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Title: Systematic evaluation of allergic phenotypes of rhinitis in children and adolescents

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Key message: Local forms of airway allergy (dual and local allergic rhinitis) are relevant differential diagnosis in pediatric rhinitis. An allergological work-up exclusively based on atopy tests (skin prick test and serum allergen-specific IgE) misdiagnoses >1/3 of children and adolescents referred to specialized units with chronic nasal symptoms. Several clinical and demographic features help select non-atopic rhinitis patients with high chances of testing positive in a nasal allergen challenge.

Abstract (249 words)

Background: Three allergic phenotypes of rhinitis have been described in adults: allergic rhinitis (AR), local allergic rhinitis (LAR) and dual allergic rhinitis (DAR, coexistence of AR and LAR). Nevertheless, most centres follow a diagnostic approach only based on skin prick test and serum allergen-specific IgE (collectively called atopy tests, AT). This approach prevents the recognition of LAR and DAR, the diagnosis of which requires a nasal allergen challenge (NAC). Here, we investigate the existence of LAR and DAR phenotypes in children and adolescents, and the misdiagnosis rate associated with a work-up exclusively based on AT.

Methods: Clinical data was obtained during physician-conducted interviews, and AT and NAC were systematically performed in 5-18 year-old patients with chronic rhinitis. The misdiagnosis rate was defined as the proportion of cases where AT and NAC results were discordant.

Results: 173 patients (mean age 15.1 years, 39.9% male) completed the study. AR (positive AT and NAC), LAR (negative AT and positive NAC), DAR (positive AT and NAC for some allergens and negative AT and positive NAC for other allergens) and non-allergic rhinitis (negative NAC) were diagnosed in 45.7%, 24.9%, 11.6% and 17.9% of individuals, respectively.

The clinical profile was comparable among allergic phenotypes, but allergic patients had a significantly earlier rhinitis onset, higher conjunctivitis prevalence and more severe disease than NAR individuals. A diagnostic work-up exclusively based on AT, misclassified 37.6% of patients.

Conclusions: LAR and DAR represent relevant differential diagnosis in pediatric rhinitis. NAC increases the diagnostic accuracy of clinical algorithms for rhinitis in children and adolescents.

Introduction

Allergic rhinitis (AR) is a prevalent disease during childhood and adolescence. Among the different treatments for AR, allergen immunotherapy (AIT) is the only disease-modifying intervention with a sustained effect persisting after therapy discontinuation. An early diagnosis of AR with subsequent initiation of AIT has the potential to both control the symptoms and prevent disease progression, especially asthma onset (1). Therefore, when administered during early stages of the disease, AIT is a cost-effective intervention.

Besides AR, children and adolescents can suffer from non-allergic rhinitis (NAR) (2). The differentiation between these phenotypes has classically been based on the results of skin-prick test (SPT) and serum allergen-specific IgE (sIgE) (collectively called atopy tests) (3). However, these tests only denote sensitization (4), whereas the confirmation of the allergic triggers of

rhinitis may require a nasal allergen challenge (NAC). The NAC is a safe and reproducible technique (5) that should be monitored by both subjective and objective parameters (6). The later include measurements of nasal patency by active anterior rhinomanometry (7) or acoustic rhinometry, among other methods. Interestingly, the atopy-based division of chronic rhinitis is being progressively replaced by an allergy-based classification grounded on the pattern of nasal allergen-specific reactivity. In this regard, AR patients are defined as those showing positive NAC, SPT, and/or serum sIgE results (2, 3). Conversely, the NAR phenotype comprises a heterogeneous group of diseases, who only share the negativity of the NAC (8). Importantly, an atopic patient who does not experience nasal symptoms upon exposure to any allergen including those testing positive in SPT, should be considered a NAR individual. Moreover, some non-atopic rhinitis subjects also display positive NAC results, and this phenotype is termed local allergic rhinitis (LAR) (9, 10). No population study has investigated to date LAR prevalence and several works focusing on selected populations report a variable frequency of the disease (0-67%) in children and adolescents (11-18). A systematic review of these studies revealed a 16.1% prevalence of LAR in non-atopic rhinitis patients <18 years (19). To further complicate the field, nasal reactivity to aeroallergens with and without positive SPT can coexist in the same individual ("coexistence of AR and LAR") (20). This phenotype has been called dual allergic rhinitis (DAR), and its occurrence in pediatric populations remains uninvestigated. Importantly, AIT can control the symptoms and increase the quality of life of adult LAR patients (reviewed in 21). Therefore, AIT might be also useful in children and adolescents with local airway allergy. Nevertheless, the scarce implementation of the NAC impairs the recognition of LAR and DAR individuals and the subsequent initiation of specific interventions.

This study analyzed the clinical characteristics of rhinitis phenotypes in a large group of children and adolescents referred to specialized centers for chronic nasal symptoms. A systematic evaluation including atopy tests and NAC was conducted in every individual, and the rate of misdiagnosis associated with a diagnostic work-up exclusively based on atopy test was calculated. The results were also used to generate a clinical predictive model for LAR in children and adolescents and to propose a diagnostic algorithm for pediatric rhinitis.

Methods

Subjects and study design

We recruited prospectively children and adolescents with chronic rhinitis from the Pediatric and Allergy Departments of Hospital Regional Universitario de Malaga (Malaga, Spain) between January 2017 and December 2019:

Inclusion criteria: 5-18 year-old subjects with ≥ 2 -year history of chronic rhinitis.

Exclusion criteria: conditions severely affecting nasal patency (choanal atresia, septum perforation/deviation, obstructive adenoids, etc.), malignancies, severe or unstable cardiovascular, pulmonary, immunologic, non-atopic skin, or metabolic disease, FEV1<70%, pregnancy or breast-feeding for female adolescents, and inability to cooperate (6). No procedure was conducted within the 4 weeks following respiratory infections.

The study was approved by Local Ethics Committee and performed according to the Declaration of Helsinki. All subjects ≥ 16 years and the guardians of younger individuals provided signed informed consent.

Demographic and clinical data

After inclusion, the responsible physician interrogated the patient and/or guardians about relevant clinical parameters for rhinitis. A detailed description of the questionnaire can be found at the supplementary material. This study used the classification of rhinitis persistence and severity proposed by ARIA (22) (see supplementary material for details).

Atopy evaluation

SPT were performed with all relevant aeroallergens in our area and according to international recommendations (4). Serum sIgE was determined by fluoroenzyme immunosorbent assay (UNICAP; Pharmacia Diagnostics, Uppsala, Sweden). Cutoff for positivity was ≥ 0.35 kUA/l. A complete list of the aeroallergens tested can be found in the supplementary material.

The number of sensitizations detected by atopy tests defined three atopy states: non-atopic (all tests negative), mono-atopic (1 allergen positive), and poly-atopic (>1 allergen positive).

Allergy evaluation

Allergy was evaluated by NAC with a single allergen per session (NAC-S) in atopic individuals, and with multiple allergens per session (NAC-M) in non-atopic patients (14, 23). All patients were

challenged with 5 different allergens, grass-mix pollen, *Olea europaea* (OE), *Alternaria alternata*, *Parietaria judaica* (all 30 HEP/mL) and *Dermatophagoides pteronyssinus* (DP) (100 HEP/mL). Additionally, individuals exposed to pets were also provoked with cat and dog (both 30 HEP/mL) (LETI, S.L. Tres Cantos, Madrid, Spain). Four increasing allergen concentrations (1/100, 1/10, 1/2, and undiluted) and the maximal allergen concentration (undiluted) were administered during NAC-S and NAC-M, respectively. NAC was monitored by visual analogue scale (VAS) of nasal-ocular symptoms and acoustic rhinometry (SRE 2000 rhinometer, Rhinometrics, Lynge, Denmark) (24, 25). The test was considered positive if an increase $\geq 30\%$ in the total VAS and a bilateral decrease $\geq 30\%$ in the total sum of vol. 2-6 cm (patients ≥ 160 cm-tall) or 2-4 cm (patients < 160 cm-tall) of the acoustic rhinometry, compared with baseline, occurred (6). A detailed description of NAC procedure can be found in the supplementary material.

The number of allergens testing positive in the NAC defined three allergy states: non-allergic (NAC negative for all allergens), mono-allergic (NAC positive for 1 allergen), and poly-allergic (NAC positive for > 1 allergen).

Clinical phenotypes

Based on the previous results, four phenotypes were defined:

AR: at least one allergen positive in NAC, and positive SPT and/or serum sIgE for all NAC-positive allergens.

LAR: at least one allergen positive in NAC, and negative SPT and serum sIgE for all allergens.

DAR: positive NAC and positive SPT to at least one allergen, and positive NAC and negative SPT to at least one allergen.

NAR: negative NAC for all allergens, regardless of the result of SPT and serum sIgE.

Statistical analysis

The diagnostic accuracy of atopy tests for the identification of allergy was calculated as the rate of concordance (determined by kappa index) between the results of atopy tests and the results of NAC (gold standard). The misdiagnosis rate was calculated as the sum of the rate of false positive results plus the rate of false negative results. Statistical analysis was performed with SPSS version 15.0 (Chicago, IL). Continuous data were expressed as mean+standard deviation or median+interquartile ranges, and categorical data as percentages. Comparisons were performed by Chi² analysis (categorical data), parametric test (ANOVA, Student T-test or Mann–Whitney U test for unrelated samples) non-parametric test (Student T-test or Wilcoxon-test for related samples),

and Bonferroni correction for multiple comparisons. A multivariate backward logistic regression of clinical-demographic data was performed to generate a predictive model. P values <0.05 were considered statistically significant.

Results

Demographic and clinical characteristics

Two-thousand children and adolescents with chronic rhinitis were prospectively evaluated, and 173 individuals accepted to participate and provided signed informed consent. The mean age at inclusion was 15.1 ± 2.2 years, and 104 subjects were female (60.1%). The mean age at rhinitis onset was 10.0 ± 4.0 years. The demographic and clinical characteristics of included patients are shown in Table S1.

Atopy evaluation

From 173 patients included, 101 (58.4%) displayed at least one positive SPT, thus being classified as atopic. Six patients with positive SPT displayed negative serum sIgE results, whereas all subjects with detectable serum sIgE had consistent positive SPT. Conversely, 72/173 individuals (41.6%) were negative for both tests, thus being labelled as non-atopic. Among atopic individuals, 80.2% (81/101) and 19.8% (20/101) were poly-atopic and mono-atopic, respectively. Moreover, 51.5% of atopic individuals (52/101) were sensitized to both seasonal and perennial allergens, whereas 28.7% (29/101) and 19.8% (20/101) of them were sensitized to seasonal and to perennial allergens only, respectively. OE pollen was the main sensitizer in atopic patients (40.5%), followed by DP (27.2%) (Figure S1).

Allergy evaluation

Three-hundred and nine NACs yielded a positive result in 142 patients (82.1%). Therefore, these individuals were classified as allergic, whereas the 31 (17.9%) remaining subjects were labelled as non-allergic (Figure 1). The main allergens eliciting a positive NAC were OE pollen (47.3%) and DP (46.7%) (Figure S1). Overall, 97 patients were poly-allergic and 45 were mono-allergic (56.1% and 26.0% of the whole group, respectively).

Rhinitis phenotypes

Table 1 shows the comparative features between allergic and non-allergic subjects. Allergic patients had an earlier onset of rhinitis and conjunctivitis, together with a significantly higher

prevalence of family history of atopy, seasonal symptoms, and moderate-to-severe rhinitis, and a more frequent evolution towards the clinical worsening. Moreover, allergic patients suffered from nasal itching, sneezing, conjunctivitis and atopic dermatitis significantly more often than non-allergic individuals. Allergic patients identified the exposure to house dust, vegetation and furry animals as triggers for rhinitis in a significantly more frequent manner than non-allergic individuals. Conversely, irritants smells were reported as rhinitis triggers more often by non-allergic subjects.

Regarding allergic phenotypes, 79/173 (45.7%), 43/173 (24.9%) and 20/173 (11.6%) children and adolescents fulfilled diagnostic criteria for AR, LAR, and DAR, respectively. No significant differences were found among allergic phenotypes for clinical or demographic parameters. Conversely, AR patients reported an earlier conjunctivitis onset (9.4 ± 4.2 vs 12.5 ± 2.2 ; $P=0.008$), higher prevalence of seasonal symptoms (25.3% vs 3.2%; $P=0.008$), nasal itching (69.6% vs 25%; $P<0.001$), sneezing (81.0% vs 45.2%; $P<0.001$), and conjunctivitis (59.6% vs 22.6%; $P<0.001$) than NAR individuals. Moreover, compared with NAR, LAR subjects reported a higher prevalence of family history of atopy (62.8% vs 29.0%; $P=0.004$), and moderate-to-severe symptoms (81.4% vs 34.5%; $P<0.001$) and identified more frequently the exposure to house dust (60.5% vs 12.9%; $P<0.001$) and vegetation (51.2% vs 6.5%; $P<0.001$) as rhinitis triggering factors. Finally, DAR patients displayed a worse evolution during the previous 2 years (45.0% vs 12.9%; $P=0.019$), reported less often nasal obstruction/congestion (45.0% vs 93.5%; $P<0.001$), and identified irritant smells as rhinitis triggers less frequently (5.0% vs 48.4%; $P=0.001$) than NAR subjects (Table 2).

Figure 2 shows the distribution of nasal reactivity to individual allergens among allergic phenotypes. OE pollen was the main trigger for DAR (100%) and AR (44.3%), whereas DP was the main elicitor of LAR (48.8%). The comparison between allergic phenotypes showed a higher prevalence of allergy to OE pollen in DAR (100%, $P<0.001$), compared with AR (44.3%) and LAR (20.9%). Similarly, allergy to OE pollen was less common in LAR than in AR ($P=0.010$) or DAR ($P<0.001$).

Misdiagnosis rate of atopy tests

The ability of atopy tests to detect allergic subjects was assessed by analysing the concordance between SPT and NAC results. Sixty-five individuals (37.6%) showed a mismatch between the SPT and NAC results (43 LAR, 20 DAR, and 2 atopic NAR) (Table S2). Clinically relevant allergens that atopy tests failed to identify in a more frequent manner were DP and *Alternaria*

alternata in LAR patients (48.8% and 10.4%, respectively), and *Parietaria judaica* and DP in DAR individuals (30.0% each) (Figure 2).

Clinical predictive model

A multivariate backward logistic regression model was generated to facilitate LAR diagnosis in non-atopic patients. Table S3 shows the risk factors and odds ratios of the four parameters showing a significant association with LAR in non-atopic subjects, 5-18 years-old, with chronic rhinitis. Because of this association, these four parameters were used to generate the model: presence of moderate-to-severe nasal symptoms (MSS) and nasal symptoms triggered by exposure to house dust (HD-TF), vegetation (veg-TF), and irritant smells (irritant-TF). The mathematical expression of the model is:

$$\text{Logit}(p) = -2.305 + 2.851 \text{ MSS} + 2.475 \text{ HD-TF} + 4.164 \text{ veg-TF} - 3.079 \text{ irritant-TF}$$

The discriminative power (area under the receiver operating characteristic (ROC) curve) was 0.944 (C.I. 95% 0.888-0.999, $p < 0.001$), and *Logit* (p) values > 0.228 displayed 97.8% sensitivity and 72.2% specificity. Figure 3 shows the ROC curve and the prognostic probability of the model.

Diagnostic algorithm for pediatric rhinitis

Based on our results, we propose to conduct NAC in individuals ≥ 5 years suffering from moderate-to-severe persistent rhinitis and displaying negative/discrepant SPT results (Figure 4).

Discussion

Our study constitutes the first systematic analysis of atopy and allergy in children and adolescents referred to Pediatric and Allergy Units for chronic rhinitis. Unlike previous studies focusing on difficult to treat non-atopic patients (14) or specific allergens (11, 17), NAC was conducted in every subject with a panel of allergens which included the triggers of most airway allergy cases in our area (26).

In our work, 58.11% of non-atopic patients suffered from LAR. This proportion is in line with previous reports from Western populations (29-67%) (11-14). Conversely, studies from Asian countries report a significantly lower prevalence (0-19%) (15-18). Whether these differences relate to ethnic specificities, methodological aspects, or other factors needs further investigation. Moreover, in our study, 19.8% of atopic children and adolescents fulfilled criteria for DAR diagnosis (20). Importantly, this finding represents the first demonstration of the existence of DAR phenotype among pediatric patients. Collectively, these results confirm that allergic mechanisms are involved in 82.3% of cases of pediatric rhinitis referred to specialized units. On the other hand, a population study would be required to investigate the prevalence of the distinct phenotypes in the general population. Still, our work highlights the need to include local forms of airway allergy in the differential diagnosis of chronic nasal symptoms during childhood.

Our study also shows that the allergic phenotypes of rhinitis share a common clinical and demographic profile, probably reflecting similar pathological mechanisms. Conversely, significant differences exist between allergic and non-allergic patients. Severe symptoms triggered by house dust and vegetation, a trend towards clinical worsening over time, and coexistence of conjunctivitis prevailed in allergic patients, whereas milder symptoms elicited by irritant smells were commoner among non-allergic individuals. These data allowed generate a predictive model to identify non-atopic patients with high probability of testing positive in NAC. Our mathematical model displays an excellent discriminative power and might help select candidates for NAC in the clinical practice. Nevertheless, the model needs further validation in future studies.

Importantly, our work also shows that a diagnostic work-up exclusively based on atopy tests fails to identify all relevant allergic triggers in 37.6% of children and adolescents with rhinitis, thus

incurring in a significant rate of misdiagnosis. In this regard, although it is well-established that atopy tests only denote sensitization (4), most clinical centers do not perform NAC routinely. While clinical history often suffices to establish the relevance of IgE-sensitizations, NAC is indispensable to identify LAR and DAR patients (27). Therefore, we propose a diagnostic algorithm including the performance of NAC in patients with discrepancies between clinical history and results of atopy tests. This approach will pave the way for a holistic and personalized therapeutic strategy (28). Of note, a comprehensive intervention including both avoidance measures and AIT (21, 29) during childhood might prevent rhinitis progression and asthma onset during later periods of life (30).

In summary, our study demonstrates that DAR and LAR are relevant differential diagnosis in pediatric rhinitis. An allergological work-up exclusively based on atopy tests misdiagnoses >1/3 of children and adolescents referred to specialized units with chronic nasal symptoms, thus emphasizing the need to implement NAC in the clinical algorithms of rhinitis during childhood.

References

1. Valovirta E, Petersen TH, Piotrowska T, Laursen MK, Andersen JS, Sørensen HF, Klink R. Results from the 5-year SQ grass sublingual immunotherapy tablet asthma prevention (GAP) trial in children with grass pollen allergy. *J Allergy Clin Immunol*. 2018;141(2):529-538.e13.
2. Papadopoulos N, Aggelides X, Stamataki S, Prokopakis E, Katotomichelakis M, Xepapadaki P. New concepts in pediatric rhinitis. *Pediatr Allergy Immunol*. 2021 Online ahead of print.
3. Rotiroti G, Roberts G, Scadding GK. Rhinitis in children: common clinical presentations and differential diagnoses. *Pediatr Allergy Immunol*. 2015;26(2):103-10.

4. Roberts G, Ollert M, Aalberse R, Austin M, Custovic A, DunnGalvin A, Eigenmann PA, Fassio F, Grattan C, Hellings P, Hourihane J, Knol E, Muraro A, Papadopoulos N, Santos AF, Schnadt S, Tzeli K. A new framework for the interpretation of IgE sensitization tests. *Allergy*. 2016;71(11):1540-1551.
5. Augé J, Vent J, Agache I, Airaksinen L, Campo Mozo P, Chaker A, Cingi C, Durham S, Fokkens W, Gevaert P, Giotakis A, Hellings P, Herknerova M, Hox V, Klimek L, La Melia C, Mullol J, Muluk NB, Muraro A, Naito K, Pfaar O, Riechelmann H, Rondon C, Rudenko M, Samolinski B, Tasca I, Tomazic P, Vogt K, Wagenmann M, Yeryomenko G, Zhang L, Mösges R. EAACI Position paper on the standardization of nasal allergen challenges. *Allergy*. 2018;73(8):1597-1608.
6. Eguiluz-Gracia I, Testera-Montes A, González M, Pérez-Sánchez N, Ariza A, Salas M, Moreno-Aguilar C, Campo P, Torres MJ, Rondon C. Safety and reproducibility of nasal allergen challenge. *Allergy*. 2019;74(6):1125-1134
7. Cilluffo G, Zicari AM, Ferrante G, Malizia V, Fasola S, Duse M, De Castro G, De Vittori V, Schiavi L, Brindisi G, Petrelli P, La Grutta S. Assessing repeatability and reproducibility of Anterior Active Rhinomanometry (AAR) in children. *BMC Med Res Methodol*. 2020;20(1):86.
8. Rondón C, Bogas G, Barrionuevo E, Blanca M, Torres MJ, Campo P. Nonallergic rhinitis and lower airway disease. *Allergy*. 2017;72(1):24-34.
9. Rondon C, Campo P, Togias A, Fokkens WJ, Durham SR, Powe DG, Mullol J, Blanca M. Local allergic rhinitis: concept, pathophysiology, and management. *J Allergy Clin Immunol*. 2012;129(6):1460-7.
10. Beken B, Eguiluz-Gracia I, Yazıcıoğlu M, Campo P. Local allergic rhinitis: a pediatric perspective. *Turk J Pediatr*. 2020;62(5):701-710.
11. Fuiano N, Fusilli S, Incorvaia C. A role for measurement of nasal IgE antibodies in diagnosis of *Alternaria*-induced rhinitis in children. *Allergol Immunopathol (Madr)*. 2012;40(2):71-4.
12. Zicari AM, Occasi F, Di Fraia M, Mainiero F, Porzia A, Galandrini R, Giuffrida A, Bosco D, Bertin S, Duse M. Local allergic rhinitis in children: Novel diagnostic features and potential biomarkers. *Am J Rhinol Allergy*. 2016;30(5):329-34.
13. Krajewska-Wojtys A, Jarzab J, Gawlik R, Bozek A. Local allergic rhinitis to pollens is underdiagnosed in young patients. *Am J Rhinol Allergy*. 2016;30(6):198-201.

14. Tsilochristou O, Kyriakakou M, Manolaraki I, Lakoumentas J, Tiligada E, Maragkoudakis P, Douladiris N, Papadopoulos NG. Detection of local allergic rhinitis in children with chronic, difficult-to-treat, non-allergic rhinitis using multiple nasal provocation tests. *Pediatr Allergy Immunol.* 2019;30(3):296-304.
15. Cheng KJ, Xu YY, Liu HY, Wang SQ. Serum eosinophil cationic protein level in Chinese subjects with nonallergic and local allergic rhinitis and its relation to the severity of disease. *Am J Rhinol Allergy.* 2013;27(1):8-12.
16. Jang TY, Kim YH. Nasal provocation test is useful for discriminating allergic, nonallergic, and local allergic rhinitis. *Am J Rhinol Allergy.* 2015;29(4):e100-4.
17. Buntarickpornpan P, Veskitkul J, Pacharn P, Visitsunthorn N, Vichyanond P, Tantilipikorn P, Jirapongsananuruk O. The proportion of local allergic rhinitis to *Dermatophagoides pteronyssinus* in children. *Pediatr Allergy Immunol.* 2016;27(6):574.
18. Tao XY, Ng CL, Chen D, Lin ZB, Wu SL, Liang MJ, Li CW, Xu R. Clinical Characteristics and Allergen Sensitization Patterns of Patients with Local Allergic Rhinitis in Southern China. *Int Arch Allergy Immunol.* 2018;175(1-2):107-13.
19. Hamizan AW, Rimmer J, Alvarado R, Sewell WA, Kalish L, Sacks R, Harvey RJ. Positive allergen reaction in allergic and nonallergic rhinitis: a systematic review. *Int Forum Allergy Rhinol.* 2017;7(9):868-877.
20. Eguiluz-Gracia I, Fernandez-Santamaria R, Testera-Montes A, Ariza A, Campo P, Prieto A, Perez-Sanchez N, Salas M, Mayorga C, Torres MJ, Rondon C. Coexistence of nasal reactivity to allergens with and without IgE sensitization in patients with allergic rhinitis. *Allergy.* 2020.
21. Eguiluz-Gracia I, Ariza A, Testera-Montes A, Rondón C, Campo P. Allergen Immunotherapy for Local Respiratory Allergy. *Curr Allergy Asthma Rep.* 2020;20(7):23.
22. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, T Zuberbier, C E Baena-Cagnani, G W Canonica, C van Weel, I Agache, N Aït-Khaled, C Bachert, M S Blaiss, S Bonini, L-P Boulet, P-J Bousquet, P Camargos, K-H Carlsen, Y Chen, A Custovic, R Dahl, P Demoly, H Douagui, S R Durham, R Gerth van Wijk, O Kalayci, M A Kaliner, Y-Y Kim, M L Kowalski, P Kuna, L T T Le, C Lemiere, J Li, R F Lockey, S Mavale-Manuel, E O Meltzer, Y Mohammad, J Mullol, R Naclerio, R E O'Hehir, K Ohta, S Ouedraogo, S Palkonen, N Papadopoulos, G Passalacqua, R Pawankar, T A Popov, K F Rabe, J Rosado-Pinto, G K Scadding, F E R Simons, E Toskala, E Valovirta, P van Cauwenberge, D-Y Wang, M Wickman, B P Yawn, A Yorgancioglu, O M Yusuf, H Zar, I Annesi-Maesano, E D Bateman, A Ben Kheder, D

- A Boakye, J Bouchard, P Burney, W W Busse, M Chan-Yeung, N H Chavannes, A Chuchalin, W K Dolen, R Emuzyte, L Grouse, M Humbert, C Jackson, S L Johnston, P K Keith, J P Kemp, J-M Klossek, D Larenas-Linnemann, B Lipworth, J-L Malo, G D Marshall, C Naspitz, K Nekam, B Niggemann, E Nizankowska-Mogilnicka, Y Okamoto, M P Orru, P Potter, D Price, S W Stoloff, O Vandenplas, G Viegi, D Williams, World Health Organization; GA(2)LEN; AllerGen. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy*. 2008;63 Suppl 86:8-160.
23. Rondon C, Campo P, Herrera R, Blanca-Lopez N, Melendez L, Canto G, Torres MJ, Blanca M. Nasal allergen provocation test with multiple aeroallergens detects polysensitization in local allergic rhinitis. *J Allergy Clin Immunol*. 2011;128(6):1192-7.
 24. Eguiluz-Gracia I, Testera-Montes A, Salas M, Perez-Sanchez N, Ariza A, Bogas G, Bartra J, Torres MJ, Rondon C. Comparison of diagnostic accuracy of acoustic rhinometry and symptoms score for nasal allergen challenge monitoring. *Allergy*. 2021;76(1):371-375.
 25. Hilberg O, Pedersen OF. Acoustic rhinometry: recommendations for technical specifications and standard operating procedures. *Rhinol Suppl*. 2000;16:3-17.
 26. Ojeda P, Ibáñez MD, Olaguibel JM, Sastre J, Chivato T; investigators participating in the National Survey of the Spanish Society of Allergology and Clinical Immunology Alergológica 2015. Alergológica 2015: A National Survey on Allergic Diseases in the Spanish Pediatric Population. *J Investig Allergol Clin Immunol*. 2018;28(5):321-329.
 27. Eguiluz-Gracia I, Tay TR, Hew M, Escribese MM, Barber D, O'Hehir RE, Torres MJ. Recent developments and highlights in biomarkers in allergic diseases and asthma. *Allergy*. 2018;73(12):2290-2305.
 28. Santos AF, Borrego LM, Rotiroti G, Scadding G, Roberts G. The need for patient-focused therapy for children and teenagers with allergic rhinitis: a case-based review of current European practice. *Clin Transl Allergy*. 2015;5(1):2.
 29. Eguiluz-Gracia I, Mathioudakis AG, Bartel S, Vijverberg SJH, Fuertes E, Comberiati P, Cai YS, Tomazic PV, Diamant Z, Vestbo J, Galan C, Hoffmann B. The need for clean air: The way air pollution and climate change affect allergic rhinitis and asthma. *Allergy*. 2020;75(9):2170-2184.
 30. Campo P, Eguiluz-Gracia I, Plaza-Serón MC, Salas M, José Rodríguez M, Pérez-Sánchez N, González M, Molina A, Mayorga C, Torres MJ, Rondón C. Bronchial asthma triggered by house dust mites in patients with local allergic rhinitis. *Allergy*. 2019;74(8):1502-1510.

Accepted Article

Table 1. Demographic and clinical comparison between allergic and non-allergic children

Total patients (n = 173)	Allergic (n= 142)	Non-allergic (n=31)	OR (95%CI)	P value
Age in years, mean (SD):				
at evaluation	15.15 (2.08)	15.23 (2.57)	(-0.96-0.79)	0.850
rhinitis onset	9.70 (3.99)	11.36 (3.69)	(0.03-3.28)	0.046
conjunctivitis onset	9.63 (4.19)	12.50 (2.24)	(1.24-4.51)	0.001
onset of asthma symptoms	9.15 (4.79)	9 (4.67)	(-3.29-3.00)	0.927
Family history of atopy, N (%)	87 (61.3)	9 (29)	3.87 (1.66-9.01)	0.001
Female gender, N (%)	86 (60.6)	18 (58.1)	1.11 (0.50-2.44)	0.797
Smoking habit, N (%)	4 (2.8)	0 (0)	0.82 (0.76-0.88)	0.344
Pet-keeping habit, N (%)	58 (40.8)	12 (38.7)	1.09 (0.49-2.42)	0.826
Urban dwellers, N (%)	91 (64.1)	24 (77.4)	1.92 (0.77-4.77)	0.154
Rural dwellers, N (%)	51 (35.9)	7 (22.6)		
Respiratory diseases, N (%):				
conjunctivitis	93 (65.5)	7 (22.6)	6.51 (2.62-16.17)	<0.001
asthma	59 (41.5)	10 (31)	1.49 (0.66-3.40)	0.338
otitis	22 (15.5)	6 (19.4)	0.76 (0.28-2.08)	0.597
Other allergic diseases, N (%):				
atopic dermatitis	39 (27.5)	3 (9.7)	3.53 (1.01-12.29)	0.036
food allergy	5 (3.5)	1 (3.2)	1.10 (0.12-9.72)	0.935
drug allergy	5 (3.5)	0 (0)	0.82 (0.76-0.88)	0.289
Nasal symptoms, N (%):				
itching	90 (63.4)	8 (25.8)	4.98 (2.08-11.92)	<0.001
sneezing	112 (78.9)	14 (45.2)	4.53 (2.01-10.23)	<0.001
rhinorrhea	85 (59.9)	23 (74.2)	0.52 (0.22-1.24)	0.135
obstruction/congestion	71 (50)	29 (93.5)	0.07 (0.02-0.30)	<0.001
Persistence, N (%):				

intermittent	30 (21.1)	7 (22.6)	1.09 (0.43-2.77)	0.858
persistent	112 (78.9)	24 (77.4)		
Seasonality, N (%):				
perennial	111 (78.2)	30 (96.8)	8.38 (1.10-63.91)	0.016
seasonal	31 (21.8)	1 (3.2)		
Severity, N (%):				
mild	30 (21.1)	20 (65.5)	6.79 (2.93-15.71)	<0.001
moderate-to-severe	112 (78.9)	11 (34.5)		
Evolution, N (%):				
similar-better	90 (63.4)	27 (87.1)	3.9 (1.29-11.77)	0.011
worse	52 (36.6)	4 (12.9)		
Specific triggers, N (%):				
house dust	78 (54.9)	4(12.9)	8.23 (2.74-24.74)	<0.001
vegetation	59 (42)	2 (6.5)	10.31 (2.37-44.89)	<0.001
furry animals	36 (25.4)	0 (0)	0.77 (0.71-0.85)	<0.001
Non-specific triggers, N (%):				
irritant smells	19 (13.4)	15 (48.4)	0.17 (0.07-0.39)	<0.001
temperature changes	33 (23.2)	11 (31)	0.55 (0.24-1.27)	0.156

CI: confidence interval; N: number; OR: Odd ratio; SD: standard deviation. P < 0.05 values appear in bold.

Figures legends:

Figure 1: Flow-chart of the study. SPT: skin prick test; sIgE: allergen-specific IgE; NAC: nasal allergen challenge; AR: allergic rhinitis; DAR: dual allergic rhinitis; LAR: local allergic rhinitis; NAR: non-allergic rhinitis.

Figure 2: Distribution of sensitization (atopy tests) and specific reactivity (NAC) to aeroallergens among allergic phenotypes of rhinitis. SPT: skin-prick test; sIgE: serum allergen-specific IgE; NAC: nasal allergen challenge; AR: allergic rhinitis; LAR: local allergic rhinitis; DAR: dual allergic rhinitis; D.: *dermatophagoides*.

Figure 3: Receiver operating characteristics curve of the multivariate logistic predictive model for local allergic rhinitis. AUC: area under the curve; CI: confidence interval.

Figure 4: Clinical algorithm for pediatric rhinitis. *Alternatively, serum allergen-specific IgE can be quantified; #With the allergen/s that would explain the symptom pattern; \$: mixed rhinitis implies the coexistence of allergen and non-allergic mechanisms in the same rhinitis patient; grey color refers to diagnostic tests and orange color to rhinitis phenotypes.

Figure S1: Frequency of sensitization and specific reactivity to aeroallergens among study subjects.

Table 2. Demographic and clinical characteristics of pediatric rhinitis phenotypes

Total patients (N = 173)	AR (N= 79)	LAR (N=43)	DAR (N=20)	NAR (N=31)	P value
Demographic data					
Age in years, mean (SD)					
at evaluation	15.51 (1.72)	15 (2.09)	14.19 (2.87)	15.07 (2.57)	0.089
rhinitis onset	9.52 (3.95)	10.26 (3.72)	9.21 (4.74)	11.36 (3.69)	0.161
conjunctivitis onset	9.44 (4.18)	10.30 (3.77)	9.08 (5.13)	12.50 (2.24)	0.105
P value [£]	0.008*	1.017**	0.192***		
onset of asthma symptoms	9.07 (5.06)	10.33 (4.20)	6.75 (4.60)	9 (4.67)	0.368
Female gender, N (%)	47 (59.5)	29 (67.4)	10 (50)	18 (58.1)	0.597
Family history of atopy, N (%)	49 (62)	27 (62.8)	11 (55.5)	9 (29)	0.011
OR (95% CI)	3.99 (1.63-9.81)	4.13 (1.53-11.12)	2.99 (0.92-9.66)		
P value [£]	0.002*	0.004**	0.064***		
Smoking habit, N (%)	2 (2.5)	1 (2.3)	1 (5)	0 (0)	0.708
Pet-keeping habit, N (%)	32 (40.5)	18 (41.9)	8 (40)	12 (38.7)	0.995
Urban dwellers, N (%)	54 (68.4)	26 (60.5)	11 (55)	24 (77.4)	0.299
Comorbidities					

Respiratory diseases, N (%)					
conjunctivitis	55 (59.6)	26 (60.5)	12 (60)	7 (22.6)	< 0.001
OR (95% CI)	7.86 (2.98-20.70)	5.24 (1.85-14.84)	5.14 (1.51-17.57)		
P value [£]	< 0.001*	0.001**	0.007***		
asthma symptoms	35 (44.3)	16 (37.2)	8 (40)	10 (31)	0.677
otitis	11 (13.9)	8 (18.6)	3 (15)	6 (19.4)	0.865
Other diseases, N (%)					
atopic dermatitis	20 (25.3)	13 (30.2)	6 (30)	3 (9.7)	0.185
food allergy	3 (3.8)	2 (4.7)	0 (0)	1 (3.2)	0.818
drug allergy	2 (2.5)	2 (4.7)	1 (5)	0 (0)	0.626
Rhinitis characteristics					
Nasal symptoms, N (%)					
itching	55 (69.6)	26 (60.5)	9 (45)	8 (25.8)	<0.001
OR (95% CI)	6.59 (2.58-16.81)	4.40 (1.60-12.01)	2.35 (0.71-7.76)		
P value [£]	<0.001*	0.003**	0.156***		
sneezing	64 (81)	33 (76.7)	15 (75)	14 (45.2)	0.002
OR (95% CI)	5.18 (2.10-17.79)	4.01 (1.47-10.99)	3.64 (1.06-12.52)		
P value [£]	<0.001*	0.005**	0.036***		
obstruction/congestion	41 (51.9)	21 (48.8)	9 (45)	29 (93.5)	<0.001

OR (95% CI)	0.07 (0.02-0.33)	0.07 (0.01-0.31)	0.06 (0.01-0.30)		
P value [£]	<0.001*	<0.001**	<0.001***		
rhinorrhea	49 (62)	27 (62.8)	9 (45)	23 (74.2)	0.219
Persistence, N (%)					0.600
intermittent	15 (19)	12 (27.9)	3 (15)	7 (22.6)	
persistent	64 (81)	31 (72.1)	17 (85)	24 (77.4)	
Seasonality, N (%)					0.039
perennial	59 (74.7)	34 (79.1)	18 (90)	30 (96.8)	
seasonal	20 (25.3)	9 (20.9)	2 (10)	1 (3.2)	
OR (95%CI)	10.17 (1.30-79.46)	7.94 (0.95-66.39)	3.33 (0.28-39.43)		
P value [£]	0.008*	0.028**	0.315		
Severity, N (%)					< 0.001
mild	17 (21.5)	8 (18.6)	5 (25)	20 (65.5)	
moderate-to-severe	62 (78.5)	35(81.4)	15 (75)	11 (34.5)	
OR (95%CI)	6.63 (2.67-16.48)	7.96 (2.75-23.04)	5.46 (1.56-19.06)		
P value [£]	< 0.001*	< 0.001**	0.006***		
Evolution, N (%)					0.030
worse	26 (32.9)	17 (39.5)	9 (45%)	4 (12.9)	
OR (95%CI)	3.31 (1.05-10.46)	4.41 (1.31-14.88)	5.52 (1.40-21.75)		
P value [£]	0.035*	0.018**	0.019***		

Triggers					
Specific triggers, N (%)					
house dust	42 (53.2)	26 (60.5)	10(50)	4(12.9)	<0.001
OR (95%CI)	7.66 (2.45-23.94)	10.32 (3.06-34.80)	6.75 (1.72-26.51)		
P value [£]	< 0.001*	< 0.001**	0.004***		
vegetation	31 (39.2)	22 (51.2)	6 (30)	2 (6.5)	<0.001
OR (95%CI)	9.37 (2.09-42.07)	15.19 (3.22-71.76)	6.21 (1.11-34.80)		
P value [£]	< 0.001*	< 0.001**	0.045***		
furry animals	22 (27.8)	6 (14)	8 (40)	0(0)	0.001
OR (95%CI)	0.65 (0.56-0.76)	0.54 (0.44-0.68)	0.28 (0.17-0.45)		
P value [£]	0.001*	0.037**	<0.001***		
Non-specific triggers, N (%)					
Irritants smells	12 (15.2)	6 (14)	1 (5)	15 (48.4)	<0.001
OR (95%CI)	0.19 (0.08-0.49)	0.17 (0.06-0.53)	0.06 (0.01-0.47)		
P value [£]	0.001*	0.002**	0.001***		
temperature changes	21 (26.6)	7 (16.3)	5 (25)	11 (31)	0.307

AR: allergic rhinitis; CI: confidence interval; DAR: dual allergic rhinitis; LAR: local allergic rhinitis; NAR: non-allergic rhinitis; OR: Odd ratio; P value^f: Comparative analysis between AR vs NAR (*), LAR vs NAR (**), and DAR vs NAR (***), significant differences appear in bold; SD: standard deviation

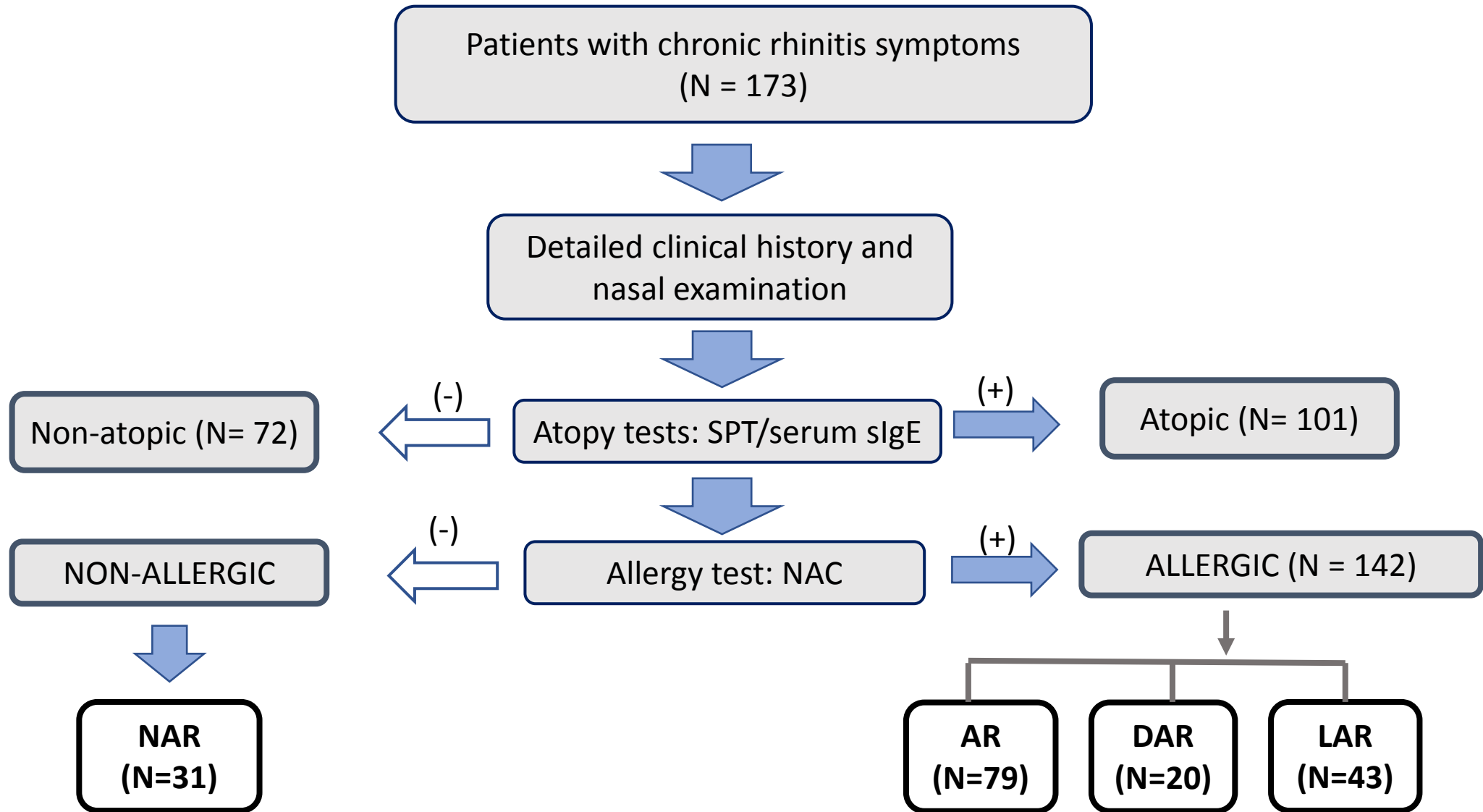
Figure 1.

Figure 2.

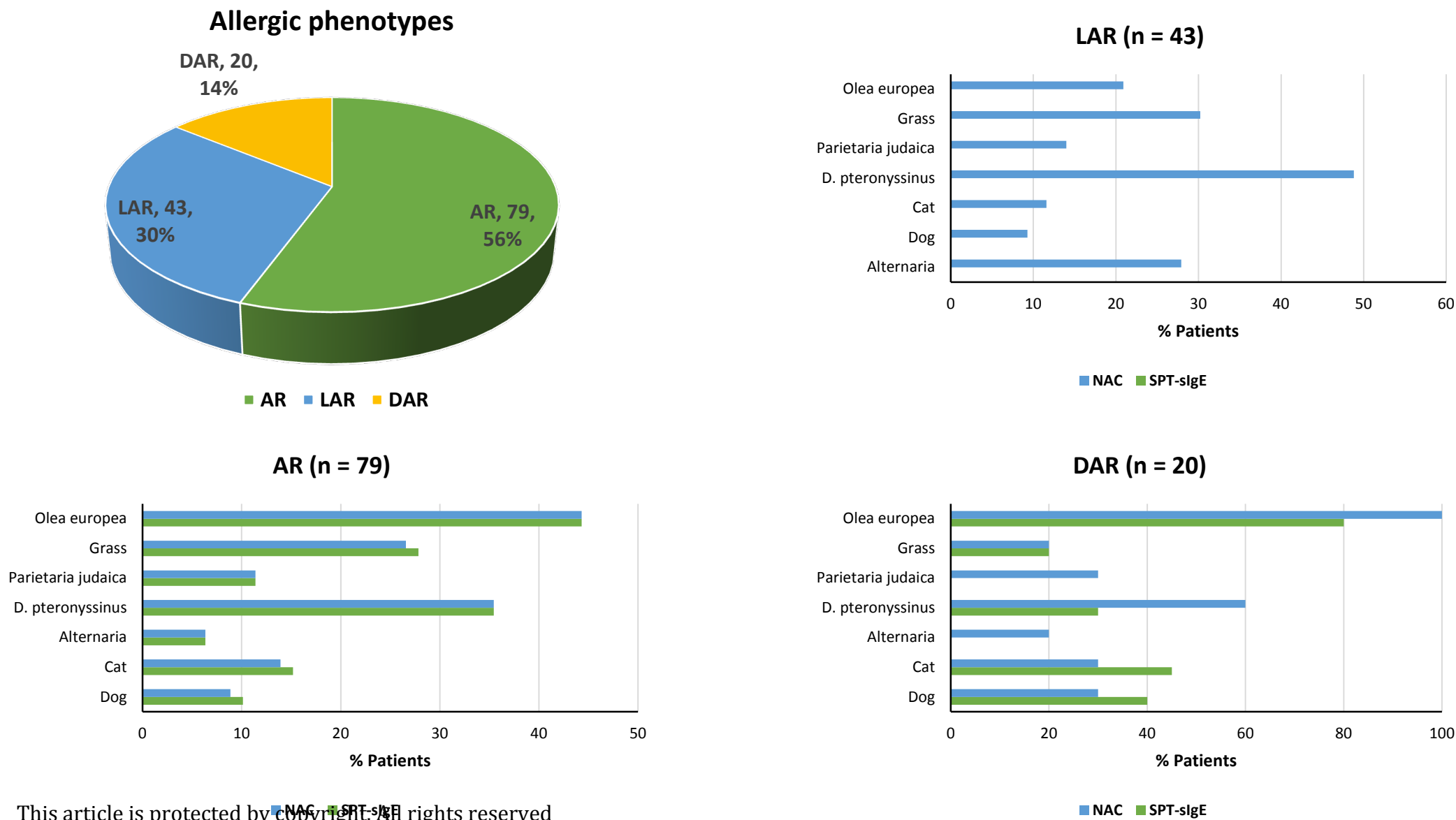
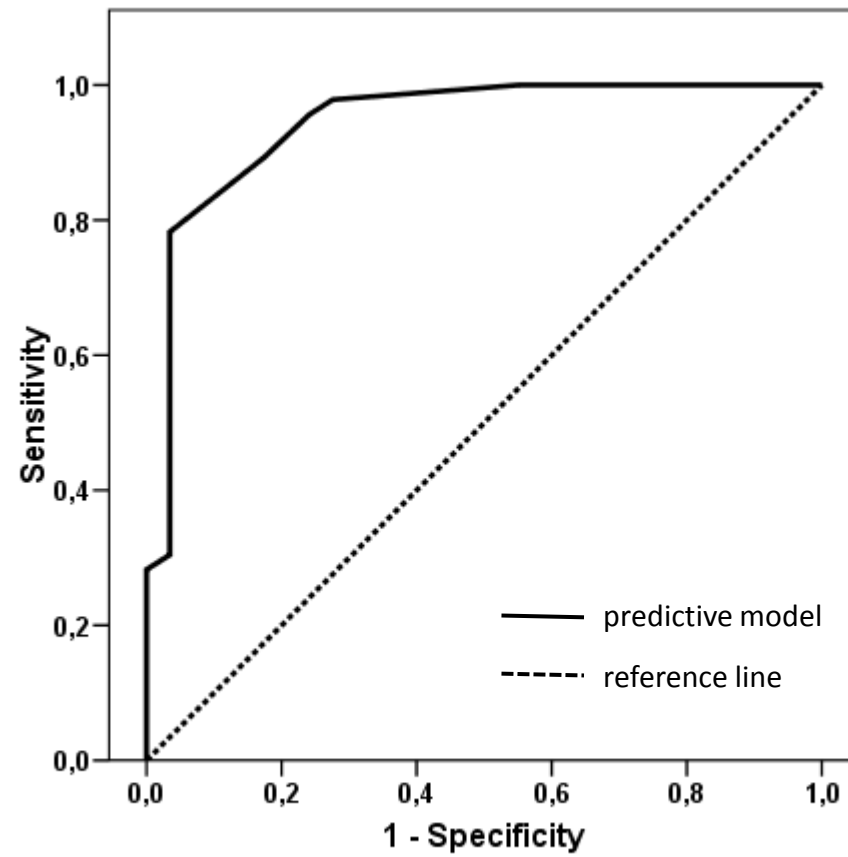


Figure 3.

AUC	95% CI	P value
0.944	0.888-0.999	<0.001

Figure 4

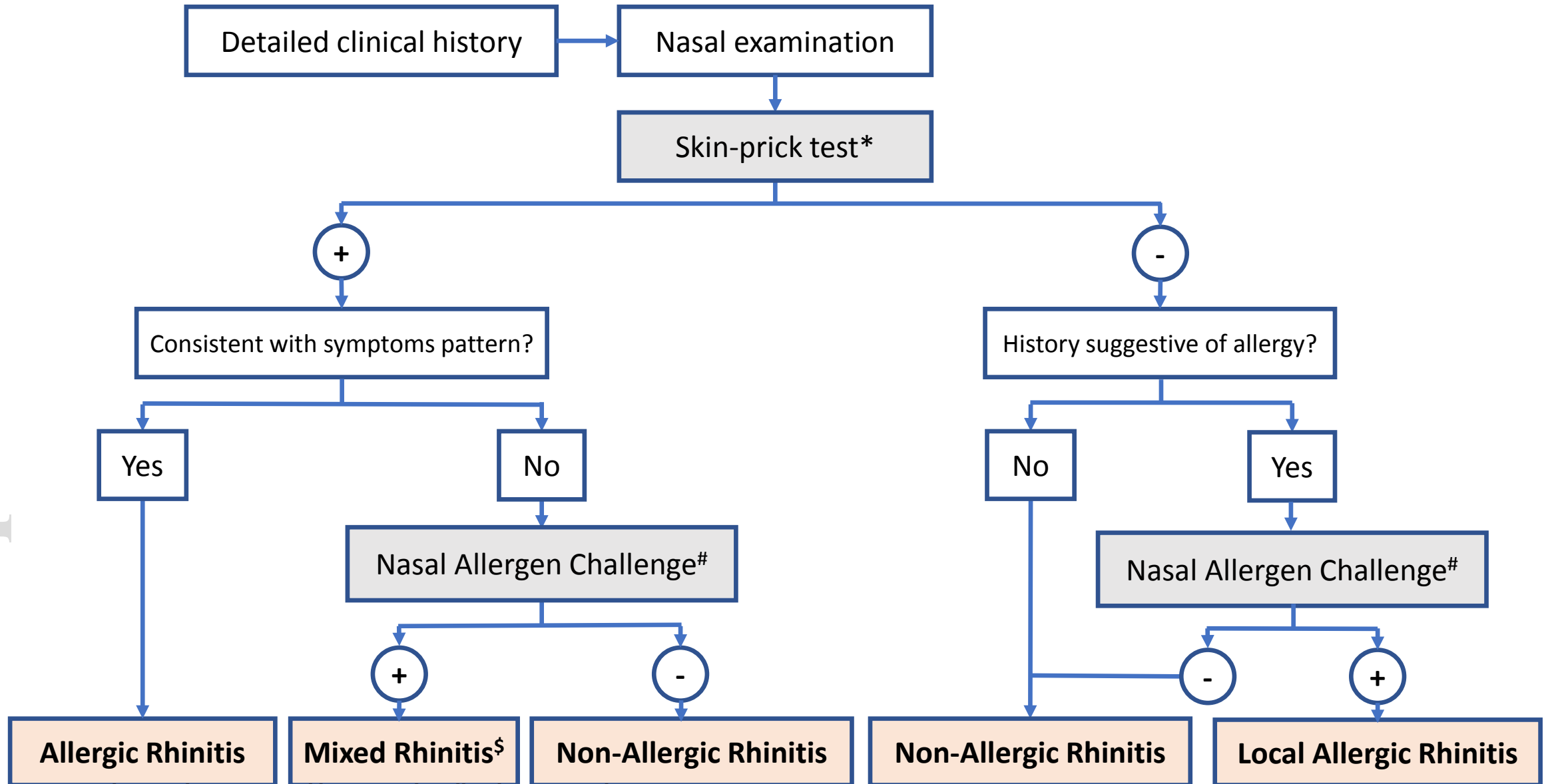
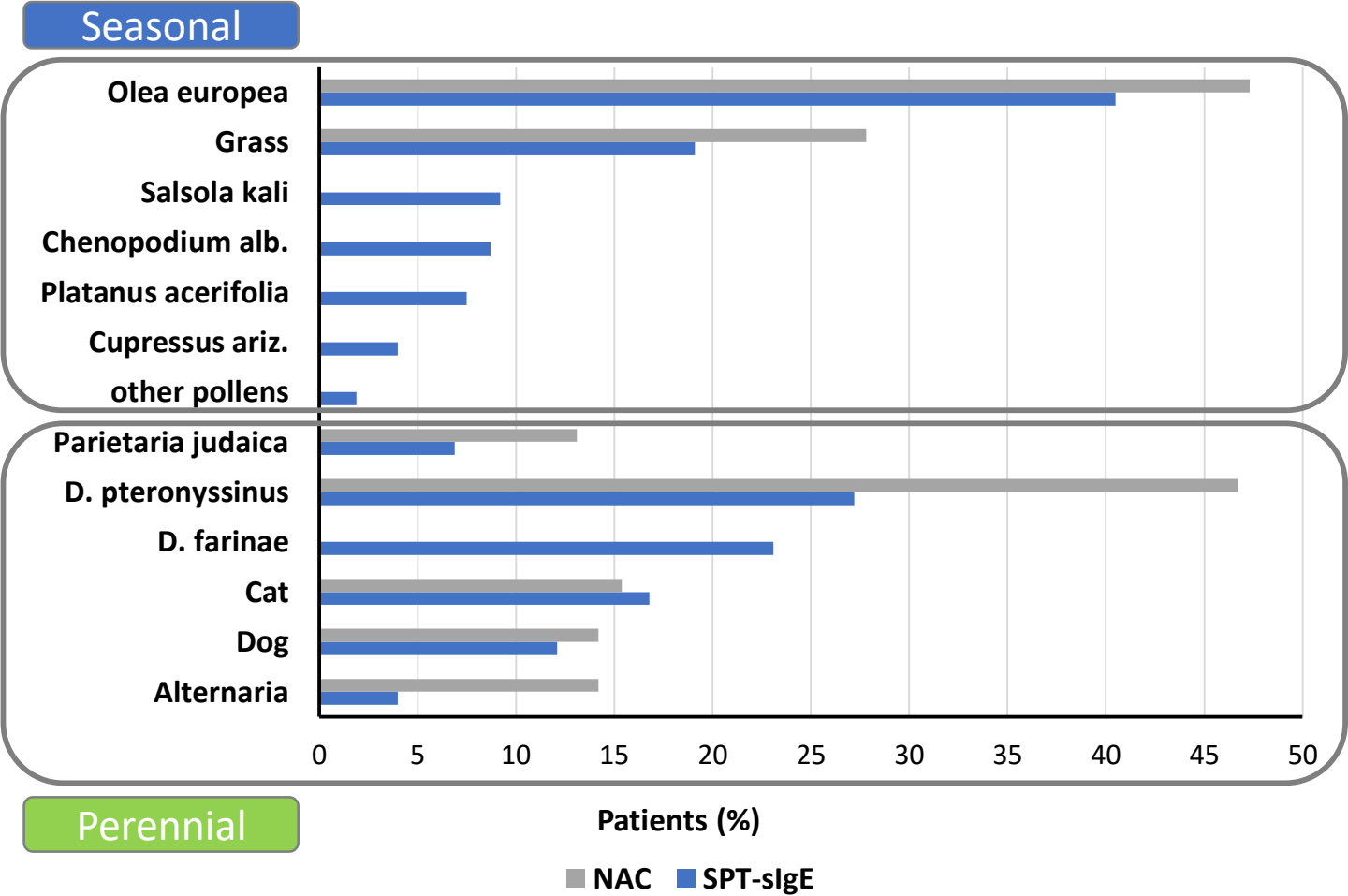


Figure S1

N= 173



Online supplementary material

Title: Systematic evaluation of allergic phenotypes of rhinitis in children and adolescents

Running title: Allergic phenotypes of pediatric rhinitis

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Methods

Patient selection

During the selection visit, all children and adolescents underwent a nasal examination including visual inspection, facial and nasal palpation, and anterior rhinoscopy using a light source and a Killian nasal speculum in order to assess for exclusion criteria related to nasal anatomy.

Clinical and demographic data

Prior to the performance of any test, the responsible physician conducted an interview with the patients and their guardians. During these interviews the following demographic and health-related information was collected: gender, age, urban/rural dwelling, family history of atopy, pet-keeping and smoking habits, rhinitis features (age at onset and seasonality, persistence and severity during the previous 2 years), rhinitis evolution (changes in rhinitis severity during the previous 2 years) and existence of respiratory and allergic comorbidities. Moreover, patients were asked about specific and unspecific triggers of their nasal symptoms. The former included exposure to house dust, vegetation (e.g. trips to the countryside) or furry pets, whereas the later comprised exposure to irritant smells and sudden changes of temperature.

This study used ARIA classification (1). Rhinitis persistence was classified as intermittent (symptoms <4 days/week or for <4 consecutive weeks) or persistent (symptoms >4 days/week or for >4 consecutive weeks). Rhinitis severity was graded according to the number of compromised items from the following: daily/sport/leisure activities, work/school performance, sleep, and presence of troublesome symptoms. Rhinitis was classified as mild or as moderate-to-severe if no item or 1-4 items were compromised, respectively.

46 Atopy evaluation

47 Skin-prick tests (SPT) were performed with all relevant aeroallergens in our area:
48 *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Poa pratense*, *Phleum*
49 *pratense*, *Lolium perenne*, *Cupressus arizonica*, *Platanus acerifolia*, *Olea europea*,
50 *Chenopodium spp*, *Artemisia artemisifolia*, *Parietaria judaica*, *Salsola kali*, *Alternaria*
51 *alternata*, *Aspergillus fumigatus*, *Cladosporium spp*, *Penicillium spp*, cat and dog (LETI,
52 S.L. Tres Cantos, Madrid, Spain). Serum allergen-specific IgE was determined for the
53 same aeroallergens tested in SPT.

54 Nasal allergen challenge procedure

55 Children and adolescents were instructed to avoid systemic corticosteroids, nasal
56 corticosteroids, systemic or nasal antihistamines, and topical vasoconstrictors for 12, 4,
57 2, and 1 weeks, respectively, before the nasal allergen challenge (NAC) session (2, 3).
58 NAC were performed using two different standardized and validated protocols for
59 bilateral provocation: NAC with a single allergen/session administered at four
60 progressively increasing concentrations (1/100, 1/10, 1/2, and undiluted) (NAC-S) (2-
61 5), or NAC with five allergens/session (NAC-M) administered undiluted in a sequential
62 manner (6, 7). In both cases, allergens/allergen solutions were administered with a 15-
63 minute interval. Before the application of the allergen, all children and adolescents were
64 subjected to a bilateral nasal challenge with 100 µL of isotonic saline serum in order to
65 rule out nasal hyperreactivity (8). In case of positive saline challenge, NAC was
66 rescheduled for a later visit with a minimum 1-week interval. Patients displaying two
67 consecutive positive saline challenges (read *NAC monitoring*) were excluded from the
68 study. The individuals with negative saline challenge were subsequently administered
69 100 µL/nostril of lyophilized/freshly reconstituted allergens previously brought to room

temperature. The consecutive allergens/allergen solutions were applied at 15-minute intervals onto the head of the inferior turbinate of both nostrils using a micropipette (3-5). During allergen administration, individuals remained seated and held their breath to prevent laryngeal aspiration (2, 3).

NAC monitoring

The outcome of the NAC was evaluated using both objective and subjective parameters. The nasal patency was objectively assessed by acoustic rhinometry (SRE 2000 rhinometer, Rhinometrics, Lynge, Denmark) following the Guidelines of the Standardization Committee on Acoustic Rhinometry (9) and nasal-ocular symptoms (nasal obstruction, rhinorrhea, nasal itching, sneezing, and ocular symptoms) were subjectively evaluated by means of a visual analogue scale (VAS) of 100 mm for each symptom. The total VAS was the sum of the five symptom-specific VASs (range 0-500 mm) (1). Measurements of both parameters were made at baseline, 15 minutes after saline challenge, 15 minutes after every allergen/allergen solution administration, and one hour after the last allergen dose (3, 6). In case of symptomatic rhinitis (total VAS >300 mm) at baseline, the procedure was stopped, and NAC was rescheduled for a later visit with a minimal 1-week interval (2, 3). Patients displaying symptomatic rhinitis at baseline in two consecutive visits were excluded from the study. NAC-S was stopped before the administration of the last allergen dose if a positive response with lower concentrations occurred (3). In case of positive result after the administration of the second allergen in the NAC-M, the procedure was stopped and separated NAC-S were scheduled for every allergen (6). In case of positive result 15 minutes after the administration of the first allergen in the NAC-M, the procedure was stopped and a NAC-M with the remaining allergens was scheduled. The NAC was considered positive when a bilateral decrease $\geq 30\%$ in the volume 2-6 cm (patients ≥ 160 cm-tall) or in the volume 1-4 cm

(patients <160 cm-tall) of the acoustic rhinometry (8, 9) and an increase $\geq 30\%$ in the total VAS occurred (3). Children and adolescents were discharged if no reaction occurred during the hour following the administration of the last allergen/allergen dose. In case of positive NAC, patients were kept under observation for a minimal period of 2 hours or until symptoms resolved (2, 3).

References

1. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, T Zuberbier, C E Baena-Cagnani, G W Canonica, C van Weel, I Agache, N Ait-Khaled, C Bachert, M S Blaiss, S Bonini, L-P Boulet, P-J Bousquet, P Camargos, K-H Carlsen, Y Chen, A Custovic, R Dahl, P Demoly, H Douagui, S R Durham, R Gerth van Wijk, O Kalayci, M A Kaliner, Y-Y Kim, M L Kowalski, P Kuna, L T T Le, C Lemiere, J Li, R F Lockey, S Mavale-Manuel, E O Meltzer, Y Mohammad, J Mullol, R Naclerio, R E O'Hehir, K Ohta, S Ouedraogo, S Palkonen, N Papadopoulos, G Passalacqua, R Pawankar, T A Popov, K F Rabe, J Rosado-Pinto, G K Scadding, F E R Simons, E Toskala, E Valovirta, P van Cauwenberge, D-Y Wang, M Wickman, B P Yawn, A Yorgancioglu, O M Yusuf, H Zar, I Annesi-Maesano, E D Bateman, A Ben Kheder, D A Boakye, J Bouchard, P Burney, W W Busse, M Chan-Yeung, N H Chavannes, A Chuchalin, W K Dolen, R Emuzyte, L Grouse, M Humbert, C Jackson, S L Johnston, P K Keith, J P Kemp, J-M Klossek, D Larenas-Linnemann, B Lipworth, J-L Malo, G D Marshall, C Naspitz, K Nekam, B Niggemann, E Nizankowska-Mogilnicka, Y Okamoto, M P Orru, P Potter, D Price, S W Stoloff, O Vandenplas, G Viegi, D Williams, World Health Organization; GA(2)LEN; AllerGen. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy*. 2008;63 Suppl 86:8-160.
2. Dordal MT, Lluch-Bernal M, Sánchez MC, Rondón C, Navarro A, Montoro J, Matheu V, Ibáñez MD, Fernández-Parra B, Dávila I, Conde J, Antón E, Colás C, Valero A; SEAIC Rhinoconjunctivitis Committee. Allergen-specific nasal provocation testing: review by the rhinoconjunctivitis committee of the Spanish Society of Allergy and Clinical Immunology. *J Investig Allergol Clin Immunol*. 2011; 21(1): 1125- 12.
3. Auye J, Vent J, Agache I, et al. EAACI Position paper on the standardization of nasal allergen challenges. *Allergy*. 2018; 73(8): 1597- 1608.
4. Pepper AN, Ledford DK. Nasal and ocular challenges. *J Allergy Clin Immunol*. 2018 May;141(5):1570-1577.
5. Rondón C, Fernández J, López S, Campo P, Doña I, Torres MJ, Mayorga C, Blanca M. Nasal inflammatory mediators and specific IgE production after nasal challenge with grass pollen in local allergic rhinitis. *J Allergy Clin Immunol*. 2009; 124(5): 1005- 1011.
6. Rondón C, Campo P, Herrera R, Blanca-Lopez N, Melendez L, Canto G, Torres MJ, Blanca M. Nasal allergen provocation test with multiple aeroallergens detects polysensitization in local allergic rhinitis. *J Allergy Clin Immunol*. 2011;128(6):1192.
7. Tsilochristou O, Kyriakakou M, Manolaraki I, Lakoumentas J, Tiligada E, Maragkoudakis P, Douladiris N, Papadopoulos NG. Detection of local allergic rhinitis in children with chronic, difficult-to-treat, non-allergic rhinitis using multiple nasal provocation tests. *Pediatr Allergy Immunol*. 2019;30(3):296-304.
8. Van Gerven L, Steelant B, Hellings PW. Nasal hyperreactivity in rhinitis: A diagnostic and therapeutic challenge. *Allergy*. 2018;73(9):1784-1791.
9. Hilberg O, Pedersen OF. Acoustic rhinometry: recommendations for technical specifications and standard operating procedures. *Rhinol Suppl*. 2000;16:3-17.

170 **Table S1. Demographic and clinical characteristics of patients**

Demographic data	Total group N=173
Age in years, mean (SD)	
at evaluation	15.14 (2.16)
rhinitis onset	10 (3.98)
conjunctivitis onset	9.99 (4.11)
at onset of asthma symptoms	9.12 (4.73)
Years of evolution, mean (SD)	5.17 (3.48)
Female gender, N(%)	104 (60.1)
Family history of atopy, N(%)	96 (55.5)
Smoking habit, No, N(%)	169 (97.7)
Pet-keeping habit, N(%)	70 (40.5)
Dwelling, N(%)	
urban	115 (66.5)
rural	58 (33.5)
Multimorbidity	
Respiratory diseases, N(%)	
Conjunctivitis	100 (57.8)
Asthma symptoms	69 (39.9)
Otitis	28 (16.2)
Chronic rhinosinusitis	0
Other diseases, N(%)	
atopic dermatitis	42 (24.3)
food allergy	6 (3.5)
drug allergy	5 (2.9)
Rhinitis characteristics	

Persistence, N(%)	
Intermittent	37 (21.4)
persistent	136 (78.6)
Seasonality, N(%)	
perennial	141 (81.5)
seasonal	32 (18.5)
Severity, N(%)	
mild	49 (28.3)
moderate-to-severe	124 (71.7)
Evolution last 2 years, N(%)	
better	18 (10.4)
similar	99 (57.2)
worse	56 (32.4)
Triggers	
Specific triggers, N (%)	
house dust	82 (47.4)
vegetation	61 (35.3)
furry animals	36 (20.8)
Non-specific triggers, N (%)	
Irritant smells	34 (19.7)
temperature changes	44 (25.4)

N: number; SD: standard deviation

178 **Table S2. Diagnostic accuracy of atopy tests**

Atopy tests	AR (n=79)	LAR (n=43)	DAR (n=20)	NAR (n=31)	Diagnostic accuracy	FP	FN	Diagnostic error
SPT	101 (58.4%)	0 (0%)	0 (0%)	72 (41.4%)	108 (62.4%)	2/101 (2%)	63 (36.4%) LAR+DAR	65 (37.6%)
SPT+sIgE	101 (58.4%)	0 (0%)	0 (0%)	72 (41.4%)	108 (62.4%)	2/101 (2%)	63 (36.4%) LAR+DAR	65 (37.6%)
sIgE	95 (54.9%)	0 (0%)	0 (0%)	78 (45.1%)	110 (63.6%)	0 (0%)	63 (36.4%) LAR+DAR	63 (36.4%)

179 AR: allergic rhinitis; DAR: dual allergic rhinitis; FN: false negative; FP: false positive;

180 LAR: local allergic rhinitis; NAR: non-allergic rhinitis; SPT: skin-prick test; sIgE:

181 serum allergen-specific IgE

182

183 **Table S3. Multivariate logistic regression model: Potential predictive factors for**

184 **LAR diagnosis in non-atopic children and adolescents**

Variable	B	OR	95% CI	P value
MSS	2.851	17.310	2.716-110.308	0.003
HD-TF	2.475	11.886	2.034-69.456	0.006
Vegetation-TF	4.164	64.322	5.282-783.304	0.001
Irritant-TF	-3.079	0.046	0.005-0.448	0.008
Constant	-2.305			

185 CI: confidence interval; OR: odds ratio; MSS: moderate-severe rhinitis symptoms; HD:

186 house dust; TF: triggering factor

187