Allergen-Specific Nasal Provocation Testing: Review by the Rhinoconjunctivitis Committee of the Spanish Society of Allergy and Clinical Immunology

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■ **Abstract**

Specific nasal provocation testing (NPT) consists of eliciting a response from the nasal mucosa by controlled exposure to allergens. It is indicated in the diagnostic confirmation of allergic rhinitis and when discrepancies arise or difficulties exist in the assessment of a patient's medical history and the results of skin and/or serological tests. The technique is also applied to evaluate sensitivity to the allergen, the efficacy and safety profile of treatment, and in research on the pathophysiological mechanisms of nasal response to allergens. NPT also provides information on the etiology of occupational respiratory diseases of allergic origin. Although there have been many studies and publications on the use and standardization of bronchial provocation tests with allergen, few analyze specific NPT. In this review, the Rhinoconjunctivitis Committee of the Spanish Society of Allergy and Clinical Immunology discuss the methodology, monitoring, and assessment of allergen-specific NPT in order to provide a practical and up-to-date review of the technique.

■ **Resumen**

Antecedentes: La provocación nasal específica (PNE) consiste en reproducir de forma controlada la respuesta de la mucosa nasal a la exposición a alérgenos. Está indicada en la confirmación diagnóstica de la rinitis alérgica, cuando existen discrepancias o dificultades en la valoración de la historia clínica y las pruebas cutáneas y/o serológicas, en la evaluación del grado de sensibilidad del paciente frente al alérgeno, en estudios de investigación de los mecanismos fisiopatológicos implicados en la respuesta nasal a alérgenos, en la valoración de la eficacia y seguridad de los fármacos empleados en el tratamiento de la rinitis, y en el estudio etiológico de enfermedades respiratorias alérgicas de origen ocupacional. Han sido múltiples los estudios y publicaciones realizadas sobre el uso y estandarización de la provocación bronquial con alérgenos en contraste con las pocas publicaciones realizadas al respecto sobre la prueba de provocación nasal. En esta revisión del Comité de Rinoconjuntivitis de la Sociedad Española de Alergia e Inmunología Clínica se revisará la metodología de la provocación nasal específica con alérgenos, en un intento de ofrecer una visión práctica y actualizada de esta técnica.

1. Introduction

Allergic rhinitis is an inflammation of the nasal mucosa caused by an immune reaction mediated by immunoglobulin (Ig) E antibodies. Clinically, it is characterized by sneezing, rhinorrhea, nasal obstruction, and itching of the nasal membranes, pharynx, and soft palate. Allergic rhinitis is the most frequent allergic disease and is often associated with bronchial asthma and, especially, ocular symptoms [1].

Several studies have examined the use and standardization of bronchial provocation tests with allergens. In contrast, only a few publications have analyzed nasal provocation testing (NPT), despite the high prevalence of rhinitis (5%-20%) in the general population. In an epidemiological study of more than 4000 patients performed by the Spanish Society of Allergy and Clinical Immunology, 55% of patients consulted for rhinitis and 28% for bronchial asthma [2].

The first data on NPT were reported in 1873 by Blackley [3], who experimented by placing grains of pollen directly on the nasal mucosa. It was not until 1958 that Aschan and Drettner [4] used posterior rhinomanometry to study the effect of antihistamines on response to NPT with allergens and demonstrated the possibilities of the technique.

The 1970s saw increasing interest in NPT in daily clinical practice as a way to reproduce the allergic reaction in the nasal mucosa under controlled conditions and to study the pathophysiological, immunological, and pharmacological aspects of allergic rhinitis. In the 1990s, NPT was used primarily to study the pathophysiological mechanisms implicated in the nasal reaction and response to therapeutic agents.

Since then, a number of scientific societies have published guidelines and consensus statements on the methodology and diagnostic uses of NPT [5-13]. The 2008 update of the Allergic Rhinitis and its Impact on Asthma document [14] also dedicates a section to NPT with allergens. In the present article, we discuss in detail the methodology of allergen-specific NPT with the aim of providing a practical and up-to-date review of the technique.

2. Concept and Indications

Specific NPT consists of eliciting an allergic response from the nasal mucosa by controlled exposure to allergens. This response is characterized by itching, sneezing, rhinorrhea, and edema of the nasal mucosa with increased resistance to airflow.

Specific NPT is indicated in the diagnostic confirmation of allergic rhinitis, primarily as a means of evaluating the clinical significance of individual allergens in multisensitized patients. Specific NPT is also indicated when discrepancies arise or difficulties exist in the assessment of a patient's medical history and the results of skin and/or serological tests [9-14]. NPT is important in the evaluation of the patient's sensitivity to the allergen (study of the nasal response to allergen dose), in the study of immediate and delayed responses, and in research on the pathophysiological mechanisms of nasal response to allergens (eg, cells involved, mediators). NPT is used to assess the efficacy and safety profile of drugs used to treat rhinitis by evaluating the efficacy of the drug against individual symptoms and any change in inflammatory mediators that appear after allergen-specific NPT when the study drug is administered. Similarly, NPT has been used as a laboratory technique in the follow-up and monitoring of clinical response after the administration of specific immunotherapy in patients with allergic rhinitis. NPT is also indicated in the etiologic study of occupational respiratory

diseases of allergic origin, due to the legal implications of these conditions [15-17].

The main drawbacks of NPT are the broad methodological variability (mode of application and method of interpretation), the risk of adverse effects (ear, nose, throat, and bronchi), and the absence of any comparison with “natural” allergen exposure.

3. Preliminary Considerations

3.1 Patient-related Conditions

3.1.1 The patient should sign a written informed consent document before undergoing NPT.

3.1.2 The patient should be asymptomatic, ie, testing should be performed outside the pollen season or, in the case of perennial allergens, when only mild symptoms that do not interfere with the test results are present. Postpone for at least 2-4 weeks after exacerbation of allergic rhinitis [9,11,12].

3.1.3 Any drugs that can modify nasal response should be discontinued before testing, as follows:

- Oral antihistamines: 48 hours to 1-2 weeks, depending on the drug
- Topical antihistamines: 4-5 days
- Nasal corticosteroids: 48-72 hours
- Oral corticosteroids: 2-3 weeks
- Sodium cromoglycate: 1-3 weeks
- Nasal decongestants in general: 2 days
- Tricyclic antidepressants: 2-3 weeks
- Nonsteroidal anti-inflammatory drugs (NSAIDs): 1 week
- Reserpine-type or clonidine-type antihypertensives: 3 weeks

3.1.4 The patient should avoid smoking and alcohol intake for 24-48 hours before the test.

3.1.5 Postpone NPT for 4 weeks after a viral or bacterial respiratory tract infection [18].

3.1.6 Postpone NPT for 6-8 weeks after nasal surgery; this diminishes nasal reactivity [11].

3.1.7 Avoid NPT during pregnancy. 3.1.8 Despite the reported low risk of this test, NPT is not recommended in patients with uncontrolled asthma or severe chronic obstructive pulmonary disease, or in patients with cardiopulmonary disease in whom epinephrine is contraindicated.

3.1.9 NPT is not recommended in patients with septal perforation or total or very intense nasal obstruction, because objective assessment of nasal obstruction is very difficult and test results are hard to interpret.

3.2 Room-related Conditions

3.2.1 Temperature and humidity should be kept at a constant 20ºC-22ºC with 40%-60% humidity: temperatures above 35ºC and a high degree of humidity (80%-90%) can alter the immediate response, due to a reduction in histamine release and vascular and neural response [19].

3.2.2 The patient should become acclimatized by waiting in the room for 20-30 minutes to prevent nonspecific reactions due to environmental conditions.

3.2.3 NPT should be performed preferably in the morning to avoid the irritant effect of the usual

daily stimuli (eg, tobacco smoke, contamination, spicy foods, coffee, physical exercise).

3.3 Conditions Related to the Personnel Performing the Test

3.3.1 Personnel should have adequate knowledge of test methodology.

3.3.2 Personnel should have adequate knowledge of the technique that will be used to assess the results (eg, rhinomanometry, acoustic rhinometry, nasal nitric oxide [NOn]).

3.3.3 Personnel should have knowledge of and access to the necessary therapeutic measures in case the test is positive.

4. Characteristics of the Allergen

Standardization of allergenic extracts is fundamental for ensuring the precision, safety, and reproducibility of any diagnostic procedure.

A lyophilized allergen extract can be diluted on the day of the test to maintain equivalent potency between lots; alternatively, a ready-to-use solution of allergen in buffered saline, with or without human seralbumin, can be used. The glycerinated extracts used in skin prick tests should be avoided, because glycerin can produce a nonspecific reaction in the nasal cavities.

The initial allergen concentration applied will depend on the patient's sensitivity, the local environmental pressure of the allergen, and the characteristics and potency of the extract. The dose used to initiate nasal provocation can be calculated from the dose used in skin prick tests. Some authors propose the concentration necessary to produce a 3-mm papule in a skin prick test or 1/100 of the concentration that elicits a positive skin prick test [9].

NPT with standardized allergens can generally be started at an initial concentration of 1:1000 and then increased by a factor of 10 (in research studies, increments by a factor of 3 are recommended) [20]. In the case of less well-known and occupational allergens, endpoint titration should be performed to identify the initial dose. In the case of occupational allergens, the irritant concentration limit for each substance must also be considered.

The expiry date of commercial extracts is provided by the manufacturer. Otherwise, reconstituted lyophilized extract generally expires after 3-6 months and, once prepared, dilutions can be used for 1 to 60 days. In cases of doubt, the manufacturer should be consulted.

5. Allergen Application Techniques

Unlike the bronchial tract, the nose is very accessible. Several forms of application are possible, depending on the allergen formulation, application site, and mode of application.

- Application of micronized powder encapsulated with lactose using an inhaler, particularly with allergens that are insoluble in organic solvents.

- Application in solution (the most common form):
  - Spraying the allergen on the head of the inferior turbinate (0.1 mL/puff). This method is easy to use and reproducible. The dose dispensed varies, although it falls within acceptable margins.
  - Application of small disks impregnated with a preset amount of allergen to the area of the inferior and middle turbinates. This method allows secretions to be collected for

studying cells and mediators.

- Allergen nebulization. While this method has been used for many years, it carries the risk of depositing the allergen in the lower airways. An apparatus is necessary, as is the active cooperation of the patient (who has to sustain expiration during nebulization).
- Instillation of the allergen solution on the inferior turbinate using a syringe, pipette, or dropper. This approach is accompanied by a risk of depositing the allergen in the pharynx and upper airway. Use of a micropipette and a small amount of solution (0.1 mL) is preferable.

Ideally, the application method should be safe and offer good reproducibility, ie, little variability in the amount of allergen used in different applications. The main advantages and disadvantages of the allergen application methods are summarized in Table 1.

Table 1. Allergen Application Techniques: Advantages and Disadvantages

	Method	Advantages	Disavantages
Syringe	0.1 mL	Easy	Unpredictable distribution area, possible laryngeal-bronchial aspiration, mucociliary transport into Eustachian tube
Nose dropper	Number of drops in dosimeter	Easy	Unpredictable distribution area, possible laryngeal-bronchial aspiration, mucociliary transport into Eustachian tube
Micropipette	0.1 mL into the inferior turbinate	Exact volume, less leakage into bronchi	Some technical difficulty
Nasal Spray	0.1 mL/puff onto head of inferior turbinate	More delivery to nasal cavity, less leakage into bronchi, easy and reproducible	Consider device variability
Impregnated cotton	Application to middle/inferior turbinate	Localized application, less allergen used	Some technical difficulty, risk of sinus disease
Impregnated disk	4 mm diameter, 10 µL, inferior turbinate	Localized application, less allergen used	Some technical difficulty

The allergen can be applied unilaterally or bilaterally; bilateral application is considered to be more physiological, whereas unilateral application should be reserved for research studies. In any case, the evaluation of the nasal response should always be bilateral, because the parasympathetic reflex mechanism of the opposite nasal cavity must be taken into account [21].

NPT starts with the application of an inert substance (the same diluent used to prepare the solutions, eg, physiological saline solution with phenol 0.4%, Ringer lactate solution). Fifteen minutes later, the nasal response is assessed (eg, symptom score, rhinoscopy, rhinometry). If the nasal response is within pre-established reproducibility values (generally 10%-20% depending on the technique used), the test proceeds with the serial application of different concentrations at intervals of 15 to 60 minutes (depending on the allergen and the patient's sensitivity). Although a single dose of allergen is applied in some research studies, some authors consider that the application of a single dose does not provide more information than a skin test for routine clinical diagnosis. The serial application of different concentrations is also recommended as a way of evaluating the dose-response relationship and the patient's sensitivity to the allergen, which is

useful for assessing the evolution of sensitization over time and for evaluating possible modifications after specific therapy.

The patient should remain seated and hold his or her breath during application in order to prevent the allergen from entering the larynx and lower respiratory tract. Nasal response can be assessed every 15-30 minutes after application, although the possible occurrence of a delayed reaction with new symptoms hours after the test concludes should also be taken into consideration. The patient must be kept under observation for 2 hours and should be informed that symptoms may appear later at home. Measures should be taken to ensure that the patient has treatment for any eventual symptoms.

Baseline forced spirometry is recommended at the beginning and end of NPT, even for nonasthmatic patients.

In order to avoid the priming effect between several NPTs, a minimum interval of 1 week must be left between tests. Testing of only 1 allergen per day is advised.

The main causes of false-positive results are as follows: high allergen concentration; infectious or allergic process in the previous 2-4 weeks; extract pH, temperature, and osmolarity; and excipients, such as phenol, glycerol, or benzalkonium chloride. False-negative NPT results may be due to the use of contraindicated drugs, nasal surgery in the previous 8 weeks, atrophic rhinitis, and specific immunotherapy.

6. Assessment of Nasal Response

In many publications, the interpretation of the response to NPT is based exclusively on the symptom score (rhinorrhea, obstruction, itching, and sneezing). An arbitrary semiquantitative score is assigned to each symptom, and a minimum sum is set for a response to be considered positive. However, since symptoms are a subjective criterion, many authors think that the assessment of symptoms should be accompanied by more objective measurements. Several techniques are available for assessing changes in nasal airflow resistance, patency, and nasal cavity geometry, as well as in parameters indicative of inflammation (eg, inflammatory mediator concentration, cytological variations). These techniques include the following:

• Changes in nasal airflow resistance/patency/geometry
– Measurement of nasal peak inspiratory flow (PIFn)
– Measurement of nasal airflow and resistance to airflow: rhinomanometry
– Measurement of the nasal surface area: acoustic rhinometry

• Changes in parameters indicative of inflammation
– Cytological changes, inflammatory mediator concentrations
– Changes in the blood flow, temperature, and pH of the nasal mucosa: optical rhinometry

The tools used to assess nasal response to allergen-specific NPT and their advantages and disadvantages are discussed below.

6.1 Clinical Examination

- Anterior rhinoscopy: inspection of the mucosa after NPT and observation of mucosal variations with respect to the previous examination (eg, appearance, edema, rhinorrhea). This evidently simple method can be carried out by any professional, but the assessment is highly subjective

and varies greatly depending on the observer. Considerable variability is also observed in repeated observations from the same investigator.

- Quantification of the weight and volume of the nasal secretions: Although more objective than simple anterior rhinoscopy, in clinical practice, interpretation can be difficult if the fluid is highly viscous or has been partially swallowed, or if the volume of the secretions is small [11]. It can be considered to be a rough technique that provides only partial information and is somewhat laborious.

6.2 Clinical Symptoms Score

This score is based on a visual analog scale (mild response, 1-3 cm; moderate response, 4-7 cm; intense response, 8-10 cm) [21] or on a score for sneezing, rhinorrhea, itching in different areas (nasal, ocular, velopalatal), and nasal obstruction. Clinical assessments by scoring are inherently semiquantitative and subjective. Several published scales exist (Tables 2 and 3) [22,23].

Table 2. Lebel Symptom Score Scale (J Allergy Clin Immunol. 1988;82 [5 Pt 1]:869-77)

Symptoms	Severity	Score (Points)
Sneezes	0-2	0
	3-4	1
	≥5	3
Itchiness	Nose	1
	Ear or palate	1
Rhinorrhea	Anterior	1
	Posterior	1
Nasal obstruction	Breathing difficulty	1
	1 nasal cavity	2
	2 nasal cavities	3
Ocular symptoms		1

Positive if ≥5 (maximum possible score 11 points).

Table 3. Linder Symptom Score Scale (Clin Allergy. 1988;18:29-37)

Symptoms	Severity	Score (Points)
Sneezes	0-2	0
	3-4	1
	≥5	3
Itchiness	Nose, palate, ear 1 point each	
Rhinorrhea		0-3
Nasal obstruction		0-3
Ocular symptoms		1

Positive if ≥5 (maximum possible score 13 points).

6.3 Assessment of Nasal Airflow: Measurement of PIFn

Nasal peak expiratory flow (PEFn) and PIFn are techniques for measuring nasal resistance to airflow. PEFn is used less often due to the obvious drawback of potential contamination by secretions. Airflow is measured using a specially adapted peak flow meter. The technique is easy to perform and inexpensive, but less exact than rhinomanometry in evaluating NPT results.

The studies by Holmström et al [24] and Jones et al [25] demonstrate that PIFn values correlate with airway resistance and that PIFn is as good an indicator of objective nasal obstruction as active anterior rhinomanometry. A good correlation has also been demonstrated between PIFn and the subjective sensation of nasal obstruction [26].

PIFn is useful in the follow-up of NPT and in the long term evaluation of nasal response, for example, to drug therapy [27,28]. The use of PIFn may be difficult in cases of intense rhinorrhea. The main disadvantage of PIFn is that it is partially dependent on lung capacity [29], which can affect reproducibility. This is important in patients with asthma or associated positive bronchial response.

6.4 Assessment of Nasal Airflow Resistance: Rhinomanometry

Rhinomanometry is used to assess nasal resistance by measuring airflow (cm3/s) at specific pressures (100/150/300 Pa). It calculates the difference between external pressure and pressure in the nasal choana by means of a pressure gauge and the flow rate per time unit between 2 points. Rhinomanometry can be either anterior or posterior, depending on the placement of the measurement instruments, and either active or passive, depending on whether the measurement is performed with the patient breathing or holding his/her breath.

In 1984, the Committee on Standardization of Rhinomanometry [30] recommended active anterior rhinomanometry (AAR) as an easy-to-execute, physiological, and reproducible technique.

In AAR, the nasal cavity where the pressure is measured is sealed (eg, adhesive tape, nozzle) and a cannula connected to a pressure gauge is introduced. Airflow through a mask fitted to the face is measured in the contralateral nasal cavity. AAR evaluates each nasal cavity separately. The readings are represented on mirror-image coordinate axes in which flow is shown on the y-axis and pressure on the x-axis. Airflow is measured at a specific pressure (generally 150 Pa) and resistance is calculated with the equation $r=\Delta p/v$.

The technique is sensitive and highly specific, but cannot be used in cases of perforated septum, intense rhinorrhea, or nasal obstruction [31]. In addition, the patient's cooperation is necessary, and this can be complicated in certain age groups.

6.5 Assessment of Changes in Nasal Cavity Geometry: Acoustic Rhinometry

Described by Hilberg et al in 1989 and Hilberg in 2002 [32,33], acoustic rhinometry is a noninvasive technique for studying the geometry of the nasal cavity.

It consists of the measurement of cross-sectional areas of the nasal cavity in relation to the distance of the section from the nostril. The physical principle is based on reflection of a continuous or pulsed sound wave. The incident wave is compared to the reflected wave, and the time interval between the 2 waves and the speed of sound is used to calculate the distance from the nostril at which a given cross-sectional area is found and the changes that occur in this area.

The standardization committee of the International Rhinologic Society has prepared a user's guide for this technique with instrument specifications [10].

The recording of the cross-sectional areas in relation to the distance from the nostril can be depicted on a linear or logarithmic scale.

Three notches are visible, as follows:

- C1, or the I-notch (isthmus) corresponds to the ostium internum and is located approximately 1.3 cm from the nostril.
- C2, or the C-notch (conchal notch), corresponds to the head of the inferior turbinate and is located 2-3 cm from the nostril.
- C3, or the third notch, corresponds to the head of the middle turbinate and is located about 4-6 cm from the nostril.

The 2 main parameters assessed using the data compiled are the minimum cross-sectional area (MCA) and the volume of the first 5-6 cm of the nasal cavity, since nasal volume measurements beyond 6 cm are affected by the openings of the paranasal sinuses, mainly the maxillary sinus [34]. In a population of normal subjects [35], the MCA was found at the level of the I-notch in 42% of the population and at the C-notch in 58% (mean 0.68 ± 0.13 cm^2).

No absolute values of normality exist. Sex, age, and height have no influence, but cranial circumference and race (black more than Asian and Asian more than white) affect acoustic rhinometry measurements [36]. Measurements are made using special anatomic nosepieces with a 60° cutting angle. These nosepieces are available in several sizes and are specific for each nasal cavity (left/right). A gel or sealant is applied around the nosepiece to prevent leakage between the nosepiece and nostril. The patient should remain in the place where the measurement is going to be made for about 30 minutes to acclimatize before the test starts. It is also important to control noise levels (<60 db), ambient temperature (24ºC-26ºC), and humidity (45%), which should be constant.

The patient is asked to hold his/her breath and the tube with the nosepiece is sealed with petroleum jelly around the nostril without deforming the nostril. Several quick measurements are made. Erroneous curves are rejected, and the mean of the selected curves is taken as the result.

Of all the factors that can affect readings, the training of personnel and the use of gel to seal the nosepiece have the greatest impact on the speed and precision of the technique [37].

When assessing changes in NPT, a nasal cavity volume between 2 cm and 6 cm is the most important parameter, because it corresponds to the head of the turbinate. A nasal cavity volume between 6 cm and 10 cm provides information about the sinuses and ostia. The intrinsic bias of the nasal cycle should not be overlooked; consequently, the cross-sectional areas and volumes of the nasal cavities should be measured after NPT [38].

Acoustic rhinometry is easy to perform and reproducible. It requires little cooperation from the patient, which makes it very useful for children, and it is not affected by the presence of rhinorrhea or intense nasal obstruction. However, it cannot be applied in cases of septal perforation.

6.6 Assessment of the Inflammatory Response

Several methods allow us to evaluate the inflammatory changes that take place in the nasal

mucosa after NPT with allergens. Nasal irrigation, nasal brushing, and nasal biopsy allow the cells and mediators that participate in the allergic response to be characterized. In addition, determination of NOn provides an indirect measure of the inflammation of the nasal mucosa.

• Nasal irrigation

Nasal irrigation is a relatively simple technique that has frequently been used in research studies. It provides information on activity in the lumen of the nasal airway.

The data recorded reflect the processes taking place in the underlying tissues.

The technique for nasal irrigation was initially described by Naclerio et al [39]. The patient's head is flexed 30°-45° backwards and 2.5 mL to 5 mL of saline solution preheated to 37°C is instilled in a nasal cavity. The patient is instructed to keep the palatal velum closed and not to breathe or swallow. After 10 seconds, the fluid is collected and the maneuver is repeated in the other nasal cavity. Some patients are incapable of retaining fluid in the nasal cavity, so other techniques have been developed, such as the use of a nasal device that releases saline solution into the nasal cavity when pressed and allows the liquid to be recovered when pressing stops [40].

Nasal irrigation allows cell analysis (total count and percentage of eosinophils, basophils, monocytes, and neutrophils) [41,42], and quantification of the concentration of and variations in mediators such as histamine, tryptase, eosinophil cationic protein, leukotrienes (LTC4, LTB4), myeloperoxidase, interleukin (IL) 5, prostaglandin D2 (PGD2).

After NPT with an allergen, histamine levels in the nasal irrigation fluid increase, reaching peak concentrations about 10 minutes after NPT and returning to baseline levels in 5-10 minutes. The potential delayed response can appear up to 3-11 hours after provocation.

Together with increases in histamine, tryptase, and PGD2, increases in LTB4 and LTC4 concentrations are also detected soon after NPT, together with itchiness, sneezing, and rhinorrhea [39].

Allergen-specific NPT is accompanied by an increase in the eosinophil levels of the nasal irrigation fluid 30 to 60 minutes after the provocation, with a second peak at 6 to 10 hours that may persist for up to 24 hours. Increased eosinophil cationic protein and eosinophil peroxidase concentrations are detected [41].

Study of nasal irrigation fluid has also made it possible to demonstrate local production of specific IgE and inflammatory changes after a positive NPT with allergen in patients diagnosed with nonallergic rhinitis based on negative skin-prick tests and negative specific IgE in serum [43].

• Nasal brushing

Nasal brushing is usually performed at the level of the middle third of the inferior turbinate. The main advantages of nasal brushing are minimal trauma with no need for anesthesia, reproducibility, good sample specificity, relatively easy sampling, and lower cost than nasal biopsy. Nasal brushing also has disadvantages: interpretation depends on the sample collection technique and processing, as well as on the experience of the analyst. In addition, samples provide information on superficial cellular changes in the nasal mucosa, but not on deeper tissues.

• Nasal biopsy

Nasal biopsy is usually performed on the lower part of the inferior turbinate. The main disadvantage of the technique is that it is slightly traumatic and cannot be performed serially. Its main advantage is that it enables the examination not only of the superficial epithelium, but also of the basement membrane and submucosa. Recommendations on sample processing were published in 2003 [44]. Nasal brushing seems to detect eosinophils in the nasal mucosa before nasal biopsy–including the first half hour after NPT–and is more easily performed [45]. According to some authors [46-49], the variation in the proportion of eosinophils in nasal secretions is the best discriminator between a positive and a negative response to NPT; however, other cell types, such as neutrophils, basophils, and mucosal and epithelial cells, also undergo quantitative changes after NPT. In published studies of occupational rhinitis, a variation of 4% to 5% in the eosinophil proportion has been suggested as the cutoff point for considering NPT as positive [50,51]. It should be noted that the cell profile detected after NPT differs from that seen after natural exposure to the allergen. For instance, a local increase in neutrophils and CD4+ and CD25+ T lymphocytes is only detected after NPT; on the other hand, mastocyte migration to the nasal epithelium occurs during natural pollen exposure, but not after NPT [42].

• Assessment of NOn

Although nitric oxide was initially described as a vasodilator agent synthesized by the endothelium, it is known to participate in diverse cellular and tissular functions [52,53]. Gustaffson et al [54] first described the presence of nitric oxide in exhaled air, and Alving et al [55] reported that nitric oxide is found in much higher concentrations in the nasal cavity than in the lung. NOn is produced mainly in the paranasal sinuses [56], and levels range from 200 ppb to 2000 ppb.

Levels are characteristically low in diseases such as primary ciliary dyskinesia and cystic fibrosis, and assessment of NOn has been proposed as a diagnostic tool in screening for primary ciliary dyskinesia [57]. Levels of NOn are also lower in rhinitis with nasosinusal polyps than in rhinitis without polyps; the reduction in NOn is proportional to the size and number of polyps [58-60].

Several studies have demonstrated that NOn levels are high in patients with allergic rhinitis [61,62]. The rise in NOn levels could be due to an increase in the expression of inducible NOn synthase enzyme (iNOS) [63].

However, in other types of rhinitis, both allergic and nonallergic, disparate data have been found on the correlation between NOn levels and parameters such as the degree of inflammation, association or not with asthma, symptom intensity, and, in the case of allergic rhinitis, variations in NOn with respect to allergen exposure, whether natural or after allergen-specific NPT.

NOn has been measured after nasal provocation with allergen in several studies, including a study by Kharitonov et al [61], in which 5 patients with pollen-induced allergic rhinitis showed a decrease in NOn levels with NPT that coincided with maximal symptom intensity; within 4 hours NOn levels had returned to baseline.

In 2007, Boot et al [64] studied 20 patients with allergic rhinitis in whom serial NOn measurements were made after nasal provocation. Compared to placebo, NPT with an allergen produced a decrease in NOn at 20 minutes; at 7 hours, NOn showed a tendency to rise, which was significant 24 hours after provocation. The initial decrease in NOn is attributed to mucosal edema, which would reduce diffusion of nitric oxide from the paranasal sinuses, as occurs in nasal polyposis. Neither antihistamines nor antileukotrienes appear to modify NOn levels, whereas the use of topical corticosteroids reduces levels by iNOS downregulation [61,65].

Consequently, although NOn shows promise as a diagnostic and noninvasive management tool, its value in nasal pathology is still not clear, mainly due to the lack of standardization of the test. Different methods of measurement have been used in published studies and the results reported are not comparable.

Recommendations for measurement of nitric oxide in exhaled and nasal air were published in 2005 (ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide) [66].

6.7 Optical Rhinometry

Optical rhinometry is a spectroscopic technique for assessing edema of the nasal mucosa by measuring the changes that edema produces in blood flow and light absorption [67- 69].

An 800-nm light emitter and detector are placed on each side of the nose and the extinction of the light as it crosses the nasal tissue is measured in real time. If blood flow increases, more light will be absorbed by hemoglobin and less light will be detected by the detector.

Hemoglobin absorbs light in the near infrared range, and both blood flow volume and hemoglobin saturation influence absorption. The wavelength used in optical rhinometry (800 nm) corresponds to the isosbestic point of hemoglobin, at which the coefficients of absorption of saturated and unsaturated hemoglobin coincide and absorption is independent of saturation [70].

The patient is asked to stay still and breathe normally while a 2-minute baseline recording is made; no mask is required. The increase in optical density is a quantitative measurement of mucosal edema; a variation of 0.2 OD indicates edema of the mucosa [69].

In 2007, Wüstenberg et al [71] published a study in which NPT was carried out with allergen, xylometazoline, histamine, and saline solution in 70 patients, and the results of optical rhinometry were compared with those of active anterior rhinometry. The authors concluded that optical rhinometry had a better correlation with the sensation of congestion than active anterior rhinometry and was less uncomfortable for the patient.

In summary, optical rhinometry appears to be a promising technique, although few data are available to support this impression.

6.8 Other Techniques

Other techniques, such as the study of microcirculation using Doppler ultrasound, irrigation with xenon radioisotope and hydrogen, and mucosal colorimetry, have been used for experimental purposes [72,73].

7. Positivity Criteria in Nasal Provocation

In many published studies, NPT positivity is established using only the symptom score. We believe that symptoms alone are insufficient and that the symptom score should be combined with a technique that provides a more objective measure of the changes that take place after NPT.

- Some of the scales most often used to assess symptoms are listed in Tables 2 and 3. Positivity criteria are given in these tables.
- A fall in PIFn of ≥40% post-NPT is accepted as positive [74].
- In rhinomanometry, NPT is accepted as positive when airflow resistance increases by

100% [19].

- Generally speaking, in acoustic rhinometry, NPT is considered positive when MCA and nasal cavity volume 2 cm to 6 cm from the nostril vary by 25%-30%, although data vary from one study to another [75].

Diverse combinations of symptom scores with a percentage decrease in airflow rate (PIFn, rhinomanometry) or nasal cavity cross-sectional area (acoustic rhinometry) and/or an increase in nasal secretions or inflammatory parameters are proposed in almost all published articles.

In a study published in 2005, Gosepath et al [13] considered NPT to be positive when there is a 40% reduction in airflow at 150 Pa in active anterior rhinomanometry, regardless of the symptom score, or when there is a 20% reduction in airflow at 150 Pa with a symptom score of more than 2 (according to the scale proposed by the ENT Section of the German Society for Allergology and Clinical Immunology) [76].

Rondon et al [43] performed NPT with *Dermatophagoides pteronyssinus* in patients with chronic rhinitis and categorized as positive any NPT resulting in a 30% increase in the symptom score using a visual analog scale and a 30% reduction in nasal cavity volume by acoustic rhinometry.

Other authors consider the amount of secretion produced as an important parameter, although it can be difficult to collect and quantify. Wihl [77] considers NPT to be positive if 0.5 mL (0.5 g) of nasal secretion with 5 or more sneezes and a >20% reduction in PIFn are produced. Hytonen et al [78] propose 0.1 g nasal secretion in the first 30 minutes as the threshold value for considering unilateral NPT as positive.

Pirila and Nuutinen [79] studied NPTs in 33 cow milk– allergic patients in whom measurements were made of nasal secretion, airflow resistance (active anterior rhinometry), and variation in MCA (acoustic rhinometry). Using these parameters, the authors determined that NPT is positive when the following criteria are met: 30 minutes after NPT, 100 mg of nasal secretion with a 15% decrease in MCA and 50% increase in nasal airflow resistance; 60 minutes after NPT, 210 mg of nasal secretion with a 30% decrease in MCA and 100% increase in nasal airflow resistance.

Ganslmayer et al [80] conducted NPTs with grass pollen in 30 patients and established a 29% decrease in MCA and 26% decrease in PIFn as the cutoff points for considering NPT to be positive with 100% specificity.

8. Approach After Allergen-Specific NPT

If NPT is positive, abundant nasal irrigations are prescribed. A topical nasal decongestant and topical or systemic antihistamine should be administered as dictated by the intensity of the symptoms. Systemic reactions are treated according to the usual guidelines.

Some authors suggest that if NPT with an allergen is positive, NPT with placebo should be performed afterwards. This practice is mandatory in NPTs conducted in the context of investigational protocols.

Finally, the reappearance of nasal symptoms, especially obstruction, 3 to 12 hours after NPT should be interpreted as a delayed reaction. The patient should be advised of this eventuality and measures should be taken to ensure that suitable treatment is available to control symptoms at home. The criteria for assessing a delayed nasal response are not as well established as for bronchial response. While the immediate response is easy to demonstrate, the symptom score is

not sufficient in the case of a delayed reaction. It would be advisable to monitor nasal airflow resistance by active anterior rhinometry or at least by PIFn [24]. However, the influence of the nasal cycle on the interpretation of results must also be taken into account [81].

9. Recommendations of the Spanish Society of Allergy and Clinical Immunology Rhinoconjunctivitis Committee for Conducting NPT With Allergens

In conclusion, after reviewing the literature on NPT with allergens, the Rhinoconjunctivitis Committee of the Spanish Society of Allergy and Immunology makes the following recommendations for NPT:

1. Allergen application: bilateral

2. Method of application: Use a micropipette to deposit the allergen solution on the head of the inferior turbinate while the patient holds his/her breath.

3. Amount deposited in each nasal cavity: 100 µL.

4. Start with a concentration of 1/1000 of the concentration that elicits a positive skin prick test result (or a concentration of 1/10 000 in the case of nonstandardized allergens).

5. Apply the diluent before applying the allergen to evaluate nasal hypersensitivity. The nasal response to the diluent is considered to be anomalous in the following cases:

- The symptom score increases by ≥3 points.
- Acoustic rhinometry reveals a ≥10% reduction in MCA, nasal volume (first 2 cm-6 cm), or both.
- Active anterior rhinomanometry reveals a 20% increase in total nasal airway resistance or a decrease of 20% in total nasal airflow at 150 Pa.
- Nasal peak inspiratory flow decreases by 15%.

6. NPT monitoring (evaluation 15 minutes after allergen application). Use of a combination of the symptom score [22,23] and an objective evaluation of nasal obstruction is recommended. Techniques are ranked from the first preference: *1)* acoustic rhinometry, *2)* active anterior rhinomanometry, *3)* PIFn.

7. NPT positivity criteria. NPT is considered to be positive when the positivity criteria of an objective evaluation of nasal obstruction are satisfied, although the clinical criteria can be included by adding the symptom score to the objective evaluation.

- Symptom score [22,23]: increase in the symptom score of ≥5 points.
- Evaluation of nasal obstruction (techniques ranked from the first preference down): *1)* Acoustic rhinometry (25% reduction in the minimal crosssectional area of the nasal cavity or in the volume of the nasal cavity 2 to 6 cm from the nostril); *2)* Active anterior rhinomanometry (100% increase in total airway resistance/air flow at 150 Pa); *3)* Nasal peak inspiratory flow (≥40% reduction in airflow)

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