

Nasal allergen provocation test with multiple aeroallergens detects polysensitization in local allergic rhinitis

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Background: Patients previously given a diagnosis of nonallergic rhinitis (NAR) might have a new form of local allergic rhinitis (LAR) with local production of specific IgE antibodies and a positive response to a nasal allergen provocation test (NAPT).

Objective: We evaluated an NAPT protocol using multiple aeroallergens (NAPT-M) for the detection of polysensitization to several aeroallergens in patients with LAR.

Methods: NAPT-Ms with 2 different panels of aeroallergens for patients with perennial and seasonal rhinitis were performed in 25 patients with LAR and 25 patients with NAR whose disease was diagnosed by means of NAPT 1 year earlier. The response to nasal challenge was evaluated based on subjective (nasal-ocular symptoms) and objective (acoustic rhinometry) parameters. Nasal levels of tryptase and eosinophil cationic protein were determined by means of immunoassay at baseline, 15 minutes, and 1, 2, and 24 hours after challenge.

Results: NAPT-Ms showed 100% concordance with the gold standard of NAPT with a single aeroallergen. No false-positive or false-negative responses were detected. The use of NAPT-Ms achieved 75% reduction in the total number of visits required for final diagnosis in the NAR group (from 168 to 42 visits) and a 55% reduction in the LAR group (from 144 to 65 visits) compared with NAPT with a single aeroallergen.

Conclusions: These results demonstrate that clinically relevant polysensitization to aeroallergens in patients with LAR occurred and that the NAPT-M is a useful, specific, sensitive, reproducible, and less time-consuming *in vivo* diagnostic test for the screening of patients with LAR. (*J Allergy Clin Immunol* 2011;■■■:■■■-■■■.)

Key words: Acoustic rhinometry, local allergic rhinitis, nasal allergen provocation test, visual analog scale

Local allergic rhinitis (LAR) is a new phenotype of rhinitis characterized by nasal production of specific IgE (sIgE)

Abbreviations used

ECP:	Eosinophil cationic protein
LAR:	Local allergic rhinitis
NAPT:	Nasal allergen provocation test
NAPT-M:	Nasal allergen provocation test with multiple allergens
NAPT-S:	Nasal allergen provocation test with a single aeroallergen
NAR:	Nonallergic rhinitis
sIgE:	Specific IgE
SPT:	Skin prick test
VAS:	Visual analog scale
VOL 2-6 cm:	Volume of the nasal cavity from 2 to 6 cm

antibodies,¹⁻³ a T_H2 cell inflammatory response in nasal secretions during natural exposure to aeroallergens,²⁻⁵ and a positive response to a nasal allergen provocation test (NAPT)^{2,3,6,7} with local production of sIgE, tryptase, and eosinophil cationic protein (ECP) in the absence of systemic atopy.^{8,9} Previous studies have shown that LAR might affect more than 45% of patients previously given a diagnosis of nonallergic rhinitis (NAR).^{2,3,6}

The confirmation of the existence of a selective localized allergic response in the nasal mucosa has demonstrated the need for a thorough allergologic evaluation in the target organ. The NAPT with a single aeroallergen (NAPT-S) has proved to be an important diagnostic tool in patients with LAR,^{2,3,6} with a higher sensitivity than determination of nasal sIgE, tryptase, or ECP levels.^{2,3,8,9} However, it is a very time-consuming technique, involving more than 2 hours at the first visit plus a second visit 24 hours later for the evaluation of immediate and late allergic responses to a single aeroallergen.^{2,3,8,9} In addition, because several seasonal aeroallergens, perennial aeroallergens, or both might be involved, this process can take several days for a complete evaluation. The greater the number of allergens involved, the more time is needed to perform the study. Accordingly, its use might be limited in daily clinical practice.

In this study we developed a protocol for a NAPT with multiple aeroallergens (NAPT-M) for the screening of LAR and compared it with the NAPT-S, which is used as the gold standard in this assay. Our aim was to design a simplified method for the evaluation of patients with LAR to multiple sensitizing allergens.

METHODS

Study subjects

The study was performed from July to September 2010, during a period when the patients were free of symptoms. A total of 25 adult patients with LAR and 25 adult patients with NAR were randomly selected from the total number of patients with rhinitis seen by our allergy service (Malaga, Spain)

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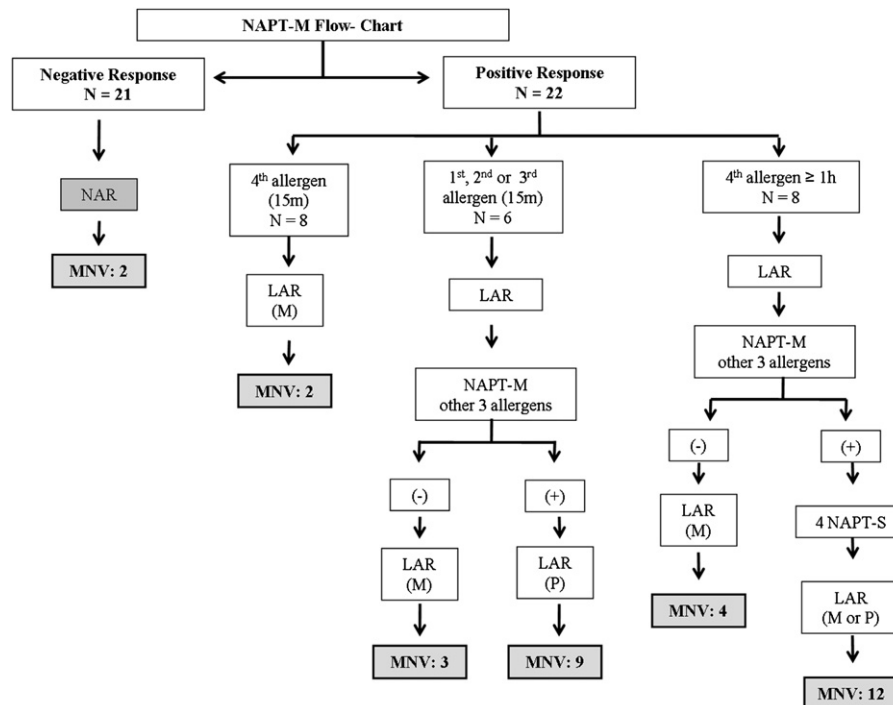


FIG 1. NAPT-M flowchart. M, Monosensitization; MNV, maximum number of visits; P, polysensitization.

the year before. All the patients had a history of at least 2 years of persistent rhinitis with negative skin prick test (SPT) responses and serum sIgE levels to the most prevalent aeroallergens, including house dust mite (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Lepidoglyphus destructor*, and *Blomia tropicalis*), pollens (grass pollen, *Cupressus* species, *Platanus* species, *Olea* species, *Chenopodium* species, *Platago* species, *Artemisia* species, *Parietaria* species, and *Salsola kali*), molds (*Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* species), and dog, cat, and hamster epithelia (ALK-Abelló, Madrid, Spain).

LAR was diagnosed by the presence of a positive response to 1 or more NAPT-Ss with *D pteronyssinus*, *Alternaria alternata*, *Olea europea*, or grass pollen and NAR by a negative NAPT response with each of these aeroallergens.

Patients with LAR and patients with NAR were screened for eligibility and randomized by means of simple random sampling with a computer-generated list in our database.

Exclusion criteria were a pregnant or breast-feeding women; patients with other immunologic diseases, chronic rhinosinusitis, and/or nasal polyposis (computed tomographic scan); or a respiratory tract infection in the previous 4 weeks. The patients were instructed to avoid treatment with systemic or nasal corticosteroids for 4 weeks, systemic or nasal antihistamines for 2 weeks, and nasal vasoconstrictors for 1 week before the study.

The study was approved by the local ethics committee, and informed consent was obtained.

Intradermal skin test

Intradermal skin testing was carried out with freshly reconstituted freeze-dried allergen solutions of the same panel of the 4 most common aeroallergens, *D pteronyssinus* (0.4 µg/mL), *A alternata* (0.25 µg/mL), *O europea* (0.6 µg/mL), and grass pollen (0.1 µg/mL; ALK-Abelló), as previously described, to confirm the absence of systemic atopy.¹⁰

Trypsin and ECP levels in nasal lavage fluid

Bilateral nasal lavage was performed by using the Naclerio method¹¹ with 8 mL of physiologic saline. Trypsin and ECP levels were determined by means

of immunoassay (UniCAP; Phadia, Uppsala, Sweden) at baseline, 15 minutes, and 1, 2, and 24 hours after challenge. The cutoff value was 1 ng/mL for trypsin and 2 ng/mL for ECP.

Nasal-ocular symptoms

Nasal obstruction, rhinorrhea, itching, sneezing, and ocular symptoms were recorded by using a visual analog scale (VAS) of 100 mm. The total VAS score was the sum of the 5 VAS scores (total range, 0-500 mm).

Acoustic rhinometry

Nasal patency was assessed by means of acoustic rhinometry with the use of an SRE 2000 rhinometer (Rhinometrics, Lyngø, Denmark) according to the guidelines of the Standardization Committee on Acoustic Rhinometry.¹² The parameter used was the volume of the nasal cavity from 2 to 6 cm (VOL 2-6 cm), the volume of the nasal cavity corresponding to the lower turbinate.

NAPT-M

The NAPT-M study was performed by a second physician who was unaware of the clinical data of the patients and carried out in a bilateral double-blind manner with a masked panel of 4 aeroallergens.

Symptom-free patients (total VAS, <60 mm) were challenged intranasally with 2 puffs (100 mL) of saline in each nostril to exclude nasal hyperreactivity. If the result was negative, 15 minutes after NAPT-M, we began administering 4 consecutive and different reconstituted freeze-dried allergen solutions of *D pteronyssinus* (4 µg/mL), *A alternata* (0.25 µg/mL), *O europea* (0.6 µg/mL), and grass pollen (0.1 µg/mL; ALK-Abelló) at 15-minute intervals. Two puffs (100 mL) of the solution at room temperature were applied in each nostril.

The response to nasal challenge was evaluated based on subjective (VAS of nasal-ocular symptoms) and objective (VOL 2-6 cm) parameters. A positive NAPT-M response was considered to be an increase of 30% or greater in the total VAS score and a decrease of 30% or greater in the sum of VOL 2-6 cm from both nasal cavities.^{2,3}

TABLE I. Clinical characteristics of patients at the first evaluation

	Patients with LAR	Patients with NAR
No. of patients	22	21
Sex, no. (%)		
Male	7 (32)	9 (43)
Female	15 (68)	12 (57)
Age (y [range])	29 (18-35)	32 (26-44)
Nasal symptoms, no. (%)		
Perennial	12 (55)	21 (100)
Seasonal	10 (45)	0 (0)
Asthma, no. (%)	6 (27)	7 (33)
Positive NAPT response (yes/no)	Yes	No
<i>D pteronyssinus</i> , no. (%)	14 (64)	NA
<i>A alternata</i> , no. (%)	2 (9)	NA
Grass pollen, no. (%)	12 (55)	NA
<i>O europea</i> , no. (%)	2 (9)	NA
Sensitization profile, no. (%)		
Monosensitive	15 (68)	NA
Polysensitive	7 (32)	
No. of sensitizations	30	0
No. of visits for final diagnosis	144	168

NA, Not applicable.

TABLE II. Concordance between NAPT-M and NAPT-S results for the diagnosis of patients with LAR and patients with NAR

		NAPT-S result	
		Positive (LAR)	Negative (NAR)
NAPT-M	Positive (LAR)	22	0
	Negative (NAR)	0	21
		Concurrence cases/total cases	Concurrence rate (%)
		P value (χ^2)	
Positive		22/22	100
Negative		21/21	100

The evaluation of the nasal response was performed at baseline, 15 minutes after saline challenge, 15 minutes after each aeroallergen challenge, and 1, 2, and 24 hours after the last aeroallergen challenge. The NAPT-M was stopped when a positive response occurred or 24 hours after challenge.

Aeroallergen panel design for NAPT-M

The 4 most common aeroallergens in our area were placed in 2 different panels, with a defined position for testing in patients with either perennial or seasonal rhinitis. This was based on previous studies and the prevalence of the allergens used.^{2,3} We followed an established order for the putative candidate from the least likely to the most likely to be involved as follows:

1. perennial rhinitis: grass, *O europea*, *A alternata*, and *D pteronyssinus*;
2. seasonal rhinitis: *A alternata*, *D pteronyssinus*, *O europea*, and grass.

NAPT-M diagnostic protocol

The diagnostic decision flowchart is shown in Fig 1.

1. If a negative response to an NAPT-M was obtained with the 4 allergens tested, the patient was given a diagnosis of NAR (2 days of evaluation).
2. If a positive response was found, 3 possible outcomes were expected:
 - A. Isolated immediate response in the first 15 minutes after the fourth allergen: The patient was given a diagnosis of being LAR monosensitive to this allergen (2 days of evaluation required).
 - B. Positive response in the first 15 minutes after either the first, second, or third aeroallergen: The NAPT-M was stopped, and the

TABLE III. Concordance between NAPT-M and NAPT-S results for identification of sensitizing aeroallergens in patients with LAR

		NAPT-S results	
		Positive	Negative
NAPT-M	Positive	30	0
	Negative	0	58
		Concurrence cases/total cases	Concurrence rate (%)
		P value (χ^2)	
Positive		30/30	100
Negative		58/58	100

patient was given a diagnosis LAR with sensitization to the allergen that induced the response (1 day of evaluation). In this situation the subject had to return after 7 days to perform another NAPT-M with the other 3 aeroallergens to complete the study. After this second NAPT-M, there could be 2 possible results:

- I. Negative response to the other 3 aeroallergens: The patient was given a diagnosis as being monosensitive (maximum of 3 days of evaluation).
 - II. Positive response to 1, 2, or the other 3 aeroallergens: The patient was given a diagnosis as being polysensitized (maximum of 9 days of evaluation).
- C. Positive response at 1 hour or more after the fourth aeroallergen: In this circumstance it was not possible to identify the sensitizing aeroallergen or aeroallergens. The patient was given a diagnosis of LAR and returned in 7 days to perform a second run of the NAPT-M with the first 3 aeroallergens. In this group there could be 2 possible outcomes:
- I. Negative response to this second NAPT-M with the 3 aeroallergens: The patient was given a diagnosis as being monosensitive to the fourth allergen (maximum of 4 days of evaluation).
 - II. Positive response to the second NAPT-M: In this case 4 single NAPT-Ms with all 4 aeroallergens needed to be performed to identify the sensitization (maximum of 12 days of evaluation).

NAPT-S

The NAPT-S was used in this study as the gold standard diagnostic test for LAR. After bilateral saline challenge to exclude nasal hyperreactivity, NAPT-S were performed with a single allergen per session at weekly intervals. The allergen concentration, criteria for positivity, and response category were the same as used in nasal challenge with multiple aeroallergens.

Statistical analysis

SPSS version 15.0 (SPSS, Inc, Chicago, Ill) was used for the statistical analysis. Continuous data are expressed as medians and ranges, and categorical data are expressed as percentages. The clinical and demographic data were compared between groups by using χ^2 analysis and the Mann-Whitney *U* test. The Wilcoxon signed-rank test was used to identify significant differences in the number of visits and tryptase and ECP levels within groups. Correlations were checked by using the Spearman rank method. All *P* values of less than .05 were considered statistically significant.

RESULTS

From the total group, 22 patients with LAR (12 with perennial LAR and 10 with seasonal LAR) and 21 patients with perennial NAR were enrolled in the study. Three patients with LAR and 4 patients with NAR who had a positive response to saline challenge were excluded from the study. The clinical

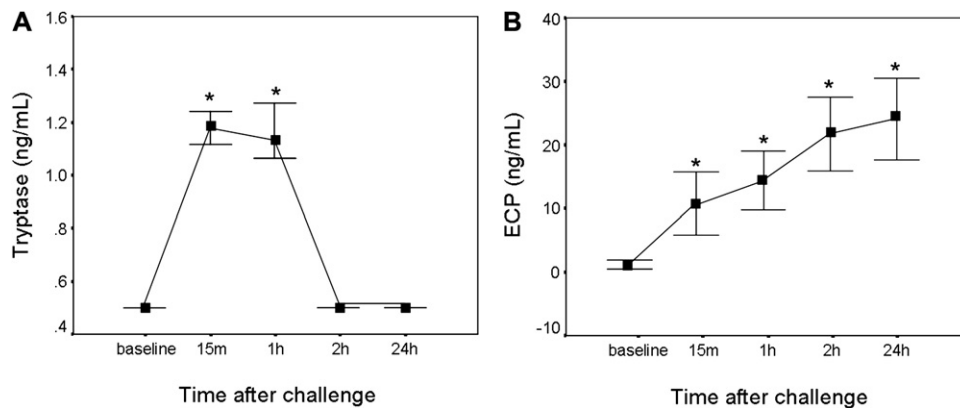


FIG 2. Nasal levels of tryptase (**A**) and ECP (**B**) in patients with LAR after NAPT-Ms. Data are expressed as medians $\pm$ ranges. Significant differences ($P < .05$) between baseline and 15 minutes and 1, 2, and 24 hours after challenge are indicated by asterisks (Wilcoxon signed-rank test).

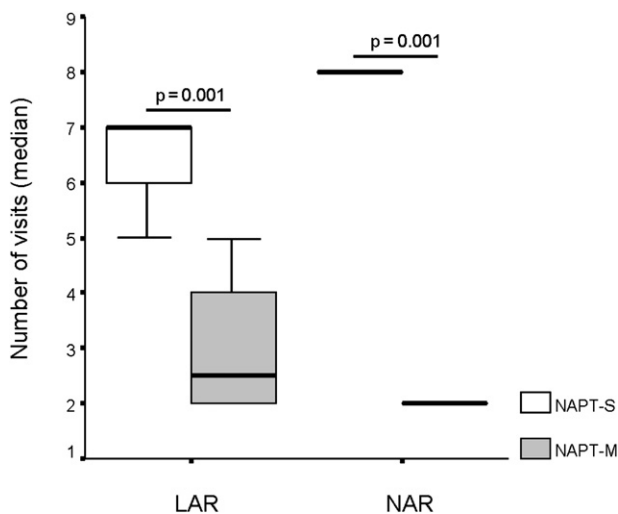


FIG 3. Number of visits required for the final diagnosis in NAPT-Ms and NAPT-Ss. Data are shown as medians $\pm$ ranges. P values refer to comparisons between values for patients with LAR and patients with NAR (Mann-Whitney U test).

characteristics of the patients are shown in Table I. All the patients with NAR and 55% of the patients with LAR reported perennial symptoms, and about 30% of each group had asthma.

The comparative study between the NAPT-M performed in this study and the NAPT-S carried out 1 year earlier showed 100% concordance (κ index = 1), both for the differential diagnosis between LAR and NAR (Table II) and for the identification of sensitizing aeroallergens in patients with LAR (30/88 aeroallergens, Table III). None of the patients with NAR reported nasal or ocular symptoms during the 24 hours after nasal challenge.

Significant increases in nasal levels of tryptase (46%, $P = .01$) and ECP (55%, $P = .05$) were detected in the patients with LAR, with peaks at 15 minutes and 24 hours after NAPT-Ms, respectively (Fig 2). No significant increases in nasal levels of tryptase or ECP were observed in the patients with NAR with a negative NAPT-M response compared with baseline values. Correlation studies between increases in VAS symptoms and nasal secretion of tryptase and ECP detected a significant positive association between the increase in tryptase and VAS nasal-ocular

symptoms ($r = 0.794$, $P = .001$) and a weak but significant association between VAS and ECP ($r = 0.419$, $P = .01$). These results are in agreement with previous data reported by our group.⁸

Comparison of the number of visits required to identify the sensitizing aeroallergens showed that use of the NAPT-M significantly reduced the number of visits in comparison with use of the NAPT-S in both groups of patients ($P = .001$, Fig 3). Overall, the use of the NAPT-M resulted in a 75% reduction in the number of visits to obtain the final diagnosis in the NAR group (from 168 to 42 visits) and a 55% reduction in the LAR group (from 144 to 65 visits).

In the LAR group the highest reduction in the number of visits was obtained in the subgroup of 15 monosensitive patients with LAR with an immediate isolated response to the last aeroallergen challenged (35 visits in the NAPT-M group compared with 105 visits in the NAPT-S group). In this group 10 subjects had a positive response 15 minutes after challenge (10 patients $\times$ 2 visits = 20 visits) and the other patients had a positive response at 1 hour (5 patients $\times$ 3 visits = 15 visits). Only 1 monosensitive patient with LAR had no positive response to the last aeroallergen challenged. No isolated late response was observed.

Although the possibility of performing the NAPT-S to identify sensitizing aeroallergens was included in the flow chart, there was no need to carry it out during the study.

A total of 30 sensitizations were identified in 22 patients with LAR according to the previous NAPT-Ss performed at the first evaluation (Table I). The majority of patients had a positive response to *D pteronyssinus* (14/22 [64%]) and grass pollen (12/22 [55%]). Nine patients with perennial LAR were monosensitive to *D pteronyssinus*, and 6 patients with seasonal LAR were monosensitive to grass pollen. A detailed summary of the NAPT-M and NAPT-S responses in patients with LAR is shown in Table IV.

DISCUSSION

Although the diagnosis of allergic rhinitis is based on clinical history, SPT responses, and positive serum sIgE responses,¹³ the performance of SPTs in the light of a structured clinical history improves the precision of the allergic rhinitis diagnosis compared with the patient's self-report or clinical history alone.¹⁴ However, this diagnostic approach is not useful in patients with LAR in whom skin test responses and serum sIgE levels are negative

TABLE IV. Individual positive response to NAPT-Ms and NAPT-Ss in patients with LAR

Patient no.	Symptoms	Allergen	NAPT-M (+)	No. of visits NAPT-M	NAPT-S (+)	No. of visits NAPT-S
1	Perennial	<i>D pteronyssinus</i>	15 min	2	1h	7
2	Perennial	Grass	15 min	5	15 min	5
		<i>A alternata</i>	15 min		15 min	
		<i>D pteronyssinus</i>	1 h		15 min	
3	Seasonal	<i>D pteronyssinus</i>	15 min	4	15 min	5
		<i>O europea</i>	15 min		15 min	
		Grass	15 min		15 min	
4	Perennial	<i>D pteronyssinus</i>		2	1 h	7
5	Perennial	<i>A alternata</i>	1 h	4	1 h	7
6	Perennial	<i>D pteronyssinus</i>	15 min	2	1 h	7
7	Seasonal	Grass	15 min	2	1 h	7
8	Seasonal	Grass	15 min	2	15 min	7
9	Perennial	<i>D pteronyssinus</i>	1 h	3	1 h	7
10	Seasonal	<i>D pteronyssinus</i>	15 min	4	15 min	5
		<i>O europea</i>	15 min		1 h	
		Grass	15 min		15 min	
11	Seasonal	Grass	1 h	3	15 min	7
12	Perennial	<i>D pteronyssinus</i>	15 min	2	15 min	7
13	Perennial	Grass	15 min	5	15 min	5
		<i>A alternata</i>	15 min		15 min	
		<i>D pteronyssinus</i>	1 h		15 min	
14	Seasonal	Grass	15 min	2	15 min	7
15	Perennial	<i>D pteronyssinus</i>	1 h	3	1 h	7
16	Seasonal	<i>D pteronyssinus</i>	15 min	3	15 min	6
		Grass	15 min		1 h	
17	Perennial	<i>D pteronyssinus</i>	1 h	3	1 h	7
18	Seasonal	Grass	15 min	2	15 min	7
19	Perennial	<i>D pteronyssinus</i>	1 h	3	15 min	7
20	Seasonal	Grass	15 min	3	15 min	7
21	Perennial	<i>D pteronyssinus</i>	15 min	3	15 min	7
22	Seasonal	<i>O europea</i>	15 min	3	15 min	6
		Grass	15 min		15 min	
Total visits				65		144

and for whom NAPT and determination of nasal sIgE antibodies are needed to establish the diagnosis.^{2,3,6-9}

For this purpose, we investigated the value of a protocol for an NAPT-M monitored based on subjective and objective parameters to screen for the involvement of more than 1 aeroallergen in patients with LAR.

The NAPT-S, which is considered the gold standard test for the diagnosis of allergic rhinitis, has contributed to the study of the pathophysiology of the disease, the intensity of the allergic response, and the effectiveness of symptomatic treatment and specific immunotherapy.¹⁵⁻¹⁹ However, this test is time-consuming, which limits its use as a screening tool and in the identification of polysensitization to several aeroallergens.

The present study demonstrated that the NAPT-M is useful and reproducible, as well as being a less time-consuming *in vivo* diagnostic test for the screening of patients with LAR than the NAPT-S. In addition, the NAPT-M showed 100% concordance with the NAPT-S and an important reduction in the total number of visits required to confirm the diagnosis (from 168 to 42 visits in patients with NAR and from 144 to 65 in patients with LAR) compared with the NAPT-S.

The response to nasal challenge was evaluated by means of both subjective (VAS of nasal-ocular symptoms) and objective (acoustic rhinometry) parameters to obtain a good level of

accuracy and according to the recommendations for experimental research.²⁰ Furthermore, an additional objective parameter (nasal release of inflammatory mediators) was also determined. Based on the previous studies,^{8,9} none of the subjects with a negative NAPT-M result showed a significant increase in tryptase or ECP levels after challenge.

A saline challenge was performed before the NAPT to exclude patients with nasal hyperreactivity. Glycerinated control or extract solutions were avoided because glycerin can produce a nonspecific reaction in the nasal airways.^{13,20}

To avoid failing to detect patients with LAR with a late response to nasal challenge, as has been reported in previous studies,^{2,3,7-9} we conducted the evaluation of the nasal response after each allergen challenge in all subjects at 15 minutes and 1, 2, and 24 hours after challenge.

The results obtained with NAPT-Ms in patients with LAR and NAR showed that the administration of several aeroallergens in the same session did not produce false-positive responses because of a nonspecific or irritant effect in the nasal airways. In addition, the possible occurrence of a nasal priming effect, defined as an increase in the reactivity of the nasal mucosa after repeated exposures to inhalant allergens to which the subject was previously sensitized,²¹ did not occur in this study, in which patients with LAR were challenged with different allergens and not with

increasing concentrations of an allergen to which the subject was already sensitized.

In a previous study Wierzbicki et al²² attempted to use a different model of the NAPT with multiple aeroallergens to diagnose LAR in a sample of 20 subjects with perennial NAR, but they found 7 false-positive responses to NAPT in 5 subjects. The lack of positive results of this study might be due to several methodological aspects: the use of a glycerinated solution as a control solution, which, as mentioned, can produce a nonspecific or irritant effect in nasal cavities and alter the response to aeroallergens^{13,20}; use of unilateral nasal challenge rather than bilateral challenge, which is considered more physiological; use of total score symptoms as the sole NAPT criterion of positivity; lack of evaluation of late responses; and finally, use of a low concentration of *D pteronyssinus* (1000 AU/mL, which is equivalent to 0.4 µg/mL Der p 1), although previous studies have detected positive responses to *D pteronyssinus* in a range from 0.04 to 4 µg/mL (100–10,000 AU/mL) in NAPT, both in patients with systemic allergic rhinitis²³ and patients with LAR.^{2,9}

One important aspect of our study was the definition of an appropriate and realistic panel of aeroallergens. This proved a key point to reduce the number of visits required to identify monosensitization and polysensitization in patients with LAR. In fact, the best results were obtained when the sensitizing allergen was challenged last, followed by cases in which it was administered first. These results reflect the importance of conducting a detailed clinical history that includes triggering factors, as well as allergen sources to which the patient is exposed, before designing the panel of allergens that are to be used in NAPT-Ms.

Although the NAPT-M has been useful in this study to differentiate between LAR and NAR and to identify monosensitized and polysensitized patients with LAR, the intensity of sensitization and allergic response cannot be measured with NAPT-Ms. This requires performing NAPT-Ss with increasing concentrations of a single allergen, which allow us to establish the threshold concentration that induces the allergic response and correlates with the clinical history and the nasal-ocular symptoms during natural exposure in studies of patients with LAR.^{2,3}

In conclusion, the data from this study demonstrate that the NAPT-M with an appropriate aerallergen panel is very useful, specific, sensitive, and reproducible, as well as being a less time-consuming *in vivo* diagnostic test for the screening of patients with LAR. This approach will facilitate and improve the evaluation of patients with LAR, a common entity in our population.

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Clinical implications: The use of the NAPT-M in the screening of LAR will be a very useful diagnostic tool for patients with polysensitization.

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