

Nasal Provocation Tests With Allergens: Just a Research Tool or Suitable for Everyday Clinical Practice?

Paloma Campo, MD, PhD

Esther Barrionuevo, MD, PhD

Ibon Eguiluz, MD, PhD

María Salas, MD, PhD

M. José Torres, MD, PhD

*Carmen Rondón, MD, PhD**

Address

*Allergy Unit, Regional Hospital of Málaga-IBIMA, Pabellón 6, 1ª planta. Plaza del Hospital Civil s/n, 29009, Málaga, Spain
Email: carmenrs61@gmail.com

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Opinion statement

Nasal provocation test (NPT) with allergens is a simple and safe technique recommended in different diagnostic and research settings. In the daily practice, NPT has proved to be very useful when there are discrepancies between patient's symptoms and the results of skin or blood testing, for confirming the clinical relevance of a certain allergen in polysensitized patients and also for the diagnosis of new rhinitis phenotypes such as local allergic rhinitis (LAR). In the research field, nasal provocation test with allergens has been widely used in the study of mechanisms of inflammation and allergic response in subjects with allergic rhinitis (AR) and LAR and the study of pathophysiological mechanisms of response allergens to evaluate the therapeutic effect of drugs or immunotherapy in controlled clinical trials. There are key aspects necessary to achieve the best safety and reproducibility of the test, such as the characteristics of the allergen, application techniques, and measurement of the response that must be as objective as possible. The use of short protocols and simple methods of measurement allows the use of NPT in the daily practice as a diagnostic aid and not only a research tool reserved for clinical trials.

Introduction

Diagnostic test in allergic diseases has evolved over the years, being currently more precise and non-invasive (e.g., molecular diagnosis). However, the demonstration of the inflammatory response in the nasal mucosa after a controlled exposure to an allergen is still a very useful approach in the diagnosis and the understanding of the underlying mechanisms of such response.

This controlled exposure with either allergens or certain drugs can be done by means of the nasal provocation (or also called challenge) test (NPT). NPT has multiple applications and has been recommended in different diagnostic and research scenarios. This test offers an objective proof of the clinical relevance of an allergen/

drug and the clinical symptoms and severity [1•]. Also, it is a test of high value to evaluate the therapeutic responses in both clinical trials and daily practice. There are an ample number of substances, type of measurements, and evaluation techniques for performing nasal provocations.

In this review article, the usefulness of the test, the different technical aspects, and the applications in the clinical and research settings are discussed. This work will be focused on NPT with allergens but will mention the application of this test when performed with lysine-acetylsalicylic acid (L-ASA) in the diagnosis of the NSAID-exacerbated respiratory disease (NERD).

Indications and contraindications

Nasal provocation test with allergens (NPTA) has been designed to elicit a nasal response by controlled exposure to variable amounts of allergen [2••]. The aim of the test is to mimic the reaction occurred during a natural exposure to allergens, demonstrating the presence of allergen-specific IgE (sIgE) and the causal role of the allergen, since the presence of sIgE alone (sensitization) does not always imply a clinical relevance. The same concept is applied to the nasal challenge with certain occupational substances or with L-ASA, although in these cases, the mechanism maybe non-IgE-mediated [3••].

The main indications and contraindications are summarized in Table 1 [1•, 2••, 3••, 4, 5].

The main indications for NPT in the clinical practice are as follows:

- Accurate diagnosis of seasonal/perennial allergic rhinitis (AR) by confirming the clinical relevance of a certain allergen
- Discrepancies between patient's symptoms and the results of skin or blood testing
- Diagnosis of local allergic rhinitis (LAR)
- Identification of the clinical significance of allergens in patients with multiple sensitizations
- Help to select relevant allergens for allergen immunotherapy (AIT) in AR
- Diagnose NSAID hypersensitivity in patients with history compatible of respiratory symptoms (rhinitis and/or asthma) after NSAID intake (more often NERD patients) and avoid oral/bronchial challenge with ASA

The main indications or applications for NPT in a research setting are as follows:

- Study of mechanisms of inflammation and allergic response in subjects with AR and LAR

Table 1. Indications and contraindications of nasal provocation test (NPT)

Indications of NPT	
Clinical setting	Research setting
<ul style="list-style-type: none"> • Confirm diagnosis of seasonal/perennial AR (including IgE-mediated occupational AR) • Diagnosis of LAR • Clinical relevance of allergen (polysensitized) • Select relevant allergens for AIT • Diagnose NSAID hypersensitivity in NERD 	<ul style="list-style-type: none"> • Study mechanisms of inflammation and allergic response in AR and LAR • Study of pathophysiological mechanisms of response to L-ASA in NERD • Evaluate therapeutic effect of drugs in controlled clinical trials. • Evaluate therapeutic effect of immunotherapy in controlled clinical trials
<p>Contraindications of NPT</p> <ul style="list-style-type: none"> • Recent nasal surgery (<3 months) • Respiratory tract infection in the past 2–4 weeks • Use of certain medication (antidepressants, oral steroids, etc.) • Severe nasal polyposis • Pregnancy • Uncontrolled bronchial asthma or active severe cardiopulmonary disease 	
<p><i>AR</i> allergic rhinitis, <i>AIT</i> allergen immunotherapy, <i>L-ASA</i> lysine-acetylsalicylic acid, <i>LAR</i> local allergic rhinitis, <i>NERD</i> NSAID-exacerbated respiratory disease, <i>NPT</i> nasal provocation test</p>	

- Study of pathophysiological mechanisms of response to L-ASA in subjects with NERD
- Evaluate therapeutic effect of various drugs (antihistamines, nasal corticosteroids, etc.) in controlled clinical trials.
- Evaluate therapeutic effect of immunotherapy in controlled clinical trials
- Help to select relevant allergens for AIT in AR

Contraindications for NPT are as follows:

- Recent nasal surgery (less than 3 months)
- Respiratory tract infection in the past 2–4 weeks (needs to be postponed)
- Use of certain medication (antidepressants, oral steroids, etc. need to be postponed)
- Severe nasal polyposis
- Pregnancy
- Uncontrolled bronchial asthma or active severe cardiopulmonary disease

Technical aspects and controversies

Standardized NPT is a very sensitive, specific, reproducible, and safe diagnostic test for AR, LAR, occupational rhinitis, and hypersensitivity to non-steroidal anti-inflammatory drugs. The main aspects to be taken into account to perform NPT will be analyzed in this section.

Patient preparation

Patients should sign a written informed consent document before starting NPT [2••]. Patient must be asymptomatic, out of the pollen season in seasonal rhinitis, or with mild symptoms in perennial-persistent rhinitis [2••, 6–8]. NPT should be done preferably in the morning to avoid the effect of pollution and physical exercise [2••].

Examination room

Room temperature (20–22 °C) and humidity (40–60%) should be kept constant. High values reduce the immediate response reducing the histamine release and vascular and neural response.

Patient should wait in the examination room for 15–30 min to well adapt to the climate conditions and to prevent non-specific reactions. Mobile phones must be turned off to not interfere with acoustic rhinometers [2••].

Health personnel

Personnel should have the adequate knowledge of NPT methodology, the technique that will be used to assess the results, and the access to therapeutic measures in cases of positive response to NPT [4].

When a NPT should be postponed?

In some special daily circumstances and conditions, NPT should be postponed:

- Tobacco smoke, spicy food, coffee, alcohol intake, 24–48 h [2••]
- Viral/bacterial respiratory tract infection, 4 weeks [9].
- Nasal surgery, 6–8 weeks [6]
- Exacerbation of AR, 2–4 weeks [6, 7]
- Non-specific nasal hyperreactivity, 2–3 weeks [2••]
- Pharmacologic treatments [2••]
 - Oral antihistamines, 48 h to 1–2 weeks
 - Topical antihistamines, 4–5 days
 - Nasal corticosteroids, 48–72 h
 - Oral corticosteroids, 2–3 weeks
 - Sodium cromoglycate, 1–3 weeks
 - Nasal decongestants in general, 2 days
 - Tricyclic antidepressants, 2–3 weeks
 - Non-steroidal anti-inflammatory drugs (NSAIDs), 1 week
 - Reserpine-type or clonidine-type antihypertensives, 3 weeks

Nasal examination

Nasal examination should always be the first step of the NPT, starting with the inspection and palpation and continuing with the (anterior and posterior) rhinoscopy or better, if possible, a nasal endoscopy. Nasal endoscopy provides better visualization of nasal cavities [3••].

Key points of the NPT

Three key aspects are essential to achieve a maximum level of safety and reproducibility: characteristics of the allergen, application techniques, and measurement of the response.

Allergens

The potency of aqueous dilutions decreases rapidly, so lyophilized allergen extracts should be diluted and used rapidly, on the day of the test or in the next 2–3 weeks, and kept at $-4\text{ }^{\circ}\text{C}$ [10]. An alternative is a ready-to-use solution of allergen in buffered saline, with or without human serum albumin. The glycerinated extracts used in skin prick tests (SPTs) should be avoided (non-specific response) [7].

The initial allergen concentration depends on the patient's sensitivity, the environmental concentration of the allergen, and the characteristics and potency of the extract.

For standardized allergens, it is recommended to start with a concentration of 1:1000 of the SPT and continue with increases by a factor of 10 (clinical practice) or a factor of 3 (research studies). For less well-known and occupational allergens, endpoint titration should be performed [2••].

Allergen application techniques

Large variability of application techniques includes different delivery systems, application site, number of doses, and number of allergens tested.

- Delivery system

Different application techniques can be used for soluble allergens (Table 2). The most recommended in clinical guidelines are nasal spray and micropipette. Application of micronized powder encapsulated with lactose using an inhaler is reserved for less common non-soluble allergens [2••].

- Site of application

The allergen must be applied on the head of the inferior turbinate unilaterally or bilaterally. Bilateral application is more physiological and recommended to identify variations in nasal patency caused by the nasal cycle [11].

- Number of doses

Single dose provides diagnostic information, identifying the allergen responsible of the allergic symptoms. In contrast, increasing doses also provides information about the degree of tolerance to the allergen (dose-response), which is very useful for assessing the evolution of sensitization over time and monitoring the response to immunotherapy and pharmacotherapy [2••].

- NPT with different allergens

In polysensitized patients, occupational rhinitis patients, or possible LAR patients [12••], it may be necessary to perform NPT with different allergens. In those cases, we can perform several NPTs with a single allergen per session (NPT-S), with an interval of ≥ 1 week between tests in order to avoid the priming effect [2••] or a NPT with multiple allergens (NPT-M) sequentially administered in one session [13••].

Measurement of the response

- Subjective parameters

Table 2. Allergen application techniques and objective evaluation of nasal obstruction: advantages and disadvantages

Allergen application techniques		Characteristics	Advantages	Disadvantages
Technique				
Syringe		100 µl	Easy	Unpredictable distribution. Possible laryngeal-bronchial aspiration. Mucociliary transport into Eustachian tube
Nose dropper		Number of drops in dosimeter	Easy	Unpredictable distribution. Possible laryngeal-bronchial aspiration. Mucociliary transport into Eustachian tube
Micropipette		100 µl onto head of the inferior turbinate	Exact volume, little leakage into bronchi	Moderate technical difficulty
Nasal spray		100 µl onto head of inferior turbinate	More delivery to nasal cavity, little leakage into bronchi, easy and reproducible	Nasal spray device variability
Impregnated cotton		Application to middle/inferior turbinate	Localized application, less allergen used	Moderate technical difficulty, risk of sinus disease
Impregnated disk		4 mm diameter, 10 µl inferior turbinate	Localized application, less allergen used	Moderate technical difficulty
Objective evaluation of nasal obstruction				
Technique		Measurements	Advantages	Disadvantages
Acoustic rhinometry		Nasal cavities geometry (areas and volumes) based on the reflection of an acoustic wave Parameters: areas: minimum cross-sectional area (MCS) Volumes: volume of the first 5–6 cm of the nasal cavity (Vol. 2–5 cm, Vol. 2–6 cm) Nasal resistance (R) by measuring airflow (cm ³ /s) at specific pressures (100/150/300 Pa). Positivity criterion NAFT = ↑ R 100%	Rapid, easy, static, high reproducibility (coefficient of variation <10%) Little patient's cooperation (can be used in children) Not affected by rhinorrhea or intense nasal obstruction	Do not use in septal perforation
Anterior rhinomanometry		Nasal resistance to airflow	Sensitive and highly specific, active, and dynamic technique, high reproducibility (VC 15–30%)	Do not use in septal perforation and in intense obstruction or rhinorrhea. Difficult to use in children (patient's cooperation is necessary)
Nasal peak flow		Nasal resistance to airflow	Easy and cheap, good correlation with values of AR and ARM, useful for home monitoring: NPT and response to treatment	Less reproducible in bronchial asthma, bronchial response, and intense obstruction and rhinorrhea (it is dependent on stress/FVC and reflex changes in nasal obstruction) Needs patient's cooperation (difficult in children)

Adapted from Dordal et al. [20]

In many publications, the measurement of the response to NPT is based on nasal symptoms that can be recorded by semi-quantitative methods as score systems [14, 15] or visual analogue scale [11]. However, it is most recommended to use nasal symptoms accompanied by at least an objective parameter of nasal airway obstruction or mucosal inflammation [2••].

- Objective parameters

- Nasal airway obstruction

The main three methods used to assess nasal obstruction from highest to lowest reproducibility [2••, 16]) are acoustic rhinometry (ARN), active anterior rhinomanometry (AARM), and nasal peak inspiratory flow (NPIF). The advantages and disadvantages of these techniques are shown in Table 2.

- Nasal mucosa inflammation

Cytology and measurement of specific-IgE and pro-inflammatory mediators (histamine, tryptase, cytokine, eosinophil cationic protein (ECP), leukotrienes, etc.), in samples of nasal mucosa obtained by invasive methods (nasal brushing, scraping and biopsy) or samples of nasal secretion by non-invasive methods (nasal lavage, cotton swab, paper disk) are the most common methods used.

- Other objective measurements

- Quantification of the weights and volume of nasal secretions [6].

- Concentration of nitric oxide in nasal air [17].

- Optical rhinometry for assessing edema of the nasal mucosa [18–20].

- Doppler ultrasound to study of microcirculation [2••].

NPT procedure

The NPT starts with baseline assessment of nasal symptoms plus objective nasal obstruction measurement (basal value) followed by the application of an inert control solution (the diluents used to prepare the solutions) to identify non-specific nasal hyperreactivity (NHR). Fifteen minutes later, the response is assessed (reference value). A positive response to control solution is indicative of NHR, and NPT should be stopped [2••, 12••, 13••]

The nasal response to the control solution is considered in the following circumstances [2••]:

- Increases of symptom score (≥ 3 points) and/or
- ARN: MCR and/or vol 2–6-cm reduction $\geq 10\%$
- AARM: total nasal airway resistances (R) increase 20% or total nasal airflow reduction 20% at 150 Pa.
- Reduction of NPIF 15%

If the response is negative, the NPT proceeds with the serial application of different concentrations of the allergen or different allergens at intervals of 15–30 min. The patients should remain seated and hold their breath during application to prevent the pass of the allergen towards the larynx and lower respiratory tract. Nasal response can be assessed 15–30 min after application. The challenge ends when the last dose is administrated or a positive response is detected. The patient must be kept under observation for 1–2 h after the last dose to evaluate possible delayed responses [2••, 13••].

When a NPT is positive?

NPT is considered positive when the positivity criteria of an objective evaluation of nasal obstruction are satisfied, with/without increase in symptom score compared with reference value:

Objective nasal obstruction:

- ARN 25% reduction in the MCS or Vol 2–6 cm [2••, 4, 5, 13••]
- AARM 100% increase in total airway R at 150 Pa [2••, 4, 5]
- NPIF 40% reduction in airflow [21]

Symptom score:

- Increase in the symptom score of ≥ 5 points [14, 15].

False positive and negative response

Evaluation of the response is important to take into account the main causes of false-positive and false-negative response [2••]:

False-positive response:

- Nasal cycle
- High allergen concentration
- Inadequate extract pH, temperature, and osmolarity
- Excipients, such as phenol, glycerol, or benzalkonium chloride
- Contamination of the examination room
- Infectious or allergic process in the previous 2–4 weeks
- Previous allergen exposition (nasal priming)
- No evaluation of nasal hyperreactivity

False-negative response

- Inadequate allergen
- Too low allergen concentration
- Allergen extract expired
- Nasal flow too low already at the beginning
- No evaluation of delayed/late response (≥ 1 h)
- Nasal surgery in the previous 8 weeks
- Use of contraindicated medication
- Atrophic rhinitis
- Specific immunotherapy

NPT in everyday clinical practice

For a long time, the use of NPT has been limited to research studies or clinical trial, mainly due to its requirements in time, specialized personnel, and equipments. Fortunately, recent studies have demonstrated that NPT can be a useful diagnostic tool in everyday clinical practice:

Standardized NPT is a very safe test; the appearance of adverse reactions and the delayed positive responses are mild and extremely rare beyond 1 h after

application of the allergen, reducing the waiting time after the last application to 1 h [22, 23, 24••, 25•].

The new protocol of NPT with multiple allergens (NPT-M) has shortened the number of visits in patients who require NPT with different allergens, without producing irritant or priming effect [13••, 26••].

Nasal peak inspiratory flow (NPIF) is a well-standardized, validated, rapid, easy, and inexpensive objective technique for evaluating the nasal obstruction and can be used for monitoring the delayed response by the patient at home [21].

Also, NPT with L-ASA is widely used in the daily practice for the diagnosis of NSAID hypersensitivity in patients with NERD. Intranasal provocation with L-ASA is a good alternative for oral or bronchial challenge since it is safe and can be performed in an outpatient setting and even in patients with severe asthma [27••]. The sensitivity of aspirin nasal challenge test ranges from 60 to 80% or higher depending on the studies [27••, 28–30].

Minimum requirements for NPT in clinical practice

1. Allergen/ASA application: bilateral
2. Method of application: use nasal spray or micropipettes to deposit the allergen solution on the head of the inferior turbinate while the patient holds his/her breathe.
3. Volume instilled in each nasal cavity 100 μ l
4. Initial allergen concentration 1/1000 of the concentration that elicits a positive SPT result (or a concentration of 1/10,000 in the case of non-standardized allergens)
5. Evaluation of nasal hypersensitivity
6. Use of a combination of the symptom score and an objective evaluation of nasal obstruction to assess the response
7. NPT monitoring: evaluation 15 min after allergen application and 1 h after the last application).

Usefulness in research settings

As it has been shown throughout the document, NPT is a simple procedure with high specificity and sensitivity that has been used extensively in the investigation of the mechanisms of allergic and non-allergic rhinitis (NAR) [2••, 5]. Nasal allergen challenge can be used to assess the clinical and immunological aspects of rhinitis due to inhalant allergens, since the controlled application of allergens and the obtention at different time points of biopsies, nasal lavages or secretions, nasal brushing, or scraping of the nasal mucosa have allowed the study mechanisms of inflammation and allergic response in rhinitis [4, 6, 31].

NPT has been used with numerous allergens and allows the measurement of inflammatory mediators such as ECP, tryptase, cytokines (IL-4, IL-5, IL-10, IL-13, IFN- γ ...), leukotrienes, and others in nasal secretions after

the controlled exposure to the antigen [14, 32]. NPT has been performed with both complete and purified allergens [33, 34•]. NPT has also been widely used in studies of local IgE secretion in the nasal mucosa and helped define a new phenotype of rhinitis which is LAR [13••, 22, 25•]. Also, the clinical and immunological relevance of the allergens has been evaluated using NPT in other nasal diseases such as NAR, chronic rhinosinusitis, and nasal polyps [35]. In research settings, NPT with L-ASA has been crucial in the understanding of the underlying mechanisms of the inflammation in NERD patients [27••, 36, 37].

NPT has been also used as a valuable tool for evaluating the therapeutic effect of several drugs in controlled clinical trials, such as antihistamines or nasal corticosteroids, monitoring clinical responses to the drug, and changes in allergen challenge threshold and in inflammatory mediators [21, 38–40]. Also, NPT has been applied in the assessment of the efficacy of specific immunotherapy in numerous studies [41, 42], although recently, the design of clinical trials is more focused in the use of complete challenge chambers that seem to be more reliable [43].

Conclusions

NPT is a safe and reproducible test that is very useful for both daily practice and research applications. This technique is easy to do and can be performed in an outpatient clinic. NPT gives very useful information about the clinical relevance of an allergen in case of non-agreement between the symptoms and the skin test/sIgE, in polysensitized patients and in the diagnosis of LAR. Patients with NERD may also benefit of a challenge with lysine-aspirin to confirm hypersensitivity to NSAIDs. In the research field, the controlled exposure to allergens has been widely applied in the study of mechanisms and the therapeutic response to drugs and immunotherapy. Technical aspects such as avoidance of forbidden medication, allergen delivery, and objective assessment of nasal obstruction among others must be performed correctly in order to obtain reproducible and meaningful results.

Compliance with Ethical Standards

Conflict of Interest

Paloma Campo declares that she has no conflict of interest. Esther Barrionuevo declares that she has no conflict of interest. Ibon Eguiluz declares that he has no conflict of interest. María Salas declares that she has no conflict of interest. M. José Torres declares that he has no conflict of interest. Carmen Rondón declares that she has no conflict of interest.

Human and Animal Rights and Informed Consent

With regard to the authors' research cited in this paper, all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. In addition, all applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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