local sIgE, tryptase and eosinophil cationic protein (2,3) as well as cytokines as IL-13 and IL-5 (4).

NAPT is currently the gold standard for LAR diagnosis along with basophil activation test (BAT) and detection of sIgE in nasal secretions (1). BAT has proven to be a useful diagnostic tool in several studies on adult patients with LAR, sensitized to dust mites (*Dermatophagoides pteronyssinus*) and olive tree pollen (5,6).

In the adult population LAR is well defined, predominantly affecting young, non-smoking women with a family history of atopy. Symptoms are usually persistent, and patients often also suffer from conjunctivitis and bronchial asthma. Even though about 36% of adult patients suffering from LAR report its onset in childhood (7), there is limited research into LAR in the pediatric population (8-17).

In the present study we aimed to describe the clinical features of children with LAR and NAR and their evolution during a seven-year follow up, and to establish the potential role of BAT as a diagnostic tool for LAR in children.

METHODS

Patients

We performed a prospective study including 44 children with NAR (24 males and 20 females) under 15 years of age (median age 7 years, range 3-15 years), recruited during the years 2014 and 2015 at the outpatient Pediatric Allergy clinic at the Children's Hospital of A Coruña (Spain). They presented symptoms of rhinitis as runny nose, nasal stuffiness, sneezing and/or nasal itching, but yielded negative results on skin prick test (SPT) and presented undetectable serum sIgE to common aeroallergens in our area.

Clinical classification of rhinitis as persistent or intermittent, as well as its severity (mild or moderate-severe) was performed according to the Allergic Rhinitis and Impact of Asthma guidelines (18). To assess nasal symptom severity, a visual analogue scale (VAS) of 10cm was used. Rhinitis was accordingly categorized as "mild" (VAS:0-30cm), "moderate" (VAS: 30-70cm) or "severe" (VAS > 70cm) (19).

The study was conducted according to the principles of the Declaration of Helsinki and approved by the local ethics committees. All parents and participants over 12 years of age were informed and signed the relevant informed consent form.

Skin testing

SPT were performed with a panel of the most prevalent aeroallergens in our area, including dust mites (*Dermatophagoides pteronyssinus, Lepidogliphus destructor*), moulds (*Alternaria alternata*), pollen (*Phleum pratense, Cynodon dactilon, Plantago lanceolata, Parietaria judaica, Betula verrucosa, Platanus acerifolia*) and cat and dog dander (ALK-Abelló, Madrid Spain) following current guidelines (20).

Serum total and specific IgE

Serum total IgE and specific IgE to aeroallergens were determined by ImmunoCAP (ThermoFisher Scientific, Barcelona, Spain). A cut-off value of sIgE > 0,35kU/L was considered positive.

Nasal allergen provocation tests (NAPT)

NAPT was performed in all 44 patients with the aim to detect local allergic sensitization to the two most relevant allergens in our area: the dust mite *Dermatophagoides pteronyssinus* and the grass pollen*Phleum pratense* .

Before NAPT was carried out, patients were asked to avoid topical steroids and montelukast for at least 2 weeks and oral and intranasal antihistamines for at least 1 week.

Firstly, symptom-free patients were tested intranasally with 2 puffs (100mcl) of saline solution to exclude non-specific nasal hyperreactivity. If reaction was recorded, NAPT was carried out. Two puffs (100mcl) of freshly reconstituted freeze-dried allergen solutions (Roxall, Bilbao, Spain) of *D pteronyssinus* (Der p 1: 0,33, 3,3 and 33 mcg/ml; Der p 2: 0,09, 0,9 and 9 mcg/ml) and *Phleum pratense* (Phl p 1:0,44, 4,4 and 44 mcg/ml; Phl p 5: 0,07, 0,7 and 7 mcg/ml) were administered at 15-minute intervals with the use of a metered pump spray, alternating nostrils. Each allergen was administered on a different day at weekly intervals. If objective clinical manifestations such as nasal obstruction, rhinorrhea, itching and sneezing developed after administration of the corresponding dose of allergen, NAPT was stopped and considered positive. Oral antihistamines were administered then to alleviate those symptoms.

After finishing allergen administration, patients were observed during a 30 minutes period. If no symptoms developed, they were discharged with the indication of recording any late clinical manifestations over the next 24 hours.

Following the EAACI Task Force recommendations on nasal allergen challenges, NAPT was considered positive when there was a strong increase in subjective symptoms (symptoms > 23mm) (21).

Basophil activation test (TAB)

For flow cytometry, peripheral blood was collected in sodium heparin tubes. Aliquots were resuspended in 100 mcg HEPES calcium buffer containing IL-3 (10 ng/ml). Then, pre-activated blood samples were incubated with antigens: *Dermatophagoides pteronyssinus* , and*Phleum pratense* , OVA, and OM (Bial-Aristegui Laboratories, Bilbao, Spain) at three different concentrations (Der p: 5000 DBU/ml, 50 DBU/ml and 5 DBU/ml); Phl p: 30000DBU/ml, 3000DBU/ml and 300DBU/ml; and samples were incubated. As a positive control it was used a monoclonal anti-IgE receptor antibody (1 mcg/ml) (Bühlmann Laboratories, Allschwill, Switzerland), and as two negative controls, washing solution was used. Supernatant was discarded after erythrocytes lysis, washing, and centrifugation, and basophils were double-labeled with anti-CD63 antibody and anti-IgE FITC-labeled antibody, and samples were studied for expression and upregulation of CD63. Flow cytometric analysis of basophil activation was performed at 488 nm with a flow cytometer FACS-Canto (BD Biosciences, San José, California, USA). Mean fluorescent intensity was determined by using Diva software. Results were expressed as percentage of activated basophils among gated basophils. A positive BAT result was considered when at least one of the three concentrations showed a percentage of activated basophils over 15% and stimulation index (specific antigen response/basal response) >2. Negative control results were considered as the mean value between both negative controls. Non responders were considered those showing percentage of CD63+ basophils less than 5% in positive control and were discarded from analyzed.

Clinical evolution

Patients were followed up over a 7 year-period from their first visit. Persistence of symptoms, predominant clinical characteristics, and development of new atopic comorbidities over time were recorded. SPT and sIgE determination to common aeroallergens were performed again at this point.

Statistical analysis

Data were expressed in medians and ranges.

Clinical and demographic data were compared between patients with NAR and patients with LAR by Pearson´s Chi-squared, t-test and Spearman´s tests.

Statistical analyses were performed with SPSS (version 16.0 for Windows; SPSS Inc, Chicago, Ill).

RESULTS

Demographic and Clinical Characteristics

Epidemiologic data of LAR and NAR patients are summarized in Table I.

Seven patients out of the 44 NAR children included (16%) developed clinical manifestations during NAPT, thus confirming the diagnosis of LAR. They were three girls and four males, with median age of 10 years (range 6 to 14 years). All patients were locally sensitized to dust mites (*Der p*) and one also to grass pollen.

Median age at onset of symptoms in LAR patients was 5 years (range 2-11 years) and in NAR patients, 4 years (range, 1-13 years) with a median of 2 years of evolution before the first clinical evaluation in both groups (range, 1-12 years).

Nasal obstruction was the main symptom presented by both LAR and NAR patients (n=37, 77%), followed by rhinorrhea (n=29, 66%), sneezing (n=22, 50%) and nasal itching (n=22, 50%). There were non-significant differences in clinical manifestations between both groups, although LAR patients reported sneezing (57% vs 49%) and nasal pruritus (78% vs 46%) more frequently than NAR patients.

The seven LAR patients presented perennial symptoms, including the patient locally sensitized to grass pollen. Thirty-two (86%) of the NAR patients also referred perennial clinical manifestations. More than half of NAR patients (57%) and four out of the seven LAR patients presented persistent symptoms. Severity was similar in both groups. Most patients showed mild (43% LAR, 46% NAR) or moderate (51% LAR, 57% NAR) symptoms. Only one NAR patient presented severe rhinitis.

Three of the seven LAR patients reported associated conjunctival symptoms, proportionally more than the NAR patients (19%, 7 out of 37). Fourteen (37,7%) NAR patients presented other atopic comorbidities, most frequently bronchial asthma (n= 10). Only one LAR patient presented associated asthma. Differences regarding personal atopic background were non-significant between both groups.

NAR patients reported family history of atopy more frequently (67%) than LAR patients (57%), although this difference was non-significant.

Table II summarizes clinical characteristics, as well as clinical evolution of LAR patients.

Basophil activation test in LAR children

BAT results are summarized in Table III. Patient 7 did not respond to the anti-IgE-positive control, so her results could not be taken into analysis. Only patient 3 showed basophil activation after antigen stimulation with *Dermatophagoides pteronyssinus* at two different concentrations (5000DBU/ml and 500DBU/ml, respectively). The remaining 5 patients showed negative BAT to both *Dermatophagoides pteronyssinus* and *Phleum pratense.*

Clinical evolution

Comparison of NAR and LAR patients' clinical course is summarized in Table IV.

Thirty-three NAR and the 7 LAR patients were clinically reevaluated after a period of 7 years. Twenty-five NAR patients and all LAR patients agreed to repeat SPT and/or serum sIgE determination to aeroallergens.

The NAR patients who refused to do further evaluation reported clear improvement or even resolution of symptoms.

Nasal obstruction and rhinorrhea were the most frequent clinical manifestations reported over time by patients from both groups. The difference between them was not significant.

Eighteen (49%) NAR patients reported amelioration of symptoms, nine (24%) reported complete resolution of rhinitis and 6 patients (14%) reported worsening of symptoms over time.

Of the LAR patients, four reported worsening of allergic symptoms, two reported improved symptoms, and one reported a complete resolution.

Three of the seven initially diagnosed LAR patients presented positive SPT to dust mites, and were thus diagnosed with AR. Their clinical evolution was variable: one reported resolution, one improvement and the third worsening of symptoms. One patient reported associated conjunctival manifestations but no other comorbidities. None of them presented asthma at that moment.

Patients who remained symptomatic on follow-up reported a family history of atopy.

Of the 25 patients initially diagnosed with NAR, 10 (40%) developed systemic sensitization over time, and were thus diagnosed with AR (Table V). Seven AR patients (70%) referred clinical improvement and one reported complete resolution of symptoms. Eight were sensitized to dust mites, one to grass pollen and one to birch pollen.

Three NAR patients (20%) and four AR patients (40%) presented with associated bronchial asthma, two AR patients also presented conjunctivitis and two, food allergy. There were non-significant differences regarding atopic comorbidities between NAR patients and newly diagnosed AR patients.

Eighty percent of AR patients reported FHA and 60% of NAR did as well, being this difference was non-significant.

From the group of NAR patients (n=15, 46%), 8 (53%) reported clinical improvement, 3 (20%) resolution of symptoms, 3 (30%) persistence and 1(7%) reported worsening of clinical manifestations. Improvement was referred by 7 (70%) of AR children, persistence by 2 (20%) and resolution by 1 (10%).

Perennial symptoms were referred by 8 (53%) of NAR and 8 (80%) of AR children. Six (60%) of AR and 8 (53%) of NAR patients presented intermittent symptoms. NAR children presented mild (40%) and moderate rhinitis (53%) rather than severe rhinitis. Similarly, half of AR patients reported mild clinical manifestations, while the other half presented moderate symptoms.

DISCUSSION

Although in the last few years knowledge about LAR has increased, it is a still largely unrecognized illness, especially among children, probably due to the lack of consideration of NAPT in the diagnosis algorithm of rhinitis in many centers (21).

So far, most of the studies have been performed in adults. In Spain, prevalence of LAR in adult population has been estimated in 25.7% by Rondon et al (22), a percentage that increases to 54% among NAR patients (2). The most frequent phenotype of LAR in this age group has been described as a woman under 30 years of age, with family history of atopy (FHA), moderate to severe persistent and perennial rhinitis and an earlier rhinitis onset (22).

Up to date, prevalence of LAR among children seems to be lower than in the adult population. Bozek et al (15) reported 11% of LAR, in children between 5 and 18 years with rhinitis, slightly higher than the 3,4% reported by Ha et al (13).

Among children with NAR, the percentage of LAR varies depending on the geographical area considered, being lower in Asian countries than in Western populations. Thus, Ha et al (13) reported 7.8% prevalence in Korea, close to the 3.7% found by Buntarickpornpan et al (9) in Thai children, while Krajewska-Woijtys et al (10), reported 52.2% prevalence in Polish adolescent patients; Zicari et al (12) reported 66.7% in Italy and Prieto et al (16) 58% in Spanish children and adolescents.

In the present study of the 44 NAR patients included, 7 (16%) developed an allergic response after NAPT, and were diagnosed with LAR. This percentage was closer to the 25% reported by Duman et al (11), after evaluating 28 Turkish children suffering from NAR.

In the present study median age at onset of symptoms for LAR patients was 5 years (range 2-11 years), being 4 years, (range, 1-13 years) for NAR patients. Slightly higher mean ages at rhinitis onset in LAR children were reported by Prieto et al (16) (10.2 + 3.72) and Tsilochristou et al (17)(7+ 4.3) in contrast with the higher mean age at disease onset reported by other authors as Bozek et al (17.6 +4.8 years) (15).

In adults, women suffer from LAR more frequently than men, as described by Rondón et al (6). In childhood, however, most studies show a male preponderance (10,11,13,15,17), as we observed (4 males and 3 females). On the contrary, Prieto et al (16) found that more than half of children diagnosed of LAR were girls (67.4%).

The most frequent nasal symptom reported by both NAR and LAR patients in the present study was nasal obstruction, with no significant differences between both groups, followed by rhinorrhea, sneezing and nasal itching. Congestion has also been reported as the main clinical manifestation in NAR and LAR children by some (11,17), but not all authors. Prieto et al (16) report sneezing and itching as the most relevant symptoms (16) in LAR Spanish children, and nasal congestion as the main complaint by NAR children.

Applying the ARIA classification, we found that more than half of patients suffering from NAR (57%), and all LAR patients, showed persistent, perennial nasal symptoms. Our observation agrees with those previously reported (11,16,17), suggesting that children suffering from LAR usually present persistent symptoms. Only Ha et al (13) observed intermittent symptoms more frequently in both groups.

Conjunctivitis is the most frequently associated comorbidity in children, followed by asthma (13,15-17). This is also the case for adults (23) who also suffer from LAR. Moreover, conjunctival manifestations are more common in pediatric patients with LAR than with NAR (15-17). Accordingly, we found that three of the seven (42%) LAR pediatric patients presented associated conjunctival symptoms, a percentage proportionally higher than the 19% reported by NAR patients (7 out of 37).

Regarding asthma, most studies also observe lower prevalence of asthma in NAR children compared to LAR patients (9,15-17), while others (13) report a higher proportion of NAR children with asthma. Only one child of the seven LAR patients included in the present study presented asthma, a percentage proportionally lower, though non-signficant, compared with NAR children.

As described for LAR adult patients (22), FHA is a constant feature in pediatric LAR. FHA is reported by more than half of LAR children (13,16,17), in percentages that vary from the 57% reported by Tsilochristou et al (17) to the 80% published by Ha et al (13). In line with these, we found that 57% of LAR children presented FHA. However, NAR patients also reported FHA and they did so more frequently (67%) than LAR children, but this difference was non-significant. Contrary to what we describe, previous reports (16,17,27) indicate that FHA is present in less than half of NAR pediatric patients, and that it constitutes a predictor of developing allergic rhinitis (AR) over time (27).

In the present study, 32 NAR and the seven LAR children were followed after a period of seven years.

From the twenty-five NAR patients that agreed to repeat SPT and/or serum sIgE determination, eight (25%) showed sistemic sensitization to aeroallergens, specially dust mites, confirming thus the diagnosis of AR. This percentage is similar to the 24% reported by Rondon et al for adults who suffer from NAR in our country when reevaluated after a interval of 3-7 years (26), but lower than the 41% reported by Veskitkul et al (27) after a 3-5 year follow-up of NAR children. They observed (27) that the majority of these newly sensitized patients presented moderate-to-severe, persistent nasal symptoms, associated eye symptoms and referred family history of atopy. In line with their observations, we found that 80% of AR children presented FHA. Nevertheless, asthma was the most frequent comorbidity associated in our sample and more than half of AR patients reported improvement, along with intermitent and perennial symptoms.

We found that three of the seven (42.8%) LAR children showed systemic sensitization after the 7-year follow-up period. All of them were sensitized to dust mites. Their clinical evolution was variable: one patient reported improvement, other worsening of rhinitis with persistent symptoms and the third one, complete resolution. Although the small size of our sample limits our ability to draw conclusions, evolution of LAR into AR seems to be more common in children than in adults. Rondon et al (2) followed 194 adult patients with LAR over a 10 year period. In general terms, just 26% of those LAR patients reported worsening of their rhinitis, with an increase in persistence and severity over time. The percentage of systemic sensitization they reported (6.81%), was much lower than the percentage we found.

Achieving a correct diagnosis of LAR has relevant therapeutic implications, since these patients would not only benefit from oral antihistamines and topical nasal corticosteroids (2,8) but also from specific immunotherapy as previously reported in adult patients sensitized to dust mites (24) and grass pollen (25).

In the present study, diagnosis of LAR was performed by NAPT with the most prevalent allergens in our area: the dust mite *D pteronyssinus* (10) and the grass pollen *P pratense* . The election of both allergens for NAPT was based in our clinical observations (data not shown). Allergic children from this area at the Northwest of Spain, recognize either dust mites or grass pollen or both. They may be sensitized to other aeroallergens as well, but dust mites and/or grass pollen sensitization constitutes a constant finding.

The seven LAR patients reacted on NAPT to *D pteronyssinus* and one of them also reacted to *P pratense* . Although NAPT shows high sensitivity and specificity, it requires trained personnel, and is time-consuming. Therefore, other diagnosis tools as BAT are considered. BAT has been proven as a useful tool for LAR diagnosis in studies in adult patients with sensitization to *Dermatophagoides pteronyssinus* (6) and *Olea europea* pollen (5). Gómez et al (5) found that 8 (50%) of the 16 LAR adult patients included in their analysis, yielded positive results in BAT with *Dermatophagoides pteronyssinus* . BAT sensitivity was 50% and its specificity, 93%. Similarly, Campo et al (6) found that 8 (66.6%) of 12 LAR adult patients sensitized to olive tree pollen, had positive BAT results.

In the present study, only one of the 6 LAR children included in the analysis of BAT (16,6%), showed significant basophil activation*in vitro* after exposure to *Dermatophagoides pteronyssinus* , implying lower

sensitivity of this technique for the diagnosis of LAR in children compared to adults.

Gómez et al (6) suggested that after allergen-specific production of IgE at the nasal mucosa, locally produced sIgE could be able to reach the blood stream, with basophils as its first or only target, before its detection as free serum sIgE and skin mast cell sensitization. Given the difference observed on the percentage of positive BAT between adults and children with LAR, we might hypothesize that natural allergen exposure during months and even years would be necessary to complete that suggested pattern.

In summary, since a significant number of initially diagnosed NAR children present LAR indeed, we suggest considering NAPT as a routine tool in daily practice in NAR patients, given the benefits derived from a correct diagnosis.

Based on our observations, BAT seems to be less sensitive for the diagnosis of LAR in children than it has been described for adults. Even so, more studies in pediatric population with larger samples would be necessary to confirm, or disprove, our findings.

Almost half of LAR children and one fourth of NAR children will eventually develop AR. Follow-up and further diagnostic work up would be interesting in both groups over time.

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vi. Impact statement:

Local allergic rhinitis is no so rare in pediatric age and it should be considered in children diagnosed as non-allergic.

More than half of children with LAR refer family history of atopy. LAR children present with persistent rhinitis and associated conjunctivitis.

BAT seems to be less sensitive for the diagnosis of LAR in children than it has been reported for adult LAR patients.

Almost half of LAR children and one fourth of NAR children will eventually develop AR. Follow-up and further allergen specific IgE determination would be interesting in both groups over time.

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viii. Tables:

Table I. Demographic and clinical manifestations of children diagnosed with NAR and LAR

	NAR (N=37)	**NAR (N=37)**	**LAR (N=7)**	*p-value*
Age (y) at diagnosis				
Mean	7.8	10	10	0.09[a]
Range	3-15	6-14	6-14	
Median	7	10	10	
Age (y) at rhinitis onset				
Mean	5.2	5.6	5.6	0.77[b]
Range	1-13	2-11	2-11	
Median	4	5	5	
Gender				
Female (n%)	17 (46)	3 (43)	3 (43)	0.88[b]
Atopic comorbidities				
Asthma, n (%)	10	1	1	0.41[b]
Atopic dermatitis, n (%)	4	0	0	0.36[b]
Food allergy, n (%)	4	0	0	0.36[b]
Conjunctival, n (%)	7 (19)	3 (43)	3 (43)	0.17[b]
Family history of atopy, n (%)	25 (67)	4 (57)	4 (57)	0.53[b]
ARIA classification				
Perennial, n (%)	32 (86)	7 (100)	7 (100)	0.3[b]
Seasonal, n (%)	5 (13)	0	0	0.3[b]
Persistent, n (%)	21 (57)	4 (57)	4 (57)	0.99[b]
Intermitent, n (%)	16 (43)	3 (43)	3 (43)	0.99
Severity				

	NAR (N=37)	**NAR (N=37)**	**LAR (N=7)**	*p-value*
Mild	17 (46%)	3 (43%)	3 (43%)	
Moderate	19 (51%)	4 (57%)	4 (57%)	0.94[c]
Severe	1 (3%)			
Main nasal symptons				
Blockage, n (%)	29 (78)	5 (78)	5 (78)	0.69[b]
Rinorrhea, n (%)	24 (65)	5 (71)	5 (71)	0.74[b]
Sneezing, n (%)	18 (49)	4 (57)	4 (57)	0.68[b]
Pruritus, n (%)	17 (46)	5 (78)	5 (78)	0.22[b]

[a]t-test; [b]Pearson´s Chi-squared test of independence; [c] Spearman´s test

Table II. Demographic and clinical characteristics of patients with LAR and their evolution after 7-year follow-up period.

	Sex	**Initial Age (years)**	**Family history of atopy**	**Comorbidities**	**Time interval before diagnosis**	**Severity**	**Presentation**	**NAPT Der p**	**NAPT Phl p**	**Total previous IgE (kU/L)**	**Clinical evolution**	**Current Sensitivity**	**Cur Pr**
1	Female	8	Yes	None	1 year	Moderate	Persistent	+	-	122	Persistence	None	-
2	Female	6	No	None	1 year	Mild	Intermittent	+	-	38.4	Resolution	Yes	Mite
3	Male	10	No	Asthma	1 years	Mild	Intermitent	+	-	95.5	Persistence	None	-
4	Male	6	Yes	None	4 years	Moderate	Persistent	+	-	38	Improvement	Yes	Mite
5	Female	13	No	Conjunctivitis	2 years	Mild	Persistent	+	-	7.09	Improvement	None	
6	Male	14	Yes	Conjunctivitis	12 years	Moderate	Persistent	+	+	187	Persistence	None	-
7	Female	13	Yes	Conjunctivitis	10 years	Moderate	Intermitent	+	-		Persistence	Yes	Mite

NAPT: Nasal provocation test; *Der p: Dermatophagoides pteronyssinus; Phl p: Phleum pratense* . [a]: performed by skin prick test or elevated specific IgE (>0.35 KU/L)

Table III. Results of BAT after adding *Dermatophagoides pteronyssinus* (*Der p*) and *Phleum pratense* (*Phl p*) at 3 different concentrations in 6 pediatric patients with LAR.

Patient 7 did not respond to the anti-IgE-positive control, so he was excluded from the analysis.

Only patient 3 showed basophil activation after antigen stimulation with *Dermatophagoides pteronyssinus* at two different concentrations (5000DBU/ml and 500DBU/ml, respectively).

Patient	Negative control	Positive control	*Der p* 5000 DBU/ml	*Der p* 500 DBU/ml	*Der p* 50 DBU/ml	*Phl p* 30,000 DBU/ml	*Phl p* 3000 DBU/ml	Phl p 300 DB
1	2.7	87.1	2.7	0	2.5	1.8	0	0.9
2	5.1	81	3.1	3.5	5.7	3.3	0.4	1.9
3	2.9	19.3	34.9	15.5	4.5	6.5	7.3	6.6
4	1.3	10.3	1.6	2.6	3	1.8	0.8	0.6
5	1.3	78.5	0.8	0.2	1.2	0.6	0.4	0.4
6	1.6	7.1	5.4	2.7	2.8	2.3	2.3	1.9

Table IV. Clinical evolution of NAR and LAR children after 7-year follow-up period.

	NAR (N=33)	**LAR (N=7)**	P-value[a]
Clinical evolution (n,%)			
Unchanged	5 (14)	4 (57)	<0.05*
Worsening	1 (3)	0	0.66
Improvement	18 (49)	2 (29)	0.33
Resolution	9 (24)	1 (14)	0.56
Agreed to test (n,%)	25 (76)	7 (100)	0.07
Agreed to SPT	25 (76)	7 (100)	0.07
Agreed to s-IgE	20 (60)	7 (100)	0.02*
Positive SPT (n,%)	8 (32)	3 (43)	0.64
D pteronyssinus	4 (50)	2 (67)	0.21
L destructor	2 (25)	2 (67)	<0.005*
Other dust mites	3 (38)	3 (100)	<0.05*
P pratense	1 (13)	0	0.66
Other	3 (38)	1 (33)	0.6
Specific sIgE (n,%)	5 (25)	3 (43)	0.65
D pteronyssinus	4 (80)	1	0.79
L destructor	1 (20)	3	<0.005*
P pratense	2	0	0.53

[a]Pearson´s Chi-squared test of independence

Table V. Demographic and clinical features of non-sensitized (NAR) and sensitized (AR) patients, after a follow-up period of 7 years.

	NAR (N=15)	**AR (N=10)[1]**	*p-value*
Age (y) at rhinitis onset			
Mean	8	6.7	0.66[a]
Range	4-14	3-11	
Median	7	7	
Current age (y)			
Mean	15.4	14.3	0.24[a]
Range	12-22	12-18	
Median	14	14	
Gender			
Female, n (%)	8 (53)	3 (30)	0.36[b]

	NAR (N=15)	**AR (N=10)[1]**	*p-value*
Atopic comorbidities			
Asthma, n (%)	3 (20)	4 (40)	0.28[b]
Atopic dermatitis, n (%)	1 (7)	0	0.4[b]
Food allergy, n (%)	1 (7)	2 (20)	0.32[b]
Conjunctival, n (%)	1 (7)	2 (20)	0.33[b]
***FHA, n (%)**	9 (60)	8 (80)	0.13[b]
Symptons evolution			
Persistence, n (%)	3 (20)	2 (20)	1[b]
Worsening, n (%)	1 (7)	0	0.4[b]
Improvement, n (%)	8 (53)	7 (70)	0.4[b]
Resolution, n (%)	3 (20)	1 (10)	0.5[b]
ARIA classification			
Perennial, n (%)	8 (53)	8 (80)	0.25[b]
Seasonal, n (%)	4 (26)	1 (10)	0.25[b]
Persistent, n (%)	4 (26)	3 (30)	0.88[b]
Intermitent, n (%)	8 (53)	6 (60)	0.88[b]
Severity			
Mild, n (%)	6 (40)	5 (50)	
Moderate, n (%)	8 (53)	5 (50)	0.54[c]
Sever, n (%)	1 (7)		
Main nasal symptons			
Blockage, n (%)	6 (40)	6 (60)	0.34[b]
Rinorrhea, n (%)	5 (33)	4 (40)	0.78[b]
Sneezing, n (%)	1 (7)	1 (10)	0.78[b]
Pruritus, n (%)	1 (7)	1 (10)	0.78[b]
Sensitivity to			
Dust mites, n (%)		8 (80)	
Grass pollen, n (%)		1 (10)	
Birch pollen, n (%)		1 (10)	

*FHA: family history of atopy