Nasal specific IgE to Der p is not an acceptable screening test to predict the outcome of the nasal challenge test in patients with non-allergic rhinitis

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ABSTRACT

Objectives: Nasal specific IgE (NsIgE) is the most common marker to identify type-2 inflammation in local allergic rhinitis (LAR). However, the comparison of NsIgE in different types of rhinitis, its frequency in tropical countries, and its diagnostic performance for predicting the outcome of a nasal challenge test (NCT) has had limited study. The main objective of this study was to explore the diagnostic performance of NsIgE to *Dermatophagoides pteronyssinus* (Der p) among different types of rhinitis and control subjects in a tropical population.

Methods: We evaluated the frequency of NsIgE, systemic atopy (serum sIgE and Skin Prick Test), and nasal eosinophils, and we performed nasal challenge tests (NCTs) with Der p in 3 groups of patients; rhinitis without atopy (RWoA) (n = 25), rhinitis with atopy (RWA) (n = 25), and control subjects (n = 18).

Results: NsIgE had a low sensitivity and specificity to predict a positive NCT in the RWoA group: 48% had NsIgE, but only 28% had a positive NCT. Among the RWA group 84% had NsIgE and 80% had a positive NCT; the association of NsIgE and positive NCT was high (>80%). In the control group 27.8% had NsIgE, but none had a positive NCT.

Conclusions: NsIgE performs poorly in predicting NCT results in patients with non-allergic rhinitis. More methodical investigations are needed in this complex area of rhinitis. In patients with allergic rhinitis, NsIgE was useful in predicting a positive nasal challenge, but not superior to the systemic atopic test.

Keywords: Atopy, Mites, Nasal challenge test, Immunoglobulin E, Rhinitis

INTRODUCTION

The term chronic rhinitis refers to a set of nasal symptoms that may have different pathogenesis. In allergic rhinitis (AR), atopy against a clinically relevant allergen is demonstrated by specific immunoglobulin E
(sIgE) in serum, or by the skin prick test (SPT).

Non-allergic rhinitis is less common, but includes several entities with different mechanisms. In recent years, a new entity called local allergic rhinitis (LAR) has been proposed, characterized by the absence of systemic sensitization, but with the presence of type 2 inflammation (eg, sIgE) of the nasal mucosa and a positive nasal challenge test (NCT) to an allergen.

In AR and LAR, sIgE is the principal biomarker that defines the presence of type 2 inflammation. In AR, sIgE can be detected circulating in the serum or in the mast cells of the skin (systemic atopy), but it can also be found localized in different tissues. On the other hand, in LAR, sIgE can only be measured in the nasal mucosa (NsIgE). Other markers (eg, eosinophils, Th2 cells, and some cytokines) have been proposed, but so far none of them is superior to sIgE in detecting the allergenic trigger that induces the inflammatory response.

Few studies have evaluated the frequency of LAR in tropical cities, which is of great importance since this is where 40% of the world population lives. The tropical zone has its own environmental characteristics, so the frequency of LAR may be different from that in other regions. House dust mites (HDM) are the most frequent cause of IgE sensitization and respiratory symptoms. Dermatophagoides spp. explain 80–90% of RA in tropical countries and seem to be the main source of allergens in LAR.

Despite the fact that sIgE is useful to determine suspected allergic triggers, 10–30% of the general population have atopy but not an allergic disease; 40–70% of AR patients may have sIgE from different triggers, but usually not all are clinically relevant. Although several studies have emerged evaluating the frequency of LAR, especially in seasonal countries, little has been done about the performance of sIgE in nasal mucosa as a predictor of the outcome of an NCT (eg, false positives, false negatives).

The NCT is the gold standard test to confirm the clinical relevance of sIgE in patients, but it is a time-consuming test for doctor and patient, and also it has the risk of inducing systemic symptoms. If markers such as NsIgE or nasal eosinophils have a good association with NCT, they could reduce the need for NCTs and the subsequent risks, by serving as predictive markers for the test.

Based on these observations, the main objective of this study was to evaluate the diagnostic performance of NsIgE as a predictor of NCT results, and explore the frequency of NsIgE response to Dermatophagoides pteronyssinus (Der p) in a population located in the tropics.

**METHODS**

**Study population**

This is a cross-sectional, analytical, observational study. Patients were selected in a non-randomized manner according to their order of attendance at an allergy service during the recruitment period. The patients were selected from individuals aged between 18 and 40 years for the epidemiological peak of LAR reported in other studies, with chronic persistent moderate/severe rhinitis, defined according to the criteria of the ARIA guidelines.

We excluded patients with nasal or systemic comorbidities that could affect the interpretation of the NCT (nasal polyposis, septal perforation, pregnancy, use of medications such as oral steroids, cyclosporine, omalizumab, and immunotherapy). In addition, the control group of healthy volunteers who shared sociodemographic characteristics with patients with rhinitis was included.

**Definition of groups**

Patients with rhinitis were divided into 2 groups; a group of people with rhinitis with atopy (RWA), and a group with rhinitis without atopy (RWoA). The atopy was evaluated by SPT and serum sIgE to Der p.

We avoid the use of the terms “allergic rhinitis group”, “non-allergic rhinitis group”, or “LAR group”, since the presence of systemic or local IgE does not confirm these diagnoses until the nasal challenge test is done.

**Bioethical considerations**

The study protocol was approved by the institutional ethics committee (code IN20-2017) and is in line with the Helsinki declaration. Each of the
participants signed to indicate their informed consent.

**Atopy evaluation**

The patients underwent a skin prick test (SPT) (Inmunotek Laboratory, Madrid, Spain) using standardized extracts with Der p. The interpretation of the test was based on the presence of a wheal with a diameter greater than 3 mm compared to the negative control, according to international guidelines, and histamine was used as positive control. The Der p sIgE from serum was measured by the ImmunoCAP system. The cut-off value for the serum sIgE was 0.35 kUA/L, based on the recommendations of the instrument and previous studies.

Other common allergens in the region were also evaluated to determine the fraction of atopy to Der p among all sensitizations.

**Nasal challenge test (NCT)**

Challenge tests were performed with Der p after a rhinoscopy. The Der p extract (Laboratory Inmunotek®, Spain) at a concentration of 10,000 UB/mL was applied with a nasal spray in a measured dose of 100 μL/puff in each nostril. Previously, the presence of non-specific nasal hyperreactivity was ruled out by performing the same procedure with saline solution. The result of the test was evaluated objectively by performing acoustic rhinometry (acoustic rhometer ECCOVISION) with a reduction greater than 20% considered positive, and also subjectively with the Lebel score and the visual analog scale (VAS).

To define a positive NCT, the criteria proposed by the “European Academy of Allergy and Clinical Immunology” (EAACI) was used; we considered a positive NCT result to be achieved when the test was “clearly positive” or “moderately positive” according to the EAACI definition.

**Collection of nasal mucus for measurement of NsIgE**

Thirty minutes after the NCT, a nasal lavage was performed using the technique described by Naclerio et al. with some modifications. Briefly, 6 mL of distilled water was applied to the nostril, and 10 s later the samples were collected in 50 mL conical tubes and were centrifuged for 15 min at 1500 g and 4 °C; the supernatant was stored at −20 °C until the time of detection of IgE.

The Der p sIgE from the nasal mucus was measured by the ImmunoCAP system. For the detection of NsIgE, a calibration curve was made using the ImmunoCAP system; the value of 0.12 kU/L was considered as the cut-off point according to the mean and two standard deviations observed in the control group.

**Eosinophil count in the nasal mucus and peripheral blood**

The eosinophil count in the nasal mucus was performed 30 min after the NCT. The sample was taken by brushing the nasal mucosa in each nostril and subsequent staining according to the Hansel method, and was analyzed by light microscopy. Eosinophilia in the nasal mucus were considered present when the eosinophil count was greater than or equal to 10% of total leucocytes or more than 10 eosinophils for high power field.

The measurement of eosinophils in peripheral blood was performed following the routine methods of clinical laboratories.

**Statistical analysis**

For the descriptive analysis, absolute frequencies, relative frequencies, and summary measures, such as the median or the interquartile range, were used. The criteria of normality of age and laboratory tests were established through the Shapiro-Wilk test. To establish the relationship between the results of the NCT and NsIgE with respect to the study groups, the Pearson’s Chi square test of independence and the likelihood ratio test were applied. The Kruskal-Wallis test was applied to establish the relationship between age, laboratory test, and symptom score in the study groups; a p value < 0.05 was considered statistically significant. For the correlation between the levels of NsIgE and serum sIgE, the Spearman correlation coefficient and the coefficient of determination were used. Indicators of diagnostic accuracy of NsIgE were evaluated based on the result of the NCT (gold test) for groups of patients who were sensitized and not sensitized to mites. The statistical program STATA version 14 was used.
RESULTS

Sociodemographic characteristics

A total of 50 patients with chronic rhinitis were recruited: 25 in the RWoA group and 25 in the RWA group. In addition, 18 control subjects agreed to participate in the study (Table 1). In the RWoA group, a higher age at the time of diagnosis and higher frequency of females were observed in comparison with the RWA group.

The presence of asthma, atopic dermatitis, and conjunctivitis was significantly more frequent in the RWA group. The RWA group had a median serum sIgEs for Der p of 22.1 kUA/L (RI: 41.49), and in the RWoA group the median was 0.02 kUA/L (RI: 0.01). The correlation between serum sIgEs and NsIgE was moderate in the RWA group (r 0.5918, p < 0.05).

Sensitization to other allergenic triggers different to Der p or other mites was present in 34% and 0% of patients in the RWA and RWoA group, respectively.

| Characteristics | Categories | Study groups | P  |
|-----------------|------------|--------------|----|
|                 |            | RWoA n = 25  | RWA n = 25 | CS n = 18 |
| Sex             | Female     | 20 (80%)     | 16 (64%)   | 7 (38.9%) | 0.021 |
|                 | Male       | 5 (20%)      | 9 (36%)    | 11 (61.1%) |    |
| Age group*      | Me: 34 (RI: 6) | Me: 29 (RI: 7) | Me: 27 (RI: 9) | 0.001 |
| Age of diagnosis* | Me: 19 (RI: 16) | Me: 8 (RI: 10) | NA | <0.001 |
| Comorbidities   | Asthma     | 2 (8%)       | 13 (52%)   | NA | <0.001 |
|                 | Atopic Dermatitis | 2 (8%) | 10 (40%) | NA | <0.001 |
|                 | Conjunctivitis | 11 (44%) | 16 (64%) | NA | <0.001 |
| Cigarette smoke | Smoker     | 1 (4%)       | 3 (12%)    | 2 (11.1%) | 0.526 |
|                 | Passive smoker | 5 (20%) | 3 (12%) | 3 (16.7%) | 0.739 |
| Eosinophils count | Peripheric blood count* | Me: 140 (RI: 160) | Me: 200 (RI: 220) | Me: 95 (RI: 80) | 0.014 |
|                 | Frequency in nasal mucus | 0 | 3 (13%) | 0 | 0.041 |
| Symptom         | symptom score** | Me: 2 (RI: 2) | Me: 2 (RI: 5) | Me: 0 (RI: 0) | 0.0001 |

Table 1. Sociodemographic and clinical characteristics of patients. *The data are presented as the median (Me) and interquartile range. RWoA: rhinitis without atopy. RWA: rhinitis with atopy. CS: control subjects. NA: not applicable. **Label score.
increased, no significant differences were observed in the diagnostic performance of the test in the RWoA or RWA groups.

**Frequency of LAR and non-allergic rhinitis**

In the RWoA group, the frequency of LAR to Der p (NsIgE to Der p plus positive NCT) was found in 3 (12%) of the 25 patients. These 3 patients represented 25% of the patients with NsIgE and 42.8% of the patients with a positive NCT in the RWoA group. Non-allergic rhinitis (no sIgE, no SPT and negative NCT) was found in 18 (72%) of the RWoA group patients. Four (16%) patients without nasal or systemic sIgE had a positive NCT with Der p.

**Eosinophil count**

Eosinophil counts in peripheral blood and in the nasal mucus were higher in the RWA group (Table 1), but there was not a correlation with serum sIgE or NsIgE (data not shown). There were no eosinophils in the nasal mucus of patients from the RWoA group and the control subjects.

**DISCUSSION**

The prevalence of LAR varies among populations; in Spain it is 25.7%, while in cities in China and Korea it is 8% and 11%, respectively.26-28 The
variability of these results can be explained by multiple environmental factors, such as allergenic levels and the technique used to measure NsIgE. When measurements are made after nasal lavage detection of NsIgE in non-allergic rhinitis patients, a rate of 22–40% is found, while 42.8% test positive when the solid phase of ImmunoCAP is applied directly in the nostril. Our study shows that LAR is present in 28% of patients with rhinitis without serum sIgE, a percentage similar to that reported in other populations.

Although the pathogenesis of LAR and non-allergic rhinitis is not completely understood, these types of rhinitis usually start in the fourth decade of life; in non-allergic rhinitis, there are several mechanisms associated with an effect only on the nasal mucosa. Some results in vivo suggest that serum and nasal concentrations of IL-10 and nasal TGF-β concentrations are higher in LAR, suggesting a greater immunomodulatory property than in patients with allergic rhinitis. Because the RWoA group was made up of patients with LAR and patients with non-allergic rhinitis, this could explain the older age of patients in the RWoA group and the lower frequency of some comorbidities, such as asthma or dermatitis.

Traditionally, sIgE has been of great value in the clinical routine of the diagnostic approach to rhinitis. It allows us to identify possible environmental triggers for the patient and define the best immunotherapy and, if necessary, it guides us to which allergen to test with the NCT.

Because in LAR the conventional diagnostic approach through SPT or serum sIgE is insufficient, NsIgE has become a key tool in identifying the allergenic trigger associated with the symptoms.

Despite the high exposure to Der p in the study population and the fact that Dermatophagoides spp. has been identified as the main cause of atopic and allergy in the tropics, we observed that the frequency of positive NsIgE in the RWOA group (n = 12, 48%) was similar to in the control group (n = 5, 27.8%), and most of these patients had a negative challenge. These results support the previous data of Gelardi et al, who observed NsIgE in 50% of a healthy group, and suggested that the production of NsIgE may represent a form of spontaneous immune response and is not a specific finding of nasal symptoms.
Dermatophagoides spp. are the main cause of IgE sensitization in the tropics and are the main allergenic trigger involved in LAR.\textsuperscript{26,39} Since we investigated only one (Der p), we cannot rule out LAR due to other triggers. Additionally, the relatively small number of participants in our study could affect the reported frequency. Nevertheless, these observations do not affect our main objective, which was to explore the diagnostic performance of NsIgE to predict the outcome of an NCT. Considering that only 3 of the 12 patients with NsIgE had a positive NCT, it is clear that the presence of NsIgE in the nasal mucus is not enough to define clinical relevance. Therefore, we consider performing an NCT to confirm the suspicion of LAR as indispensable.

Four patients in the RWoA group had a positive challenge but had no NsIgE or serum slgE. We are not clear why this happened. Before performing the allergen NCT we performed a saline challenge to rule out an irritative effect. This is supported by the fact that none of the people in the control group had a positive challenge, even though 5 (27.8\%) subjects had NsIgE. All analyses were performed in duplicate, so a technical error is also unlikely. A possible explanation is that the nasal mucosa of patients with rhinitis can make them more sensitive than healthy subjects to different triggers by non-IgE mediated mechanisms, for example unspecific degranulation of mast cells. An inflammation mediated by eosinophils (eosinophilic rhinitis) was ruled out since only in the RWA group was there an increase in these cells in the nasal mucus.

In AR, there is a strong association between slgE and allergy, but 10–20\% of the general population have serum slgE without allergic symptoms.\textsuperscript{40} A similar result was found for NsIgE in the RWA group, which had a better diagnostic performance than in the RWoA group or in the control subjects. In this study, 80\% of the patients in the RWA group had a positive NCT, and an association between NsIgE and a positive NCT was observed in 17 of 25 (68\%) of the cases. These results suggest that in the case of allergic rhinitis it is not necessary to routinely measure NsIgE, and a suggestive clinical history, added to the evidence of systemic slgE in patients with chronic rhinitis, allows adequate diagnostic accuracy, reducing the need for confirmatory tests like the NCT.

Some studies suggest that allergen specific immunotherapy is a therapeutic alternative for patients with LAR.\textsuperscript{41,42} However, given the lack of clinical relevance of NsIgE in a number of patients, a better understanding of the pathogenesis of this disease is necessary before suggesting immunotherapy as a routine treatment.

In conclusion, the diagnostic performance of NsIgE is not adequate as a predictor of the response to a nasal challenge in non-allergic patients. To confirm LAR, it is always necessary to perform the nasal challenge test with the suspected allergen.

**Abbreviations**

AR: Allergic Rhinitis; LAR: Local Allergy Rhinitis; NCT: Nasal Challenge Test; NsIgE: Nasal Specific IgE; RWoA: Rhinitis Without Systemic Atopy; RWA: Rhinitis With Systemic Atopy; SPT: Skin Prick Test; slgE: Specific Immunoglobulin E

**Authorship contribution**

All the authors contributed equally to the conception, analysis, and writing of the manuscript. LS, AC and JS contributed the central idea of the article.

**Consent for publication**

All authors agree that this version be published.

**Availability of data**

The data used for this project are available to the public with the prior authorization of the authors and the ethics committee.

**Ethics approval**

The study protocol was approved by the institutional ethics committee (code IN20-2017).

**Declaration of competing interest**

The authors declare they have no conflict of interest.

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**Appendix A Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2020.100461.
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