



Safety and reproducibility of nasal allergen challenge

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Abstract

Background: The nasal allergen challenge (NAC) is a useful tool for the diagnosis of allergic rhinitis (AR) and local allergic rhinitis (LAR) and might serve to design and monitor allergen immunotherapy. Nevertheless, data about its safety and reproducibility are scarce.

Objective: To investigate the safety and reproducibility of NAC in pediatric and adult rhinitis patients with/without asthmatic symptoms, and in healthy controls.

Methods: A retrospective evaluation of the NACs conducted in our Unit for 2005–2017 and monitored by acoustic rhinometry and nasal-ocular symptoms was performed to analyze the safety of two methods for allergen application (metered spray & micropipette) and NAC protocols (NAC with single or multiple allergens/session [NAC-S & NAC-M]). The adverse events (AEs), spirometry values, and rescue medication required for AE were recorded. The reproducibility was examined by a prospective analysis of three repeated NAC-S performed at 1–2-month interval in AR, LAR and nonallergic rhinitis patients, and in healthy controls.

Results: A total of 11 499 NACs were performed in 518 children and 5830 adults. Only four local AE occurred, and 99.97% of NACs were well tolerated. The reproducibility and positive and negative predictive values of three consecutive NAC-S performed in 710 subjects were 97.32%, 100%, and 92.91%, respectively. There were no false-positive results in the 710 analyzed subjects. Safety and reproducibility were comparable between the methods of allergen application and the rhinitis phenotypes.

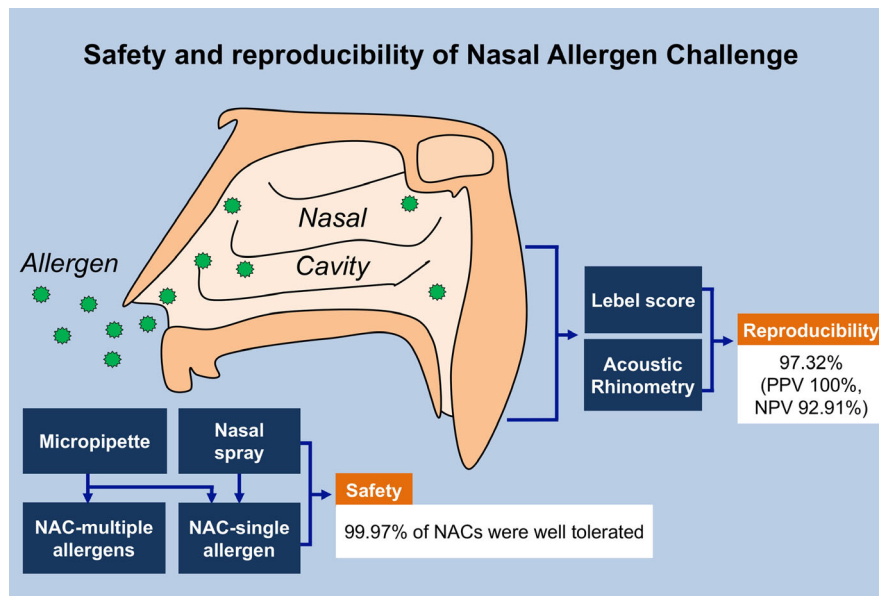
Conclusion: The NAC is a safe and highly reproducible diagnostic test ready to be used in the clinical practice in both children and adults with or without asthma.

KEYWORDS

local allergic rhinitis, nasal allergen challenge, reproducibility, safety

Abbreviations: AE, adverse event; AIT, allergen immunotherapy; AR, allergic rhinitis; DP, *Dermatophagoides pteronyssinus*; EAACI, European Academy of Allergy and Clinical Immunology; FEV1, forced respiratory volume in the first second; HC, healthy control individual; IQR, interquartile range; LAR, local allergic rhinitis; NAC-M, NAC with multiple allergens per session; NAC, nasal allergen challenge; NAC-S, NAC with a single allergen per session; NAR, nonallergic rhinitis; NHR, nasal hyperreactivity; NPV, negative predictive value; PPV, positive predictive value; sIgE, allergen-specific IgE; SPT, skin prick test; Vol 2–6 cm, volume 2–6 cm of each nostril.

Eguiluz-Gracia and Testera-Montes contributed equally to this paper.



GRAPHICAL ABSTRACT

This study evaluates 11 499 Nasal Allergen Challenges (NAC) conducted in 6348 adult and paediatric rhinitis patients and in healthy controls. The NAC is a very safe technique in children and adults and it is highly reproducible when monitored by Lebel symptoms score and acoustic rhinometry. No difference in terms of NAC safety and reproducibility were detected between different allergen application methods or NAC protocols, distinct rhinitis phenotypes, or the presence or absence of asthmatic symptoms.

PPV: Positive predictive value; NPV: Negative predictive value.

1 | INTRODUCTION

Allergic rhinitis (AR) is a chronic inflammatory IgE-mediated disorder affecting approximately 400 million people worldwide and constituting a risk factor for asthma.^{1,2} Moderate-to-severe AR negatively impacts patients' social life, school performance, and work productivity.² Thus, AR imposes a considerable burden to healthcare systems, in terms of both direct and indirect costs.¹

Historically, chronic rhinitis has been divided into the allergic and the nonallergic phenotypes, based on the classical tests of systemic atopy, namely skin prick test (SPT) and serum allergen-specific IgE (sIgE).^{3,4} Nevertheless, a proportion of healthy subjects also display positivity for either test.³ Additionally, in many AR patients it is difficult to establish the clinical relevance of IgE-sensitizations due to a lack of consistency with the symptom pattern, or due to multiple positive SPTs.⁵ In these situations, it is challenging to design the composition of allergen immunotherapy (AIT).^{6,7} Moreover, local allergic rhinitis (LAR) is a nasal disorder characterized by a localized allergic response in the absence of systemic atopy.⁸ The condition is getting greater recognition in recent years,⁹ even though a population study to clarify its prevalence and incidence during childhood is lacking. In all the above-mentioned scenarios, the nasal allergen challenge (NAC) is a helpful diagnostic tool, as it is the gold standard to establish the clinical reactivity to an allergen¹⁰ and to accurately identify AR and LAR individuals.^{8,11} Furthermore, the NAC is a useful tool for both translational and clinical research studies.¹²⁻¹⁴

The NAC aims to reproduce the response of the nasal mucosa to the allergen in a controlled manner,^{15,16} and it is a well-established technique

included in the standard diagnostic work-up for rhinitis in some centers.^{10,15,16} A recent Position Paper of the European Academy of Allergy and Clinical Immunology (EAACI) recommends the use of NAC to discriminate between the different rhinitis phenotypes (AR, LAR, nonallergic rhinitis (NAR), occupational rhinitis, etc.), and to design the composition of AIT in patients with multiple positive SPTs.¹⁶ Yet the NAC is a time- and resource-consuming technique, it is currently the only available test to confirm the nasal reactivity to allergens, and thus, it might help reduce the costs associated to the inadequate management of rhinitis patients.¹⁶

Nevertheless, the studies on NAC safety and reproducibility are scarce, and most of them include only few adult patients,^{17,18} but do not include children, and do not represent properly the different rhinitis phenotypes, the asthmatic patients or the healthy control individuals (HC). Therefore, we decided to evaluate the NACs carried out in our Unit over a period of twelve years in order to investigate the safety and reproducibility of the test. The NACs analyzed were performed in nonatopic HCs and in pediatric and adult rhinitis patients with/without asthmatic symptoms. Moreover, they were carried out both under clinical practice conditions and in the context of research studies.

2 | METHODS

2.1 | Study subjects

A retrospective study was conducted using an anonymized database including all the NACs performed in the Allergy Unit of Hospital Regional Universitario de Málaga (Málaga, Spain) between

October 2005 and December 2017 to analyze the safety of NAC. The individuals subjected to NAC were both children and adults with/without asthmatic symptoms, and nonatopic HCs. The reproducibility was examined by a prospective study analyzing three consecutive NACs with the same allergen in rhinitis patients and HCs. The study was approved by the Ethics Committee of the Hospital and performed according to the principles of the Declaration of Helsinki. Prior to the NAC, all subjects over 16 years and the parents of the younger individuals signed the corresponding informed consents.

2.2 | Definition of phenotypes

- 1 Allergic rhinitis was defined as the presence of chronic rhinitis with positive SPT, serum sIgE and NAC.^{1,2}
- 2 Local allergic rhinitis was defined as the presence of chronic rhinitis with negative SPT and serum sIgE, and positive NAC.^{4,8,11}
- 3 Nonallergic rhinitis was defined as the presence of chronic rhinitis with negative SPT, serum sIgE and NAC.¹⁹
- 4 Asthmatic symptoms were defined as chronic or chronically relapsing symptoms suggestive of bronchial hyperreactivity (cough, shortness of breath, chest tightness, or wheezing).²⁰ The design of the study did not permit the confirmation of asthma diagnosis.
- 5 Healthy control individual was defined as the absence of chronic rhinitis with negative SPT, serum sIgE and NAC response.

2.3 | Nasal allergen challenge procedure

The NACs were performed using two different standardized and validated protocols for bilateral provocation: NAC with a single allergen/session administered at four progressively increasing concentrations (1/100, 1/10, 1/2, and undiluted) (NAC-S),^{15,16,21,22} or NAC with up to four allergens/session (NAC-M) administered undiluted in a sequential manner with a 15-minute interval.²³ During the visit when the NAC was scheduled, a nasal endoscopy was conducted as a baseline evaluation, and in order to exclude anatomical and inflammatory disorders. Before the application of the allergen solution, all individuals were subjected to a bilateral nasal challenge with 100 μ L of isotonic saline serum in order to rule out nasal hyperreactivity (NHR).²⁴ The patients displaying two consecutive positive saline challenges (read NAC monitoring) with a minimum interval of 2 weeks were excluded from further NAC attempts. The individuals with negative saline challenge were subsequently administered 100 μ L/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 either a metered spray or a micropipette.^{15,16} During the allergen administration, the individuals remained seated and held their breath to prevent laryngeal aspiration.^{15,16}

2.4 | Exclusion criteria for NAC

Absolute exclusion criteria included pregnancy, uncontrolled asthma or chronic obstructive pulmonary disease (FEV1 < 70%), unstable cardiopulmonary disease contraindicating the administration of epinephrine, septum perforation, or chronic rhinosinusitis with or without nasal polyps. The NAC was not performed in symptomatic patients (Lebel score > 3) or within the 4 weeks after a respiratory infection.^{15,16,22,25} The individuals were instructed to avoid systemic or nasal corticosteroids, systemic or nasal antihistamines, and topical vasoconstrictors for 4, 2 and 1 weeks, respectively, before the NAC session.^{15,16}

2.5 | Allergen extracts

We used standardized and lyophilized allergen extracts from ALK-Abelló (Madrid, Spain): *Dermatophagoides pteronyssinus* (DP) 4 μ g/mL, *Alternaria alternata* 0.25 μ g/mL, grass pollen mix 0.1 μ g/mL, and *Olea europea* 0.6 μ g/mL, and from LETI, S.L. (Tres Cantos, Madrid, Spain): DP 100 HEP/mL, and *Dermatophagoides farinae*, *Lepidoglyphus destructor*, *Blomia tropicalis*, *Alternaria alternata*, *Cupressus arizonica*, grass pollen mix, *Olea europea*, *Parietaria judaica*, *Phelum pratense*, cat and dog epithelia 30 HEP/mL. For the NAC-M protocol, the different allergens were administered separately with a 15-minute interval.²³

2.6 | NAC monitoring

The outcome of the NACs was evaluated by 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,²⁶ and the nasal-ocular symptoms (nasal obstruction, rhinorrhea, nasal itching, sneezing, and ocular symptoms) were subjectively evaluated by the Lebel score.²⁷ Measurements of both parameters were made at baseline, 15 minutes after saline challenge, 15 minutes after every allergen/allergen solution administration, and one and two hours after the last allergen dose. The NAC was stopped before the administration of the last allergen dose if a positive response occurred. In the case of NAC-M protocol, additional NACs were scheduled if necessary to test the allergens which could not be administered during the first positive NAC. A NAC was considered positive when a bilateral decrease $\geq$ 30% in the volume 2-6 cm (vol 2-6 cm) in the acoustic rhinometry,^{22,25} and an increase of greater than or equal to 5 points in the Lebel score occurred.¹⁶ The positive NACs were categorized as immediate (positive response observed at the measurement performed 15 minutes after allergen administration), late (positive response observed at the measurement performed 1-2 hours after allergen administration), or dual (positive response at both immediate and late measurements).^{21,28} Following a positive NAC, the patients were kept under observation for 2 hours.

2.7 | Adverse events

All adverse events during NAC and the rescue medication required to treat them were recorded. The AEs were divided into local and systemic events. Symptoms/signs in the upper or lower respiratory tract (excluding the nose) were classified as local AE, and were graded as follows:

- Mild: nontroublesome upper respiratory symptoms/signs (oral or lingual pruritus/swelling, sore throat and/or asymptomatic uvula edema), or any other respiratory AE not requiring treatment.
- Moderate: troublesome upper respiratory symptoms/signs (oral or lingual pruritus/swelling, disturbing sore throat and/or uvula edema) or any other respiratory AE requiring oral antihistamines and/or corticosteroids.
- Severe: lower respiratory symptoms/signs (cough, dyspnea, wheezing, a drop > 15% of FEV1 as compared to baseline conditions) or any other respiratory AE requiring inhaled/aerosolized bronchodilators, oxygen therapy, parenteral corticosteroids or epinephrine.

Systemic AEs were defined as the onset of symptoms/signs in organs other than the airways (skin, heart or intestine, but excluding the eyes) following a NAC. The AEs were categorized as immediate or delayed, with 30 minutes after the last allergen dose as the differentiation point.

The standard protocol for AEs included physical examination, cardiopulmonary auscultation, and the performance of a flow-volume spirometry immediately after the AE, and every 30 minutes until its resolution. The spirometry was performed using a Spirobank spirometer (Medical International Research, Rome, Italy) and in accordance with the American Thoracic Society recommendations.²⁹ A drop $\geq$ 15% during the AE as compared to baseline FEV1 was considered indicative of NAC-induced bronchospasm.

After the AE, the patients remained at the Unit for at least 2 hours, or until the spirometry values returned to normal.

All patients regardless of the NAC outcome were given a phone number and instructed to call in case of delayed AE at home.

2.8 | Reproducibility

To analyze the reproducibility of NAC, we performed a prospective study comparing three repeated NAC-S performed with the same allergen and in the same environment at 1-2-month interval, in 710 subjects (230 AR, 231 LAR, 129 NAR, and 120 HC).

2.9 | Statistical analysis

Descriptive statistics were used to summarize the clinical and demographic characteristics of the study individuals. Continuous variables were expressed as mean and standard deviation or median and interquartile ranges (IQR) as indicated in the article, and categorical variables are expressed as percentages. Comparisons between groups were performed by chi-square test or Student's *t* test. The associations between AE and the NAC protocols (NAC-S vs NAC-M), and/or

allergen application method (metered spray vs micropipette) were examined by a multivariate logistic regression. The reproducibility of NAC was analyzed by calculating the ratio of equal results, the concordance among three repeated NAC-S (kappa index), and the positive and negative predictive values (PPV and NPV, respectively). The statistical analysis was performed using SPSS version 15.0.1 (IBM, Armonk, NY). *P* values < 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Characteristics of NACs

A total of 11 499 NACs were performed in rhinitis patients and HCs from October 2005 to December 2017 (1034 NACs in 518 children and 10 465 NACs in 5830 adults), with a maximum NAC/year peak of 1621 procedures in 2012. The yearly distribution of NACs is shown in Figure 1A.

From 13 308 NACs originally scheduled, 1374 procedures (10.32%) could not be performed due to the presence of NHR. The prevalence of NHR was significantly higher in patients (10.2%) than in HCs (0.3%, *P* = 0.001), but comparable among subjects with AR (14%), LAR (14.9%) and NAR (16.6%) (*P* = 0.312).

The majority of the NACs were performed in adults (91.01%), female (68%), and patients (95.34%) (Table 1). A total of 2302 NACs (21%) were conducted in individuals reporting asthmatic symptoms (Table 1). There was a higher proportion of male subjects (47.20% vs 30.50%, *P* < 0.001), and patients with asthmatic symptoms (41.94% vs 18.94%, *P* < 0.001) in the NACs performed in children, as compared to adults (Table 1).

3.2 | Characteristics of study individuals

Similarly, descriptive analyses of subjects (*n* = 6348) revealed a majority of adults (91.84%), female (68.19%), and patients (95.01%), with a higher proportion of males (48.46% vs 30.33%, *P* < 0.001), and asthmatic symptoms (39.71% vs 24.42%, *P* < 0.001) in children than in adults (Table 1).

3.3 | Allergens

Figure 1 shows the frequency distribution of allergen groups and individual allergens tested in the NACs. Eleven different airborne allergens were administered. Both the most frequently tested allergen group and individual allergen were mites (39% and 38.42%, respectively) (Figure 1B,C).

3.4 | Allergen application methods and NAC protocols

From 2005 to 2017, we used two different methods for allergen application (metered spray and micropipette) and for NAC protocol (NAC-S and NAC-M). In 2005, we started performing NACs using metered nasal spray and the NAC-S protocol. In 2009, we switched

to allergen delivery by micropipette in order to minimize the dripping to the bronchi.¹⁵ In 2011, we described the shortened NAC-M protocol,²³ and we started to use it for a rapid discrimination between LAR and NAR, and for the identification of clinically relevant IgE-sensitizations in AR patients with multiple positive SPTs. In our study, most of the allergens were administered using micropipette (82.40%) and the NAC-M protocol (62.71%). A full description is available in the repository (Table S1).

3.5 | Safety

The vast majority (99.97%) of the 11 495 NACs were well tolerated and provoked no AE. The NACs did not induce any AE in patients testing negative (including all NAR individuals and HCs) or children. Four immediate local AEs in the upper airways (0.06%) occurred in two AR and two LAR patients with positive NAC responses ($n = 6669$). These AEs were presumably due to the pharyngeal aspiration of the allergen. No delayed, local severe or systemic AEs were observed (Figure 2).

The features of the AEs and the rescue medication administered to treat them are displayed in Table 2. In the four cases, the

symptoms started 1-10 minutes after allergen administration and resolved within 15-30 minutes. The auscultation and spirometry values were all normal.

No differences in terms of safety were observed between the allergen application methods ($P = 0.151$), NAC protocols ($P = 0.146$) or between AR and LAR phenotypes ($P = 1.000$) (Table 3). Moreover, the adverse events occurred with different allergens and concentrations.

3.6 | Reproducibility

To evaluate the reproducibility of NAC, we compared the outcomes of three consecutive NAC-S performed in 710 subjects (230 AR, 231 LAR, 129 NAR, and 120 HC individuals) (Table 4A). For a positivity threshold of $\geq 30\%$ drop in vol 2-6 cm in the acoustic rhinometry and ≥ 5 points in the Lebel score after the NAC, an almost perfect concordance rate (97.35%) and a very high kappa index (0.943, $P < 0.001$) were observed. Moreover, those positivity thresholds were associated to high predictive values (PPV 100%, NPV 92.91%). There was no significant difference between metered spray and micropipette in terms of reproducibility ($P = 0.243$), PPV ($P = 1.000$) or NPV ($P = 0.482$). A more detailed description is available in Table 3.

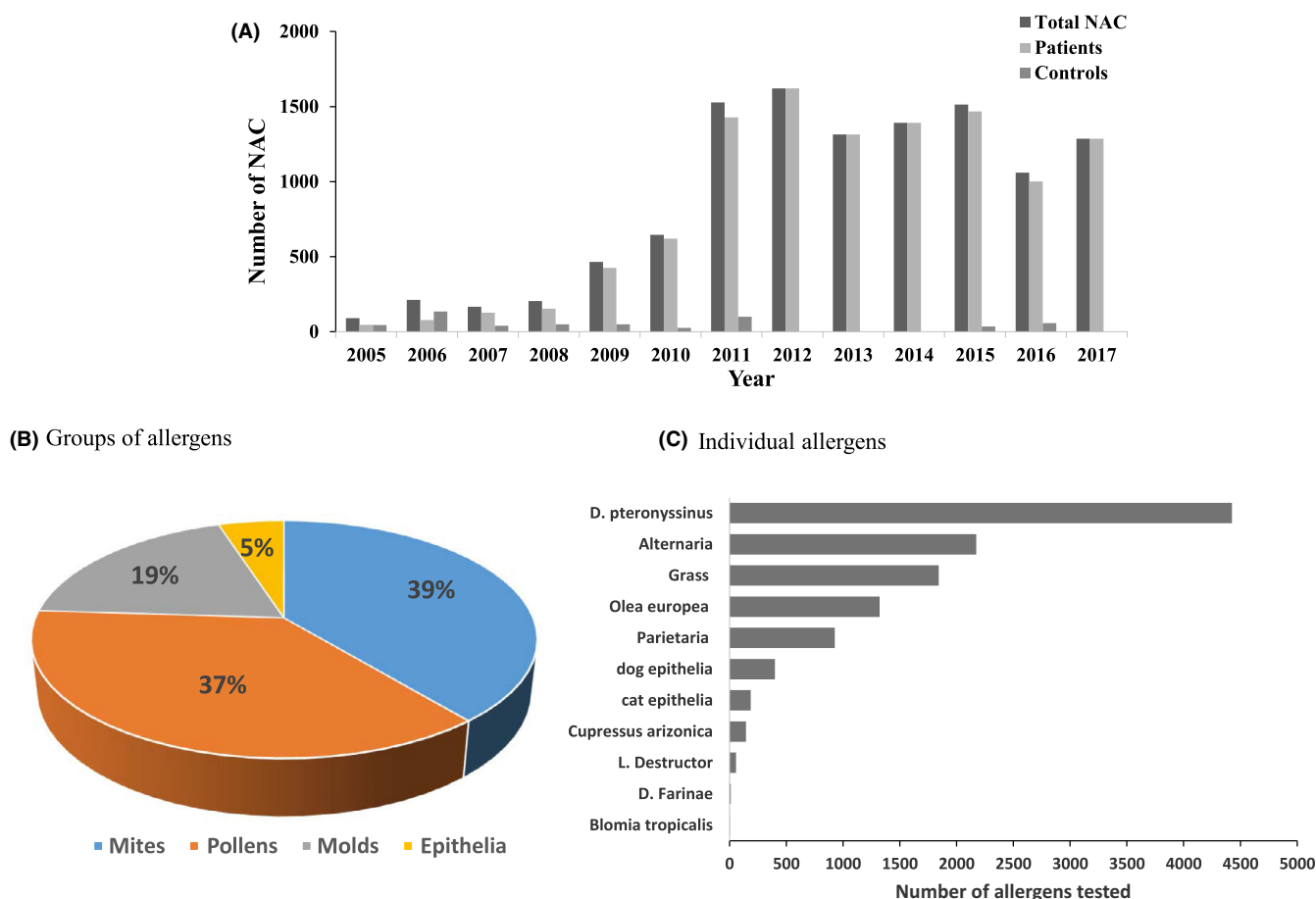


FIGURE 1 A, frequency distribution of the nasal allergen challenges (NAC) conducted in rhinitis patients and healthy control individuals from October 2005 to December 2017. Description of the aeroallergens tested in the NACs: allergen groups (B) and individual allergens (C). *D farinae*, *Dermatophagoides farinae*; *D pteronyssinus*, *Dermatophagoides pteronyssinus*; *L destructor*, *Lepidoglyphus destructor* [Colour figure can be viewed at wileyonlinelibrary.com]

Nineteen patients with allergic phenotypes of rhinitis had a false-negative result in one of the three repeated NAC-S (Table 4B), with no significant differences between AR and LAR phenotypes ($P = 0.822$). No false-positive response was detected in the 249 non-allergic (NAR and HC) subjects (Table 4B).

4 | DISCUSSION

To the best of our knowledge, this study is the largest evaluation conducted to date to assess the safety and reproducibility of NAC. For the first time, this work analyzes the performance of NAC in children, and includes a proper representation of the different rhinitis phenotypes and of patients with asthmatic symptoms.

Importantly, the study demonstrates that the NAC is a very safe and reliable technique regardless of the rhinitis phenotype, the presence of asthmatic symptoms, or the age of the patient. The inclusion of 11499 NACs (1034 challenges performed in 518 children, and 10 465 challenges performed in 5830 adults) with different allergen groups, application methods and protocols strongly supports the

validity and robustness of our results. Importantly, the NACs were carried out in a clinical setting by trained nurses, and under medical supervision.

The NAC is considered for many authors as a well-established research tool to investigate the immunopathology of airway allergy,^{12,13} or to monitor the clinical response to AIT and to other therapies during clinical trials.^{14,30,31} On the other hand, its use in the clinical practice has been scarce, possibly because NAC is a time- and resource-consuming technique. The paucity of data about NAC safety and reproducibility might also account for this low implementation. Nevertheless, the description of allergic phenotypes of rhinitis without systemic atopy (LAR)³² and the progressively increasing prevalence of AR patients with multiple positive SPTs^{3,5} indicate the need to include NAC in the diagnostic work-up of rhinitis patients.^{19,33} In this regard, our group described a shortened protocol (NAC-M) to test multiple allergens in a single session, and demonstrated its good diagnostic performance in LAR patients.²³

As highlighted by the *united airways* concept, there are important pathological links between rhinitis and asthma,^{1,34} and this fact is largely independent of the inflammatory endotype.³⁵ Therefore, the

TABLE 1 Description of the nasal allergen challenges (NAC) and the study subjects

Description of NACs relative to study subjects	All NACs	In children (<18 y)	In adults (≥18 y)	Children vs adults	
				OR (95% CI)	P value
NAC. N (%)	11 499 (100)	1034 (8.99)	10 465 (91.01)	N/A	N/A
Age, years					
Mean ± SD	36.88 ± 13.70	15.60 ± 2.63	39.77 ± 12.81	N/A	N/A
Limits	(5-91)	(5-18)	(19-91)	N/A	N/A
Gender. N (%)					
Female	7819 (68.00)	546 (52.80)	7273 (69.50)		
Male	3680 (32.00)	488 (47.20)	3192 (30.50)	2.05 (1.800-2.330)	<0.001
Study groups. N (%)					
Patients	10 963 (95.34)	980 (94.78)	9983 (95.39)	0.88 (0.656-1.170)	<0.001
Healthy controls	536 (4.66)	54 (5.22)	482 (4.61)		
Patient symptoms. N (%)					
Rhinitis	8661 (79.00)	569 (58.06)	8092 (81.06)		
Rhinitis + asthma	2302 (21.00)	411 (41.94)	1891 (18.94)	3.09 (2.697-3.543)	<0.001
Description of study subjects	All patients	Children (<18 y)	Adults (≥18 y)	Children vs Adults	
				OR (95% CI)	P value
Number (%)	6348 (100)	518 (8.16)	5830 (91.84)	N/A	N/A
Gender. N (%)					
Female	4329 (68.19)	267 (51.54)	4062 (69.67)		
Male	2019 (31.81)	251 (48.46)	1768 (30.33)	2.16 (1.802-2.589)	<0.001
Study groups. N (%)					
Patients	6031 (95.01)	486 (93.82)	5545 (95.11)		
Healthy controls	317 (4.99)	32 (6.18)	285 (4.89)	0.78 (0.535-1.138)	0.197
Patient symptoms. N (%)					
Rhinitis	4484 (74.35)	293 (60.29)	4191 (75.58)		
Rhinitis + asthma	1547 (25.65)	193 (39.71)	1354 (24.42)	2.04 (1.683-2.470)	<0.001

CI, confidence interval; N, number; N/A, not applicable; OR, odds ratio; SD, standard deviation.

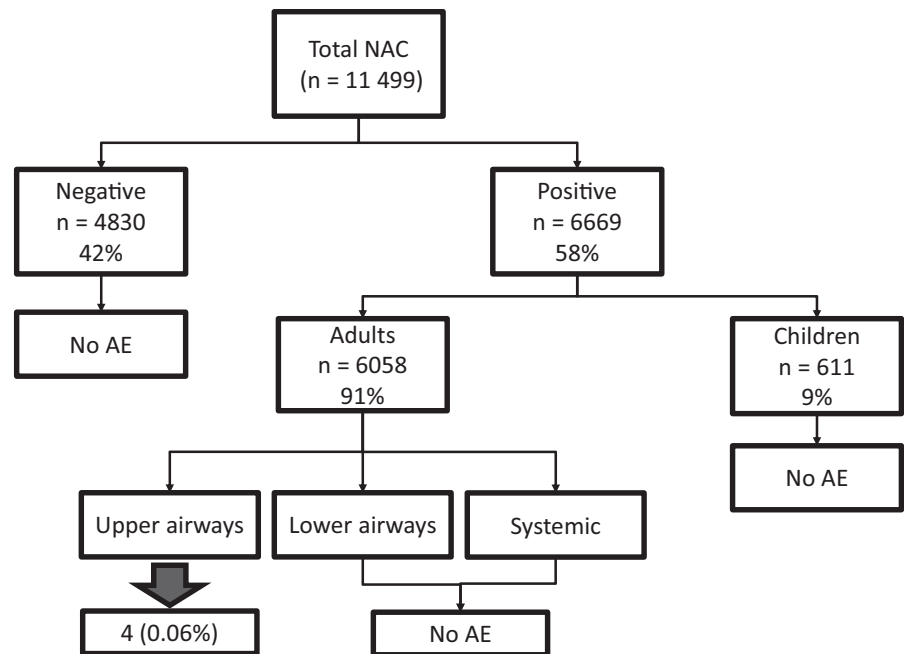


FIGURE 2 Summary of the safety evaluation of nasal allergen challenges (NAC). AE, adverse event

TABLE 2 Description of the four patients experiencing adverse events (AEs) during the nasal allergen challenges (NAC)

	Patient			
	1	2	3	4
Rhinitis phenotype	LAR	LAR	AR	AR
Age (years)	21	28	19	50
Asthmatic symptoms	No	Yes	No	No
Allergen	DP	<i>Alternaria</i>	<i>Olea europaea</i>	Grass
NAC protocol	NAC-M	NAC-S	NAC-S	NAC-S
Allergen application method	Metered spray	Micropipette	Micropipette	Metered spray
Allergen dilution	1/1	1/2	1/10	1/1
Temporality	Immediate	Immediate	Immediate	Immediate
Start (min)	5	1	10	3
Duration (min)	15	20	25	30
Severity	Mild	Moderate	Moderate	Moderate
Symptoms/signs	Palatine pruritus	Uvular edema. palatine pruritus	Uvular edema. palatine pruritus	Uvular and lingual edema. Palatine pruritus
Auscultation	Normal	Normal	Normal	Normal
FEV1 (%)				
Baseline	94.12%	85.00%	98.06%	92.05%
ΔFEV1 (%)	−7.35	1.02	−0.06	−1.62
Treatment	Not required	Oral anti-H	Oral anti-H	Oral anti-H, oral corticosteroid

ΔFEV1, change in FEV1 as compared to baseline; Anti-H, antihistamine; AR, allergic rhinitis; DP, *Dermatophagoides pteronyssinus*; FEV1, forced expiratory volume in the first second; LAR, local allergic rhinitis; NAC-M, NAC with multiple allergens per session; NAC-S, NAC with a single allergen per session.

bronchospasm-inducing potential of NACs both in asthmatic and nonasthmatic rhinitis patients warrants an investigation. Only two small studies had addressed this matter to date: a work by Schumacher et al.¹⁷ including six asthmatics and five HCs, and an article by Krzych-Falta et al. including 30 AR patients and 30 HCs.¹⁸ Yet none of these studies reported a NAC-induced AE in the lower

airways, the small number of subjects included limits the reliability of the results.

On the other hand, the present work examines 11 499 NACs conducted in 6348 individuals (1547 of them with asthmatic symptoms) and demonstrates that, when using standardized allergen extracts administered by either metered spray or micropipette, the

NAC is very safe both for pediatric and adult rhinitis patients with or without asthmatic symptoms. The NACs induced only four local mild or moderate immediate AEs which did not correlate with the allergen, application method, extract concentration or rhinitis phenotype. Interestingly, no delayed AE occurred. This finding together with the low frequency of isolated late positive responses in NAC^{23,28,36} suggests that the observation period after the test can be shortened to one hour. This change might help implement the NAC in the clinical practice.

The limitations of our study include the partially retrospective design, and the lack of a confirmation diagnosis for asthma in the included individuals. Nevertheless, the NAC did not induce AEs in the lower airways in any of the 2034 NACs conducted in 1547 patients with bronchial symptoms, clearly demonstrating the safety of the technique regardless of the asthma status.

The good performance of the acoustic rhinometry for the objective evaluation of the nasal patency was first described by Hilberg et al³⁷ in 1989. Thereafter, several studies showed that this technique is a simple, noninvasive and safe method in HC, AR and occupational rhinitis patients.²⁶ Nevertheless, the reproducibility of repeated NACs has not been explored in depth. A study by Wierzbicki et al³⁸ reported seven false-positive responses in five nonatopic rhinitis patients who were subjected to two repeated unilateral NACs with a 3-5-month interval. Nevertheless, several methodological aspects might account for this low reproducibility: A glycerinated solution was used for the control challenge, the allergen was administered unilaterally, no objective method to assess the nasal patency was included, and the symptoms were evaluated only at baseline and 15 minutes after the allergen application.¹⁵ In this regard, glycerinated extracts are not recommended for NAC because they have a significant irritant effect over

TABLE 3 Safety and reproducibility comparison of allergen application methods and nasal allergen challenge (NAC) protocols in patients with NAC positive results

	Allergen application method		NAC protocol	
	Nasal spray	Micropipette	NAC-S	NAC-M
Safety, N (%)				
NAC positive	1200 (18)	5469 (82)	2468 (37)	4201 (62)
Upper airway AE	2 (0.17)	2 (0.04)	3 (0.12)	1 (0.02)
Lower airway AE	0	0	0	0
Systemic AE	0	0	0	0
Reproducibility, N (%)				
Three repeated NAC-S	213 (30)	497 (70)	710 (100)	N/A
Concordance %	96.24	97.79	97.32	N/A
PPV	100	100	100	N/A
NPV	91.40	93.71	92.91	N/A

AE, adverse event; NAC-M, NAC with multiple allergens per session; NAC-S, NAC with a single allergen per session; NPV, negative predictive value; PPV, positive predictive value.

TABLE 4 Analysis of nasal allergen challenge (NAC) reproducibility relative to the study groups. A, results of three repeated NACs with a single allergen per session (NAC-S) in rhinitis and control subjects. B, reproducibility analysis in allergic and nonallergic individuals

A) Results of three repeated NAC-S in patients groups and controls							
Subject groups	1st NAC		2nd NAC		3rd NAC		Total
	Positive	Negative	Positive	Negative	Positive	Negative	
AR	230	0	220	10	230	0	690
LAR	231	0	222	9	231	0	693
NAR	0	129	0	129	0	129	387
Controls	0	120	0	120	0	120	360
Total	461	249	442	268	461	249	2130
B) Reproducibility of NAC-S in allergic and nonallergic subjects							
	Subject groups						Total
	Allergic (AR & LAR)			Nonallergic (NAR & controls)			
NAC positive	442			0			442
NAC negative	19			249			268
Total	461			249			710

AR, allergic rhinitis; LAR, local allergic rhinitis; NAR, nonallergic rhinitis.

the nasal mucosa,^{1,15,16,39} and the bilateral challenge is considered more physiological than the unilateral provocation because it can detect the nasal cycle (a physiological alternation of congestion/decongestion phenomena between the two nostrils).¹⁶

Later studies using a standardized and validated methodology reported a high reproducibility (91%–100%) of bilateral NAC in both LAR patients and HCs.^{15,21,25} Nevertheless, these works included only a small number of subjects (30–40) and did not investigate the performance of NAC in patients with rhinitis phenotypes other than LAR. Moreover, in a recent 10-year follow-up study including a large number of LAR patients and HCs, an evolution bias cannot be excluded due to the long interval (5 years) between repeated NACs.³² Therefore, the results of our study are substantial to confirm the high reproducibility (97.32%) of the bilateral NAC-S when performed with standardized protocols and allergens, and when assessed by acoustic rhinometry and nasal-ocular symptoms score. Importantly, we found an almost perfect concordance, and a very high PPV (100%) and NPV (92.91%) in three consecutive NAC-S performed in 461 AR, LAR, and NAR patients, and 249 HCs. Unfortunately, we could not investigate the reproducibility of the NAC-M protocol due to the lack of a sufficient number of individuals undergoing repeated NAC-M with the same allergen combination.

The present study is the first evaluation of NAC reproducibility including patients with different rhinitis phenotypes, comparing two methods of allergen application (metered spray and micropipette), and using a short interval between NACs in order to prevent the evolution bias. In our work, the proportion of false-negative results was very low (19 patients), and the application by micropipette slightly outperformed the metered spray in terms of both reproducibility (97.79% vs 96.24%) and NPV (93.71% vs 91.40%), yet the differences were not significant.

In summary, this study demonstrates that, when performed by trained nurses under medical supervision, the bilateral NAC is a safe and reproducible diagnostic test in children and adults. Metered nasal spray and micropipette are equally safe and reproducible methods for allergen administration. The absence of delayed AEs and NAC-induced bronchospasm regardless of the presence of asthmatic symptoms provides additional safety guarantees and facilitates the implementation of the NAC in the clinical practice. Moreover, the absence of false-positive results in nonallergic subjects (NAR and HC) clearly demonstrates the suitability of NAC for LAR diagnosis, as it excels in discriminating the allergic and nonallergic phenotypes of rhinitis, regardless of the atopic status of the patient.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

CR, MJT and CMA designed and coordinated the study. IEG, ATM, NP, MS, PC and CR supervised the nasal allergen challenges and collected the clinical data from the patients. MG, AA, IEG, ATM, NP

and MS created and handled the database with the clinical information. CR, PC and MJT performed the analysis of the data. IEG, CMA, PC, MJT and CR wrote the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Table E1

	Number	Percentage (%)
NAC	11499	
Results		
positive	6669	58.00
negative	4830	42.00
Protocol		
NAC-S	4288	37.29
NAC-M	7211	62.71
Allergen application		
metered spray	2024	17.60
micropipette	9475	82.40
Diagnosis		
AR	3795	33.00
LAR	5290	46.00
NAR	1878	16.33
Controls	536	4.66