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Nasal Provocation Test in the Diagnosis of Allergic Rhinitis

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1. Introduction

The specific provocation tests, since its introduction by Blackley in 1853, have been widely used in the investigation of pathophysiological mechanisms, immunological and therapeutic aspects of allergic disease, as they mimic the response to allergen exposure, under controlled conditions. However, it has not been broadly used in the diagnosis of allergic disease in clinical practice, because of the lack of standardization of the methodology and the need of other complementary diagnostic tests for monitoring specific provocation tests. Nevertheless, the importance of such test is enormous in many circumstances, since it is the only method that can establish the exact etiology of allergic disease. Although the usefulness of these tests has not been questioned, the need to standardize the methodology for monitoring the response has been stressed. In this review, these aspects will be discussed.

2. Allergic rhinitis

Rhinitis is generally subdivided into two groups: allergic and non-allergic. It has been estimated that allergic rhinitis has a high prevalence in the general population (5 to 20%), and non-allergic rhinitis alone is thought to affect more than 200 million people worldwide. So, this is a very common but under diagnosed disease. The correct diagnosis has an enormous impact in public health, since it would involve several health and economic benefits (Bousquet & ARIA Workshop Group, 2001).

Allergic rhinitis is an IgE mediated inflammatory chronic disease affecting nasal mucosa, characterized by the presence of itching, rhinorrea, sneezing and congestion (Bousquet & ARIA Workshop Group, 2001). The diagnosis of allergic rhinitis is based mostly in clinical evidence. In fact, a positive correlation between the clinical history and the allergen sensitization is usually enough to support the diagnosis of allergic rhinitis and its aetiology. However, in some circumstances (table 1), additional approaches are required to reach a correct diagnosis in allergic rhinitis patients, namely nasal provocation test (NPT). Indeed, the specific NPT is the method of choice for the reproducibility of the allergic reaction, and it is indicated when discrepancies arise in the assessment of a patient's medical history and the results of skin and/or serological tests, as reviewed by several authors (Litvyakova LI & Baraniuk JN. 2001; Loureiro, 2001; Dordal et al, 2011; Mellilo, 1997; Naclerio & Norman, 1998).

Clinical practice	• Multissensitized patients • Local allergic rhinitis • Occupational allergic rhinitis • Correlation between allergy and other morbidities
Investigational research	• Mechanisms of allergic reaction • Mechanisms of immunotherapy • Efficacy of new treatments

Table 1. Indications for NPT: clarifying the pathogenesis and diagnostic evidence, in particular situations of allergic rhinitis

2.1 Multissensitized patients

Atopic patients are frequently sensitized to multiple allergens. In some circumstances, clinical history is not clearly related to allergen specific IgE. A NPT could be performed to differentiate the relevant allergenic aetiology in multissensitized patients, since these patients need specific therapeutic approaches.

2.2 Local allergic rhinitis

Patients with allergic rhinitis have allergen-specific IgE demonstrable both systemically as well as local IgE produced in the nasal mucosa. On the other hand, the concept of non allergic rhinitis is supported by negative skin tests. However, in a subset of patients who have positive NPT to allergens despite having a negative skin prick test, it has been hypothesized that these patients have localized allergic rhinitis. Huggins made the first description of local allergic rhinitis (Huggins & Brostoff J, 1975). Recently, several studies have strengthened the existence of this allergic disorder and the immunological mechanisms involved in the immediate and late responses to NPT have been described (Kim & Jang, 2010; López S et al, 2010; Rondón et al, 2007, 2009, 2010a, 2010b). A type 2 helper T cell inflammatory pattern in nasal secretions in response to allergen exposure was demonstrated. Accordingly, local production of IgE and mast cell / eosinophil activation with its inflammatory mediators was also founded in these patients. These findings support the hypothesis of a localized inflammatory response and the concept of local allergic rhinitis. As discussed, local allergic rhinitis involves nasal production of specific IgE in the absence of atopy. Evidence of this entity is supported by suggestive clinical symptoms and a positive NPT. So it is a useful tool for detecting patients with local allergic rhinitis in previously diagnosed idiopathic / non-allergic rhinitis patients, as defended by several authors and evidenced by our group (Loureiro et al, 2011). In our experience, the specific NPT reproduced the clinical manifestations in some patients, supporting the concept of local allergic rhinitis in a subset of patients with perennial rhinitis. We studied 15 patients with an average age of 22.2±14.8 years (77.7% were female) with typical clinical symptoms of perennial rhinitis, negative skin prick test to common aeroallergens and negative specific IgE. The period of symptoms evolution was 5.37±3.9 years. A *Dermatophagoides* specific NPT (BialAristegui, Bilbao, Spain) was performed with clinical monitoring. Total nasal symptom scores were assessed using a validated questionnaire and a positive NPT was considered if a score of 5 or greater was recorded (Linder, 1988). The NPT was considered positive in 8 patients. Several studies proved that house dust mites could have a pro-inflammatory activity independent of IgE (Fujisawa et al, 2008; Gregory et al, 2009; Hammad et al, 2009; Wong et al, 2006). This fact could explain the positive result in NPT, in our study however,

all patients were negative to a non-specific NPT. Despite the few number of patients included, our data highlight the need for the most complete diagnostic approach. The correct differential diagnosis with non-allergic rhinitis is crucial for therapeutic purposes, since some of these misdiagnosed patients may benefit of specific immunotherapy. Indeed, in our findings, all the patients with the diagnosis of local allergic rhinitis were submitted to specific immunotherapy, with clinical improvement (data not published).

Because the concept of local allergic rhinitis is based in positive NPT, some authors emphasize the need to standardize this procedure to better understand its usefulness in the diagnostic approach of this new entity. It has still controversial aspects to be defined, as discussed by some authors (Alvares & Khan, 2011; Khan 2009). In a review of the studies that evaluated patients with negative skin tests using NPT, these authors argued that several aspects could explain the different data in the literature. For instance, the prevalence ranges from 0% to 100% of skin test negative individuals. This wide range in prevalence could be explained by the differences in methodology (allergen manufacturers, concentrations, and numbers of allergens tested) and, perhaps most importantly, criteria for a positive nasal challenge. In another review of the literature, the concept of entopy was also considered controversial (Forester & Calabria, 2010). In spite of this, they recognize that there are a large number of non-allergic rhinitis patients for whom current treatment regimens are suboptimal, considering the need to better understand the subjacent immunological mechanisms to achieve an optimal diagnosis and treatment in this subset of patients.

2.3 Occupational allergic rhinitis

The occupational exposure to immunogenic substances, such as chemicals and biologic products is enormous in the workplace, since it is the place were people spend more time. Despite an increasing estimated prevalence of 5 to 15%, occupational allergic diseases, namely occupational rhinitis it is still underestimated. Several factors are pointed, including the worker reluctance to complain and the failure to diagnose. More than 400 substances have been implicated as cause of occupational respiratory allergy. It is recognized that exposure to these substances can result in increased nasal hyperreactivity and can predispose to occupational rhinitis. It presents a major impact in quality of life, as well in professional performance. Further the legal impact, a correct etiologic identification in occupational allergic rhinitis as an enormous impact in the natural history of this disease. Indeed it is assumed that occupational rhinitis coexists and it may precede occupational asthma. Despite this, occupational asthma has been better evaluated than occupational rhinitis, both in epidemiological and physiopathological approaches.

The real incidence and prevalence of occupational disease is not known. Occupational disease has been recognized by physicians and epidemiologists. However, there are a few publications about occupational rhinitis. NPT is an fundamental diagnostic approach of occupational Allergic Rhinitis (Loureiro, 2008).

New allergens (high molecular weight as well as low molecular weight agents) are continuously being described in occupational asthma and/or rhinitis. Standardized extracts for skin testing are not available. A complementary diagnostic approach in occupational rhinitis, to better recognise and early diagnose this disease, includes specific NPT with clinical and functional monitoring. In fact, NPT is the ideal methodology to confirm or refute the diagnosis of occupational allergic rhinitis because it confirms the clinical symptoms and its causality. For instance, using NPT our group could reached the correct

etiologic diagnosis of the first case of fungal lipase allergy in a patient not sensitized to amylase working in the pharmaceutical industry (Loureiro, 2009). It has well known that occupational allergy to lipase has been reported in the detergent industry (Brant et al, 2004, 2006; Lindstedt et al, 2005; van Kampen et al, 2000). While the main allergenic enzyme in the pharmaceutical industry is amylase, there have been reports of lipase sensitization, albeit without clinical relevance (Park et al, 2002; Zentner et al, 1997). The NPT was the supporting approach methodology to obtain this diagnosis confirmation, in our patient. Several cases of occupational allergic rhinitis are described in the literature, based directly on positive NPT, both in confirming the diagnosis and the etiological identification. The NPT reproduces the nasal symptoms and can be performed on the workplace, or under controlled conditions in hospital environment (Gosepath et al, 2005; Hytonen & Sala 1996; Hytonen et al, 1997; Litvyakova & Baraniuk, 2001; Loureiro, 2008). In a relevant study, 507 NPT were performed in 165 patients and the authors concluded that NPT is an essential, easy and safe tool in the diagnosis of allergic occupational rhinitis (Airaksinen et al, 2007). Recently, there has been a growing scientific interest in work-related rhinitis, because the relationship to asthma has been evaluated (Vandenplas, 2010). Considerations of the epidemiology of work-related rhinitis (both occupational rhinitis and work-exacerbated rhinitis) and its medico-legal implications have stressed the need to better identify this entity. Recent consensus have been presented to better define, classify and diagnosis occupational rhinitis, emphasizing the importance of NPT (EAACI Task Force on Occupational Rhinitis, 2008; Moscato et al, 2011; Dordal et al, 2011).

2.4 Investigational research

The applicability of NPT on investigational research is widely described in the literature, namely in the study of several aspects of allergic disease, namely the mechanisms of allergic reaction, the mechanisms of immunotherapy, the efficacy of new treatments and also in the study of the link between allergy and other morbidities, namely ENT diseases.

In a prospective controlled study, the possible role of nasal allergy in chronic disease of the maxillary sinuses was evaluated using NPT combined with radiography and ultrasonography (Pelikan, 2009). It was concluded that nasal allergy might be involved in some patients with chronic sinusitis. In these patients the NPT was a useful diagnostic tool and allowed to achieve a better diagnostic of co-morbidity and, consequently, therapeutic measures.

Otitis media with effusion (OME) is a very prevalent disease, particularly in children. The OME pathogenesis is considered multifactorial, and it has been related to viral upper respiratory tract infection and eustachian tube disfunction. Allergy has been implicated in OME pathogenesis by several authors, but it is a matter still controversial. It has been assumed that there is insufficient evidence of therapeutic efficacy or a causal relationship between allergy and OME. For instance, 123 children with OME (and 141 controls) were submitted to NPT. The prevalence of the allergic rhinitis in children with OME did not differ significantly when compared to control subjects, and the abnormalities in Eustachian tube function were the same in both groups (Yeo et al, 2007). A recent review of literature pointed to a strong possibility of allergy as a risk factor for OME. Thus patients with allergic rhinitis should be evaluated for OME and patients with OME should be considered for an allergy evaluation. Allergy should be treated aggressively in these patients, because OME has important and severe sequelae (Lack et al, 2011; Skoner et al, 2009). Our group studied 34 children with diagnosis of adenoids hypertrophy with or without OME, with 7.60±1.76

years. They were submitted to skin prick test with common aeroallergens battery. 24 were sensitized to *Dermatophagoides pteronyssinus.* The link between allergy and OME was evaluated in each patient with *Dermatophagoides pteronyssinus* specific NPT (BialAristegui, Bilbao, Spain). The NPT was monitored using symptom scores and it was considered positive if a total score ≥ 5. The NPT was positive in 20.8% of the sensitized children. The therapeutic management of these patients included immunotherapy with clinical improvement, supporting the link between allergy and OME in a subset of patients.
Concerning investigational research in the yield of the allergic disease, NPT has been widely used to better understand the underlying mechanisms. Pereira C, 2009 showed that the cell response starts at an early stage, in parallel with the immediate allergic response. The IgE mediated response induces immunolymphatic involvement of the adjacent structures. This amplifies the allergic response to locoregional lymphoid organs, while leukocyte recirculation involves the primary lymphoid organs (thymus and bone marrow). These central organs are responsible for the systemic immune response induced by a focused allergen challenge, in this case, a nasal challenge.

3. Nasal provocation test

The NPT is an "*in vivo*" diagnostic method that mimics the allergen natural exposure. The allergic reaction is triggered by allergenic exposure, and symptoms are recorded. Although not standardized, it is an extremely helpful method as it has several important indications in the diagnosis of allergic rhinitis (table 1), as described above. Indeed, specific allergen challenge tests are still the gold standard for allergic diseases diagnosis, being an important tool to assess the treatment outcomes. Moreover, they have been essential in research, namely in the progressive understanding of the pathophysiology, immunology and pharmacotherapy of allergic diseases.
The first allergen challenge was performed in 1873, by Blackley, who elicited a nasal response after an application of fresh pollen to the membrane of his own nostrils (Blackley, 1873). After this first NPT, several consensus and guidelines have been published trying to achieve a better diagnostic approach of allergic disease and its knowledge (Dordal et al, 2011; Druce & Schumacher, 1990; Gosepath et al, 2005; Litvyakova & Baraniuk, 2001, 2002; Lund et al, 1994; Malm et al, 2000; Mellilo et al, 1997; Schumacher, 1992).

3.1 Methodology

The anterior section of the inferior turbinate allows direct and visible application of the allergen extract, with consequent allergic reaction development (Dordal et al, 2011; Litvyakova & Baraniuk, 2001; Melilo et al, 1997; Naclerio & Norman, 1998). Despite the availability of the published international consensus guidelines, several difficulties are described in the assessment of the technique standardization, namely the type of allergen extracts to be used (lyophilized, aqueous or paper disc), the dose of allergen (which defaults to increase the doses) and the technique of administration of allergen (drops, micropipette to extract volumes, paper disks impregnated with solutions, nebulized extracts). The NPT should only be performed after a pharmacological washout period, namely H1-antihistamines, benzodiazepines, corticosteroids and mastocyte stabilizers. It should be performed at least 4 weeks after an undercurrent infectious disease and avoidance of exercise. Room conditions of temperature and humidity must be fulfilled. Aqueous solution

and lyophilized powder are the most common commercial allergen extract presentations. In most studies it is administered unilaterally by various methods: spray (without propellant gas), instillation (pipette, dropper, syringe) or application of small pieces of cotton or paper discs with impregnated allergen. The use of different concentrations is recommended, therefore the dose-response could be evaluated and hence the real sensitivity to that allergen can be assessed. The starting dose for the NPT must be calculated from the minimum concentration used for skin prick tests that induces a wheal diameter of 3mm. Alternatively the initial concentration recommended could be 1 / 100 of the concentration that induced a positive skin prick test. After establishing the initial concentration, it should be scheduled a progressive increment of doses. All NPT should be initiated with the previous administration of saline, to evaluate a possible irritant effect. The interval between administrations of the allergen at different concentrations should be performed in 15 to 60 minutes. The terminus of the procedure occurs when there are symptoms of rhinitis or signs of mucosal inflammation. The application of the allergen must occur at the level of the previous section of the inferior turbinate, which is easily accessible, while the patient is asked a nasal expiration. The duration of expiration is not established, but the objective is to minimize bronchial inhalation. Several reviews in the literature analyse a variety of techniques and approaches, dosing and concentration of allergen extracts, delivery systems, and also the outcome-evaluation method (Dordal et al, 2011; Litvyakova & Baraniuk, 2001,2002; Tantilipikorn et al, 2010). In our experience we used commercial extracts prepared in an aqueous solution administered as a spray, directly to the anterior section of the inferior turbinate. The starting dose for the NPT was the concentration that induced a wheal diameter of 3mm in each patient.

3.2 Monitoring

The response to NPT is clinical and laboratory assessable. Several parameters could be used to evaluate the immediate and late allergic response, namely the symptoms score, the evaluation of nasal congestion (nasal Peak Inspiratory Flow Rate (nPIFR), rhinomanometry, acoustic rhinometry) and inflammatory cells / mediators analysis in nasal secretions, as reviewed in the published consensus. None of the methods that evaluate the response to NPT is standardized. In many publications the assessment of nasal response is based exclusively on symptom scores. Some authors suggested objective measurements together with symptom scoring. Thus, the response to NPT should be determined by the combination of symptom scores and / or rhinomanometry (Dordal et al, 2011; Litvyakova & Baraniuk, 2001).

3.2.1 Clinical symptom scores

Despite symptom scores is a qualitative and subjective method, it is the most used to evaluate the response to NPT, both in clinical practice and investigational research, since it mimics a spontaneous allergic response. To assess the nasal response to NPT, it could be used a score based on a visual analog scale (Bachert, 1997) or scales of semi-quantitative and subjective clinical assessments (Lebel et al, 1988; Linder, 1988). Usually our group uses the symptom scoring scaling according to Litvyakova & Baraniuk, 2001. Simple rating scales from 0 to 3 are used, for each nasal or non-nasal symptom, with defined criteria such as 0 = no symptoms, 1 = mild symptoms (symptoms that are present but not particularly bothersome), 2 = moderate symptoms (symptoms that are bothersome but do not interfere

with daily activities), and 3 = severe symptoms (symptoms that are bothersome and interfere with daily activities or disturb sleep). Even though the known individual variability and the variability between patients, several authors have been tried to standardized the symptom score. In all reports, symptom scores are compared with objective parameters, supporting the relevance of the use of the symptom score in the monitoring of NPT. For instance, 155 patients with allergic rhinitis to *Dermatophagoides* were submitted to NPT to evaluate the optimal cut-off values of symptom changes after NPT for predicting perennial allergic rhinitis, as well as the nPIFR evaluation (Chusakul et al, 2010). In another study, the symptom score change and acoustic rhinometry values were combined, before and after NPT in 208 patients with allergic rhinitis and in 222 controls (Kim & Jang, 2011).

3.2.2 Methods to evaluate nasal congestion: Nasal Peak Inspiratory flow rate, rhinomanometry and acoustic rhinometry

The assessment of nasal congestion could be evaluated by subjective parameters (symptom score) and by an objective and quantitative method. Several techniques are available for assessing changes in nasal airflow resistance, patency, and nasal cavity geometry. Such techniques provide objective measurement of nasal congestion, namely nPIFR, rhinomanometry and acoustic rhinometry. These methods provide an objective and quantitative measurement whose value is based on the comparison of results over procedures (diagnostic or therapeutic) in each individual. In spite of this, standardized methodologies assessing functional abnormalities are not sufficiently developed (Nathan et al, 2005). Comparison of results between different patients is not yet standardized. Recently, several studies have been tried to standardize these methods as they can be useful in clinical practice and applied as a diagnostic tool in allergic rhinitis (Chusakul et al, 2010; Kim & Jang, 2011).

Nasal Peak Inspiratory Flow Rate. This technique assesses the nasal airflow. It is easy to perform and inexpensive, but it is difficult to reproduce because is partially dependent on lung capacity (Wihl & Malm, 1988). Some studies demonstrated that nPIFR values correlate with airway resistance, but this is impracticable in case of intense rhinorrea (Holmstrom et al, 1990; Jones et al, 1991).

Rhinomanometry. This standardized technique measures the resistance to the airflow in nasal cavities (Schumacher, 1989). Increases in resistance in one or both nasal cavities have been considered as an objective parameter in positive responses to NPT (Clement, 1984; Kirerleri et al, 2006). The technique depends on patient cooperation and it cannot be used in cases of septum perforation, intense rhinorrhea or nasal obstruction (Nathan et al, 2005).

Acoustic rhinometry. This is a sound-based technique used to evaluate the nasal geometry, which measures nasal cavity area and volume. It has been validated by comparison to measurements with computed tomography and magnetic resonance imaging. It is used to diagnose and evaluate therapeutic responses in conditions such as rhinitis and to measure nasal dimensions during NPT (Keck et al, 2006; Kim et al, 2008; Uzzaman et al, 2006). Acoustic rhinometry is easy to perform and reproducible, but there are no reference values (Corey et al, 1998). It requires little cooperation from the patient, so it could be a useful method 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. Some interpretation caution should be made, when assessing changes in NPT. The nasal cavity volume between 2 cm and 6 cm is the most important parameter, because it corresponds to

the head of the turbinate, while the 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 both nasal cavities should be measured after NPT (Gotlib et al, 2005).
When comparing both techniques, acoustic rhinometry does not seem to be a better diagnostic method than active rhinomanometry in the monitoring of NPT (Keck et al, 2006).

3.2.3 Laboratorial measurements: Inflammatory allergic mediators and cells

The analysis of nasal cytology is essential to distinguish non-inflammatory from inflammatory nasal diseases. Additionally, the pattern of inflammatory mediators reflects the underlying immunological response. So the analysis of these inflammatory allergic mediators and cells are useful in the assessment of the response to NPT, namely in the diagnosis of allergic disease and in the evaluation of the treatment efficacy. Indeed, the NPT has been used to characterize and to clarify the immunological mechanisms involved in allergic reaction, and reciprocally, known inflammatory allergic mediators and cells have also been used to diagnose allergic rhinitis (for example local allergic rhinitis, as mentioned above) and to monitor the response to NPT. Allergic rhinitis is an allergen-induced IgE-mediated inflammatory disease of the nasal mucosa. Several inflammatory mediators (histamine, tryptase, ECP, leucotrienes, cytokines and chemokines) are involved and the cellular infiltrate is characterized of mast cells, basophils, eosinophils and T cells. The usefulness of nasal cytology depends on several factors, namely obtaining adequate specimens, appropriate samples staining, and the materials interpretation.

3.2.3.1 Methods to collect nasal samples

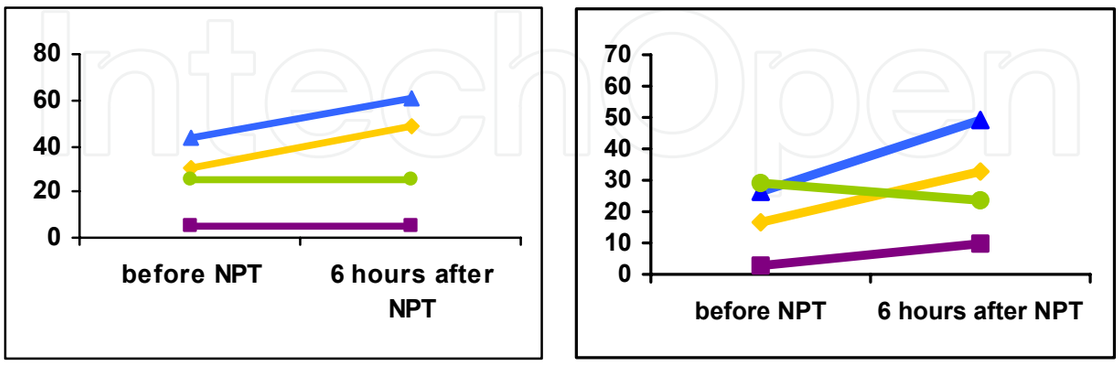
Various techniques have been used for obtaining, processing, evaluating, and interpreting nasal specimens. The different methods for collecting samples are nasal lavage, nasal swab, nasal brushing, nasal curettage, nasal biopsy and collection of nasal secretions, allowing the assay of cells and inflammatory mediators. Several comparative studies show the usefulness of these non-standardized different methods. Each technique has advantages and disadvantages, so the selection of each method must be carefully decided. Description of the different techniques was reviewed elsewhere, in detail. (Dordal et al, 2011; Howarth et al, 2005).
Nasal lavage. Naclerio first described this technique (Naclerio et al; 1983). This is a frequently used method to collect and to identify cells and inflammatory mediators. It has been used in research studies. In addition to nasal lavage, the collection of nasal secretions could be analyzed to look for both cellular and inflammatory mediators.
Nasal brushing and Nasal biopsy should be performed on the inferior turbinate, to obtain cellular samples. Nasal brushing is usually performed at the middle third of the inferior turbinate, with easy sampling of the superficial of nasal mucosa. Nasal biopsy is usually performed on the lower part of the inferior turbinate, requires anaesthesia, and reaches deeper tissues. However it cannot be systematically repeated because it is traumatic.

3.2.3.2 Difficulties in assessment of inflammatory response

These techniques helped to attain the actual knowledge about the characteristics of allergic disease. However, its usefulness in the evaluation of the response to NPT is restricted to research trials, in order to better understand immunological allergic mechanisms and effects of new therapies. In clinical practice, the assessment of these inflammatory parameters is not enough to evaluate the response to NPT.

In our Immunoallergy Department, we performed a study to evaluate the concentrations of the chemokines, eotaxin and RANTES, in nasal lavage and analyze the applicability of the determination of chemokines in nasal secretions as a parameter of immune response to specific nasal provocation test (Loureiro et al, 2003). We included 17 patients with allergic rhinitis to *Parietaria judaica* (64% male; 36.3±11.2 years old). All the patients were submitted to NPT with *Parietaria judaica* commercial extract (Leti, Madrid, Spain) outside the pollen season. Nasal lavages were performed, before, 30 minutes and 6 hours after NPT, for quantification of inflammatory mediators. NPT response was monitored through symptom score. The NPT was positive in all patients, reproducing the clinical reactivity to the allergen, with a peak in the symptom scores at the first minute with subsequent decreasing till the sixth hour. Eotaxin was not measurable in any of the nasal lavage specimens collected. The chemokine RANTES levels were 4.2±2.1pg/ ml before NPT and 3.96±0.98 pg/ml and 3.90±0.99 pg/ml in the specimens collected at 30 minutes and 6 hours after NPT respectively. These results did not correlate with symptoms progression during NPT. This could be interpreted as a discrepancy between the time of sampling and the dynamics of inflammatory mediators in response to NPT.

In the same group of patients, during the same procedure, we also analysed the tryptase and ECP levels, in nasal lavage, as immunological markers of immediate and late response in allergic reaction, respectively (Loureiro et al, 2004). Tryptase was detected in only three patients. Nasal brushings were also performed to harvest cells. Cellular phenotyping (CD3, CD4, CD8 and CD 125) was assayed by flow citometry, before and 6 hours after NPT, to recognize the cellular dynamics during NPT. Our findings showed an increase in CD3 and CD8 cells in all patients. In a subset of patients submitted to immunotherapy we observed a CD4 cells increase and a CD125 cells decrease, after NPT, while the other patients not submitted to immunotherapy does not showed any dynamic alterations in these cells (figure 1). The differences observed in each group could be explained by different therapeutic approaches in each group. However the dynamic cellular changes after NPT were not as expected. These findings could be explained by premature sampling before cellular trafficking occurred. Another possible explanation is the insufficiency of these sampling methods to harvest the sufficient cellular infiltrate.



(a) (b)

Fig. 1. Nasal cell typing before and after nasal provocation test (NPT) (% of cells): A - in a group of patients submitted to immunotherapy for one year; B - in a group of patients not submitted to immunotherapy (Legend: ▲ - CD3; ■ - CD4; ♦ -CD8; ● - CD125)

In another study conducted by our group, 21 allergic patients were submitted to *Dermatophagoides pteronyssinus* specific NPT (BialAristegui, Bilbao, Spain). Secretions, namely tears and nasal secretions, were collected after NPT and inflammatory mediators, such as interleukins and chemokines were measured (data not published). These inflammatory mediators were measurable only in 21% of the tear samples and in 71.5% of the nasal secretion samples. According to these findings, nasal secretions recovery could be acceptable to be considered as an objective tool in the evaluation of inflammatory mediators. However we could not find out a pattern of mediator release since the inflammatory mediators were inconsistently detected in the different samples.
Although the analysis of immunological parameters has been described as an objective approach to monitor the response to NPT, in our experience, these laboratorial measurements are difficult to perform because of the scheduling of sampling. Additionally, the cost-effectiveness of these procedures does not allow its implementation in the clinical practice. It should be reserved to investigational research.

3.2.4 Assessment on nasal Nitric Oxide

Determination of nasal Nitric Oxide (nNO) provides an indirect measure of the inflammation of the nasal mucosa. A decrease in nNO levels with NPT coincided with maximal symptom intensity, in five patients with pollen-induced allergic rhinitis (Kharitonov et al, 1997). Although nNO promises as a diagnostic non-invasive 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 (Dordal et al, 2011).

3.3 Criteria for positive NPT

Besides diverse combined criteria have been discussed in the literature none of them are standardized criteria to define NPT positivity. This is summarized in the Table 2.

4. NPT as a diagnostic approach in respiratory allergy

The first allergen provocation test was performed in 1873, by Blackley, who elicited a nasal response after an application of fresh pollen to the membrane of his own nostrils (Blackley, 1873). Currently, the indications of NPT are widely known. In this manuscript, the applicability of NPT as a diagnostic tool of allergic rhinitis was discussed. But the usefulness of NPT is not restricted to the diagnostic approach of allergic rhinitis. Supported by the concept of "*one airway, one disease*", several studies have pointed out that the NPT is a good alternative to Bronchial Provocation Test (BPT), even in the absence of nasal symptoms. In spite of BPT being a standardized diagnostic tool, it is not frequently used in clinical practice because of its technically and methodologically requirement. Indeed, NPT is safer and better tolerated

Reference	Assessment of nasal response	Description of positivity criteria
Hytonen et al, 1997	Symptom score	$\Delta \geq 4$, considering Δ = (obstruction score + rhinorrhea score) after NPT - (obstruction score + rhinorrhea score)
Lebel B et al, 1988	Symptom score	Lebel Symptom Score Scale: Positive if ≥ 5 (maximum possible score 11 points)

Reference	Assessment of nasal response	Description of positivity criteria
Linder A, 1988	Symptom score	Linder Symptom Score Scale: Positive if ≥ 5 (maximum possible score 13 points)
Terrien et al, 1999	nPIFR assessment	Fall in nPIFR ≥ 40%
Cimarra & Robledo, 2001	Rhinomanometry	Airflow resistance increases by 100%
Valero & Picado, 2000	Acoustic rhinometry	MCA and nasal cavity volume vary by 25-30%
Álvarez Eire et al, 2006	Combined symptom score and nPIFR	At least two of the following criteria: five sneezing, runny nose, nasal congestion documented by a decrease ≥ 20% of nPIFR
Gosepath et al, 2005	Combined symptom score and rhinomanometry	A 40% reduction in airflow at 150 Pa in active anterior rhinomanometry, regardless of the symptom score, or a 20% reduction of in airflow at 150 Pa with a symptom score of more than 2
Rondón C et al, 2007	Combined symptom score and acoustic rhinometry	a 30% increase in the symptom score using a visual analog scale and a 30% reduction in nasal cavity volume by acoustic rhinometry
Kim & Jang, 2011	Combined symptom score and acoustic rhinometry	1) symptom score change: more than 2 points in the case of nasal obstruction and more than 1 point for the case of rhinorrea or itching; 2) more than 24.5% change of total nasal volume and 3) more than 20% change of the minimal cross-sectional area.
Wihl, 1986	Combined nasal secretions amount and nPIFR	0.5 mL (0.5 g) of nasal secretion with 5 or more sneezes and a >20% reduction in nPIFR
Pirila & Nuutinen, 1998	Combined nasal secretions amount, rhinomanometry and acoustic rhinometry	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, 1999	Combined acoustic rhinometry and nPIFR	29% decrease in MCA and 26% decrease in nPIFR

Table 2. Some criteria do define NPT positivity, adapted from Dordal et al, 2011.

method in asthmatic patients than BPT (Hervás et al, 2011; Marcucci *et al*, 2007; Oddera *et al*, 1998). So, NPT has been used to the diagnosis of asthma, as reviewed by Olive Pérez, 1997. Thus, based on the *united airways disease* concept, the NPT could be considered as a model of specific provocation test that is easy and quick to perform, in the demonstration of the immediate and late phase response of type I hypersensitivity reaction. It is well known that the nose is an integral part of the upper airway, and anatomically related to several airway structures, such as ears and paranasal sinuses, and as well the eyes. There is an epidemiological relationship between rhinitis and asthma. Rhinitis and asthma are often associated, rhinitis typically precedes the development of asthma and can contribute to insufficient asthma control (Compalati et al, 2010). On the other hand, in cross-sectional and longitudinal studies, the vast majority of patients with asthma have rhinitis, and rhinitis is a major independent risk factor for asthma (Togias, 2003). Treating allergic rhinitis would probably ameliorate other associated upper airway diseases such as acute rhinosinusitis, nasal polyposis, adenoidal hypertrophy, and OME (Marple, 2010). In addition to improve allergic rhinitis outcome, the treatment of subjacent inflammatory disorder reduces asthma-associated health care consuming. A close interaction between the nose and contiguous or distant organs was described and it has been progressively clarified, supporting this epidemiological and clinical relation (Baroody, 2011). The upper and lower airways are not anatomically and functionally distinct areas (Slavin, 2008). It is currently established that the impaired function of the upper airways causing nasal obstruction, retention of secretions, and disturbed conditioning of the inspired air plays an important role in the development of lower airway symptoms (Virchow, 2005). There are important relationships between both the nose and the paranasal sinuses and asthma. Apart from the intrinsic physiological interaction, extensive evidence exists to sustain the concept that the respiratory system functions as an integrated unit (Krouse, 2008), where rhinitis and asthma are manifestations of one syndrome, the chronic allergic respiratory syndrome, in both parts of the respiratory tract (Togias, 2003). It has been described that parallel immunopathological processes involve the upper airway generally occur in conjunction with lower airway diseases, and diffuse inflammation often affects mucosal surfaces of the middle ear, nose, sinuses, and tracheobronchial tree simultaneously (Krouse, 2008). Recent studies show that the deposition of allergen into the lower respiratory tract leads to increased inflammation of the upper respiratory tract, even if the patients are only suffering from allergic rhinitis (Virchow, 2005). Additionally, studies indicate that treatment of the upper respiratory tract inflammation reduces the manifestation of allergen-associated symptoms in the lower respiratory tract, and also have preventive effects if started early on the disease evolution (Bousquet & ARIA Workshop Group, 2001). Both asthma and allergic rhinitis have now been recognized as inflammatory diseases with similar manifestations in the mucous membranes of the upper (nose and paranasal sinuses) and lower respiratory tract (Virchow, 2005). There is increasing evidence that even in patients with rhinitis who do not have asthma, sub-clinical changes in the lower airways and inflammatory mediators can be detected (Compalati et al, 2010). These and other findings support that allergic diseases have a systemic component (Virchow, 2005). The interactive mechanisms of allergic rhinitis and associated conditions highlights the relevance of a bidirectional "unified airway" respiratory inflammation model. Currently, it is accepted that IgE mediated allergic reactions are not confined to the area where the trigger occurred, inducing a secondary systemic immune response (Braunstahl, 2005, 2006; Togias, 2004). The systemic inflammation

is produced after local allergic reactions (Togias, 2003). The link between local exposure to allergen and distant response has been clarified. Although some authors defend that this systemic response could result from allergen entering in the systemic circulation from the local of exposure (Hens et al, 2007) this could activate circulating basophils, inducing an anaphylactic reaction, which is a rare condition (Togias, 2004). Both systemic cell circulation and the nervous system activation are two major ways through which local allergic reactions propagate. Mast cell mediators locally released, increase the expression of adhesion molecules on postcapillary venules. This can lead to homing of circulating leukocytes, which may infiltrate distant tissues. This cell recirculation and focalization makes the IgE mediated allergic disease a dynamic and systemic process. Pereira C showed that this cell response starts at an early stage, in parallel with the immediate allergic response (Pereira, 2009). The IgE mediated response induces immunolymphatic involvement of the adjacent structures. This amplifies the allergic response to loco-regional lymphoid organs, while circulating leukocytes recirculation compromises the primary lymphoid organs (thymus and bone marrow). These central organs are responsible for the systemic immune response induced by a localized allergen challenge, in this case, a nasal challenge (Pereira, 2009). The nervous system activation could be involved by, any or both pathways, namely neurogenic inflammation and neuronal reflexes. Neurogenic inflammation is characterized by specific neuromediators closely related to neuro-immune-endocrine system, and it is both a stimulus to and a consequence of allergic inflammation. The naso-nasal and the naso-ocular reflexes are some examples of the role of the nervous system in the propagation of the allergic disease. They seem to be predominantly mediated by parasympathetic and cholinergic pathways, respectively (Baroody et al, 1994, 2008). Histamine release during the acute response to allergen and substance P seem to have an important role in these neural mechanisms (Baroody et al, 1994, 2008; Fujishima et al 1997; Micera et al, 2008; O'Meara et al, 2005; Sheahan et al, 2005). Multiple evidences support a close interaction and influence of the nose on contiguous and distant organs via neural reflex and systemic inflammatory processes (Baroody, 2011). In summary, a local triggered allergenic inflammation is systematically extended, with the early connection of the immune central organs. Independently of the involved pathway, immediate symptoms are clinically manifested.

Besides the limitations of NPT, this is a feasible and easily method to be performed, since the nasal cavities provide easy access to specific provocation. The concept of "*One airway, one disease*" allows assuming the similarity of response to the provocation of both the upper and lower airways, so the nasal allergic reaction could be accepted as predictor of bronchial response. Supported by the concept of the bidirectional "unified airway" respiratory inflammation, a local provocation test is useful in the diagnosis of allergic respiratory disease. Concerning these aspects, the NPT is the method of choice for the reproducibility of the allergic reaction (Litvyakova & Baraniuk, 2001; Loureiro, 2001; Mellilo, 1997; Naclerio & Norman, 1998). Thus the NPT may be considered a model of respiratory provocation test, easy to perform, in the demonstration of the immediate and late phase of type I hypersensitivity reaction.

5. Characterization of NPT score symptom response

According to all the mentioned above, the clinical symptom score is widely used in clinical practice, alone or associated to objective measurements of nasal obstruction, namely nPIFR,

rhinomanometry and acoustic rhinometry. The other methods, such as immunological measurements, should be reserved to research procedures related to the investigation of inflammatory network. However, due to the lack of standardization of parameters in the monitoring of NPT response, its reproducibility remains to be defined. The main problem includes the great variability of the responses in each patient and between patients. Although this is an important limitation, concerning NPT response interpretation, the symptom score has been used in the description of positive criteria to NPT response.

As pointed out above, many authors use the symptom score as a method of monitoring and criteria for positivity in response to the NPT. According to the great variability in each patient and between patients, it has been assumed the absence of pattern of response to the NPT. The attempt to standardize this methodology was characterized by the symptom score quantification, through the use of symptoms scaling. One of the most important limitations of this symptoms scaling, is the overemphasis on nasal obstruction, since firstly not all patients value the perception of this symptom, and secondly, when it is present, it can result from concomitant obstructive and inflammatory causes. Besides there is no clinical pattern of response to NPT, our data showed a response profile, which can not be accepted as standard, but it can be useful in monitoring the NPT, namely in the evaluation of the dynamics of the response to NPT, as described bellow.

5.1 Clinical symptom score pattern

In our experience, the symptom score has supported the positivity of NPT. We analysed that the most frequent and intense symptoms occurred within the first 30 minutes after NPT, agreeing to immediate phase of allergic reaction. From all the studies conducted by our group, we did not observe a clinical score symptom pattern. However, we describe a clinical symptom score profile, which was frequent and was characterized by the presence of nasal and extra-nasal symptoms within the first 30 minutes, with a peak at 5 minutes.

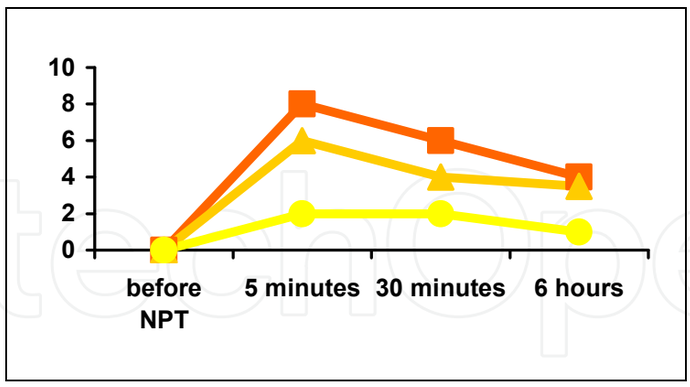


Fig. 2. Score symptoms after *Parietaria judaica* specific nasal provocation test (NPT) (legend: ■ total score; ▲ nasal score; ● non-nasal score).

Indeed, in a group of patients allergic to *Parietaria judaica*, as described above, specific NPT was performed and a symptom score was recorded. The figure 2 presents the total, nasal and non-nasal symptom scores. The higher total score of symptoms was recorded at the fifth minute with progressively decreasing symptoms till 30 minutes and then till 6 hours. Each nasal symptom followed this pattern. The non-nasal symptoms showed a different pattern, having a lower score, with similar values at both the fifth and the 30th minutes, followed by a

decline till 6 hours. Looking at the score of each nasal symptom (Figure 3), except for nasal obstruction, all of them followed the response pattern of total symptoms score, with a peak of symptoms at the fifth minute. Sneezing was the predominant symptom at the fifth minute, while nasal obstruction was the predominant symptom at the 30th minute and the sixth hour.

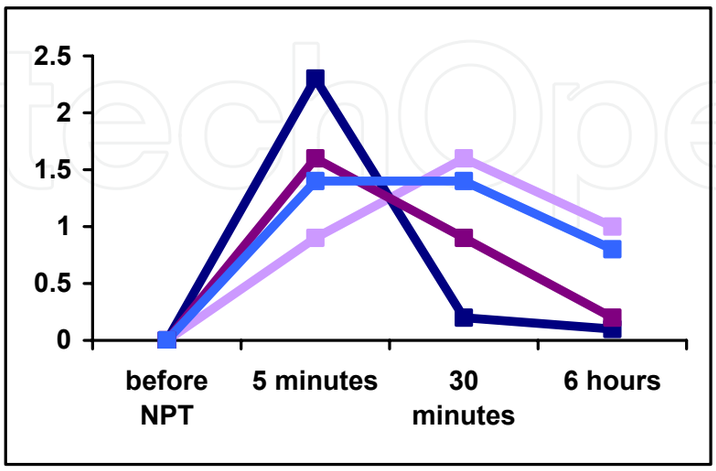


Fig. 3. Nasal symptom score after specific *Parietaria judaica* nasal provocation test (NPT): evolution of each nasal symptom (Legend: nasal congestion; pruritus; sneezing; rhinorrea).

In another study mentioned above, the *Dermatophagoides pteronyssinus* specific NPT were performed in 34 children with OME. Those who had positive NPT, showed a response dynamics characterized by a rapid increase of symptoms score till a peak at the 5th minute (monitored till 1 hour), as shown in figure 4. Looking at the score of each nasal symptom (Figure 5), except for nasal pruritus, all followed the response pattern of total symptoms score, with the peak of symptoms at the fifth minute.

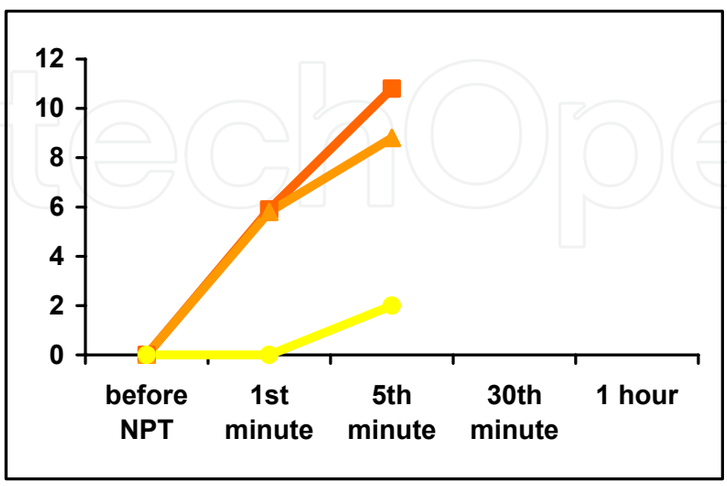


Fig. 4. Score symptoms after *Dermatophagoides pteronyssinus* specific nasal provocation test (NPT); (Legend: total score; nasal score; non-nasal score).

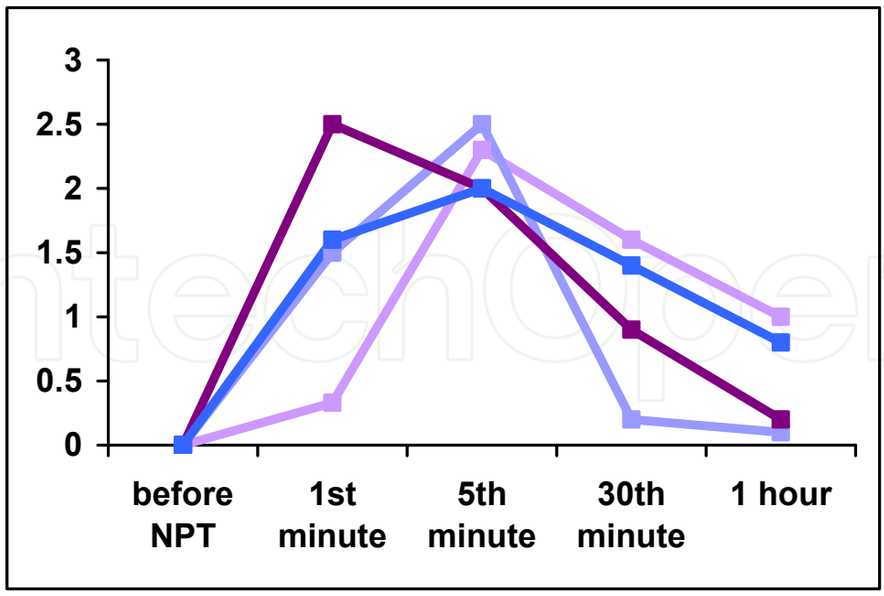


Fig. 5. Nasal symptom score after specific *Dermatophagoides pteronyssinus* nasal provocation test (NPT): evolution of each nasal symptom (Legend: nasal congestion; pruritus; sneezing; rhinorrea).

Beyond the description of symptoms score obtained during NPT, it is also important to compare them with the usual symptoms described by the patient. This looks particularly relevant in the diagnosis of local allergic rhinitis.

In our study related to local allergic rhinitis diagnosis, we included 15 patients with typical clinical symptoms of perennial rhinitis, negative skin prick test to common aeroallergens and negative specific IgE, as mentioned above (Loureiro G et al, 2011). The patients had an average age of 22.2±14.8 years, 77.7% were female. A *Dermatophagoides pteronyssinus* specific NPT was performed with clinical monitoring. Total nasal symptom scores were assessed using a validated questionnaire and a positive challenge was considered if a score of five or greater was recorded. NPT supported the diagnosis of local allergic rhinitis in a group of patients previously diagnosed with "non-allergic rhinitis". They presented a period of symptoms evolution of 5.37±3.9 years. The symptom scores reported during natural exposure and after NPT are shown in figure 6. During natural exposure, the nasal total score was 6.2±2.05. Nasal congestion was always reported and it had the highest recorded value (2.8±0.35). The highest nasal recorded value during NPT was 6.4±2.19. Nasal congestion and pruritus were always reported and this second symptom had the higher recorded value (2.4±0.5). None of the 15 patients had conjunctivitis or asthma. Furthermore, in the 8 patients that had positive NPT, extra-nasal symptoms were recorded, namely conjunctival symptoms, oropharyngeal pruritus, cough and dyspnea, although with lower values.

Concerning the occurrence of non-nasal symptoms, the major non-nasal symptoms observed were those localized in the conjunctiva, followed by oropharyngeal pruritus. Dyspnea and cough were recognized rarely. Non-nasal symptoms were documented in 20 up to 100% of the positive NPT performed, considering the different studies conducted in our Immunoallergy Department.

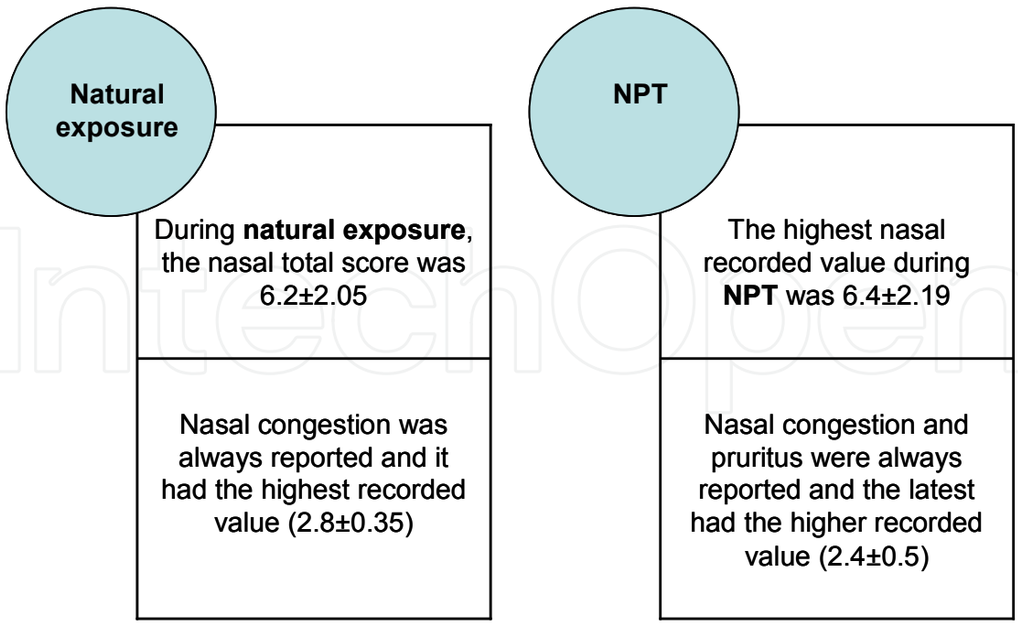


Fig. 6. Symptom scores reported during natural exposure and after nasal provocation test (NPT).

5.2 Comparison of *Dermatophagoides pteronyssinus* nasal provocation test *versus* conjunctiva provocation test

Our group and others authors have been using clinical scores to evaluate NPT response. According to our findings described previously, in respect to the symptoms scores pattern in response to NPT, we conducted a study to characterize the clinical response to NPT comparing to conjunctiva provocation test (CPT). As CPT is easy to perform and systemic reactions are uncommon, some authors have studied the concordance between NPT and CPT in the diagnosis of allergic rhinitis (Andersen et al, 1996; Leonardi et al, 1993; Malmberg et al, 1978; Petersson et al, 1986; Riechelmann et al, 2003) and asthma (Mosbech et al, 1987) using clinical score symptoms and/or objective methods. However, we are not aware of any publication describing the clinical pattern of NPT and CPT responses, neither about its comparison.

Our aim was to compare the dynamics of clinical responses induced by NPT and CPT, using a clinical score system.

5.2.1 Material and methods

5.2.1.1 Subjects

We studied two groups of voluntary adult patients, referred to our outpatient Immunoallergy Department, with *Dermatophagoides pteronyssinus* (*Dp*) allergic rhinitis/rhinoconjunctivitis with or without associated bronchial asthma, according to ARIA (Bousquet & ARIA Workshop Group, 2001) and GINA guidelines, respectively. All patients were clinically stable at the time of the study. Patients with past or ongoing immunotherapy for *Dermatophagoides*, an exacerbation of allergic disease or a respiratory tract infection in the last month, a nasal surgery in the last 3 months or nasal pathology such as polyps or a deviated nasal septum, were excluded. H1-antihistamines and costicosteroids, either nasal

or oral, were withheld for 2 weeks and 4 weeks prior to the challenge test, respectively. All patients underwent the challenge between January and February of 2009, a period of low natural exposure to mites in Portugal. A *Dp* NPT was performed in 21 patients and the conjunctival provocation test (CPT) was performed in the other 21 patients. The local ethics committee approved the study and all the participants gave written informed consent before entry. A respiratory function test (pletismography using *Master screen Body Jaeger*®) was performed by all the participants, before specific provocation tests, with all presenting a baseline $FEV_1 \geq 80\%$ and $FEV_1/FVC \geq 80$. After provocation, all patients were asked for the presence of dyspnoea, thoracic oppression, wheezing or cough.

5.2.1.2 Specific nasal and conjuctival provocation tests

A skin prick test aqueous extract of *Dp* with a 5 mg/ml concentration (23 μg/ml of *Der p* 1, BialAristegui, Bilbao, Spain), with 1/1, 1/10, 1/100 and 1/1000 dilutions were performed; negative and positive controls were performed in all patients, according to standardized procedures (Dreborg & Frew A, 1993). The concentration used to specific provocation was the minimum that induced a prick test wheal at least equal to that induced by histamine, which curiously was the 1/10 dilution in all patients. Specific NPT with *Dp* extract were performed in the morning and after an adaptation to room temperature for 30 minutes, in both groups. NPT was performed with unilateral nasal application of 2 consecutives puffs (total volume of 160 μl) of the *Dp* extract to the inferior nasal turbinate of the less congested nostril, using a nasal applicator spraying and patients were asked to perform apnoea during the allergen spraying. CPT consisted in unilateral ocular application of 1 drop (50 μl) of the *Dp* extract in the inferior and external quadrant of the bulbar conjunctiva. Nasal and eye symptoms were recorded at the 1st and 5th minutes after specific provocation tests, using a clinical score system to assess the response (Linder A, 1988).

5.2.1.3 Clinical score scaling

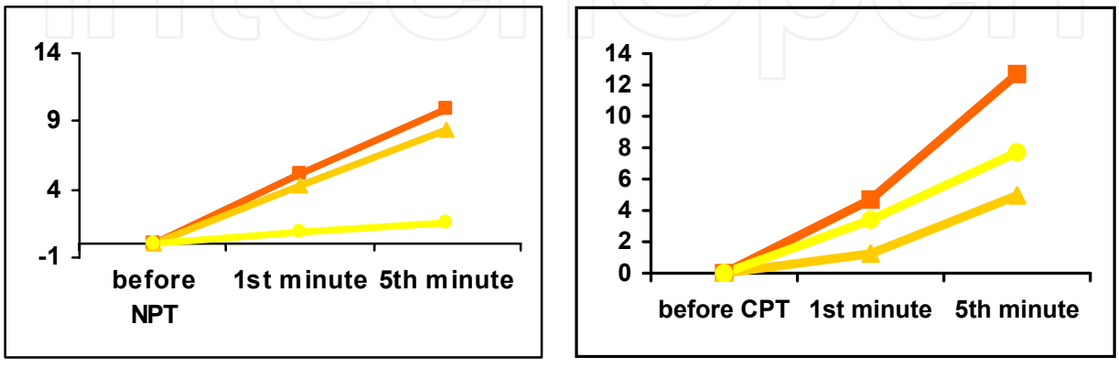
Clinical responses were evaluated using a nasal clinical score (NCS) and an ocular clinical score (OCS), at the 1st and the 5th minutes. An adaptation of the previously used NCS (Linder A, 1988) and OCS (Mortemousque, 2007) were applied. A total clinical score (TCS), representing the sum of NCS (range: 0-15) and OCS (range: 0-13) was also used, ranging from 0 to 28 points. Rhinorrhea, sneezing, itchy nose, itchy ear/throat, nasal obstruction, watery eyes, redness of eyes and burning of eyes were rated on a scale from 0 to 3 points (0, none; 1, mild; 2, moderate; 3, severe). Itchy eyes were scored from 0 to 4 points (0, none; 1, mild; 2, moderate; 3, severe; 4, very severe). A positive response to NPT was considered when NCS ≥3 (Linder A, 1988) and to CPT when OCS ≥5 (Mortemousque, 2007). Clinical evaluation was interrupted after the 5th minute to collect humours for further investigation to determine inflammatory markers within a research investigation of immunologic mechanisms in allergic disease (Pereira, 2011, *in press*).

5.2.1.4 Statistical analysis

Statistical analysis were performed using SPSS® Statistics 17.0 software. Comparisons between NPT and CPT were studied using Chi-Square test. Intra-groups differences between the 1st and the 5th minutes after provocation were analyzed using a Mann-Whitney U-test. A statistical significant difference was assumed with $p < 0.05$.

5.2.2 Results

Demographical and clinical data are presented in Table 3. Table 4 shows the number of patients that presented nasal and ocular responses at the 5th minute, induced by NPT and CPT, as well as the number of positive challenges at the 1st and the 5th minutes. A progressive increase in clinical score was observed in both provocations. The NPT progressive response was linear while for the CPT it was exponential, as shown in figures 7. CPT response was stronger than NPT at the 5th minute, achieving borderline significance (p=0.05). Clinical score results for NPT and CPT are shown in Table 5.



(a) (b)

Fig. 7. Dynamics of symptoms score in response to: A – NPT (Linear progression); B – CPT (Exponential progression) Legend: ■ - Total Symptom score; ▲ - Nasal symptom score; ● - Non-nasal symptom score)

The most frequent symptoms were nasal obstruction, itchy ear/throat and itchy nose, for NPT, and ocular hyperaemia and burning eyes, for CPT in all patients. In NPT, nasal obstruction was observed in 100% of the group. CPT induced ocular hyperaemia and burning eyes in all patients. There were neither bronchial symptoms nor systemic reactions in any of the provocation tests.

The highest scores were reached by nasal obstruction and rhinorrhea in NPT and by ocular hyperaemia in CPT. The average intensity of each sign/symptom at the 5th minute is shown in figure 8.

	NPT	**CPT**
n	21	21
Average age (years)	28.0 ± 9.0	28.1 ± 5.7
Gender ♀ (%)	57.1	66.7
Rhinitis (n) Rhinoconjunctivitis (n)	20 1	16 5
Associated asthma (%)	42.8	90.5
Cutaneous reactivity to Dp (mm) Specific IgE to Dp (KU/L)	6.5 ± 2.1 29 ± 24.9	8.6 ± 3.6 36.3 ± 37.2
Disease evolution (years)	13 ± 10	12.3 ± 8.5

Table 3. Demographical and clinical data of patients submitted to NPT and CPT (Legend: NPT – nasal provocation test; CPT – conjunctiva provocation test)

	NPT	CPT	*p*
Nasal response at 5th min	21 (100%)	20 (95.2%)	ns
Ocular response at 5th min	10 (47.6%)	21 (100%)	0.0001
Number of positive challenges:			
1st min	15	6	0.005
5th min	21	21	

Table 4. Frequency of nasal and ocular symptoms at the 5th minute and NPT and CPT outcomes at the 1st and the 5th minutes (Legend: NPT – nasal provocation test; CPT – conjunctiva provocation test; ns - not significant).

Comparing NPT and CPT, in the first one the response was faster at the 1st minute (p=0.005) while for CPT it was stronger at the 5th minute (p=0.05).
Although the inoculation of allergen was unilateral, NPT induced bilateral nasal symptoms in 100% and bilateral ocular symptoms in 47.6%. On the other hand, CPT induced unilateral ocular symptoms in 100% and bilateral nasal symptoms in 95.2%. There were neither bronchial symptoms nor systemic reactions.

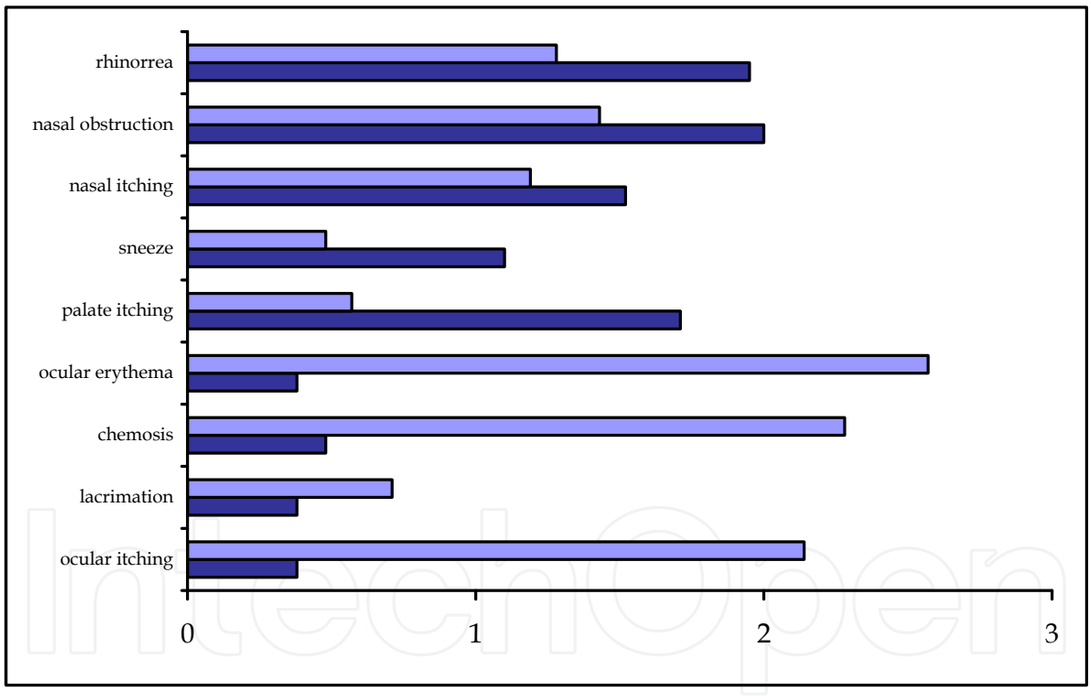


Fig. 8. The average intensity of each sign/symptom at the 5th minute; (Legend: ■ - Nasal provocation test; ■ - Conjunctiva provocation test)

5.2.3 Discussion

Although the importance of the objective monitoring of specific provocation tests is unquestionable, its applicability in clinical practice is not always possible. Usually it is limited to the evaluation of only one symptom, such as nasal patency by nasal peak flow, acoustic rhinometry and/or rhinomanometry (Nathan et al, 2005); however it is not always the most perceived symptom by patients. Clinical scoring systems, even though more subjective, reflect

	NPT	CPT	*p*
Total			
1st min	5.2 ± 3.8	4.7 ± 3.6	ns
5th min	9.9 ± 4.4	12.7 ± 4.4	ns (0.05)
5th - 1st	4.6 ± 4.57	8.0 ± 3.87	0.011
Nasal (NCS)			
1st min	4.28 ± 2.6	1.24 ± 2.1	<0.0001
5th min	8.29 ± 2.9	4.95 ± 2.8	0.001
Ocular (OCS)			
1st min	0.95 ± 1.8	3.4 ± 3	<0.0001
5th min	1.57 ± 2.3	7.7 ± 3	<0.0001

Table 5. Clinical score results for NPT and CPT (Legend: NPT – nasal provocation test; CPT – conjunctiva provocation test; ns – not significant).

all symptoms, are easy and costless to apply in clinical practice. The validity and reproducibility of CPT based on clinical score systems were demonstrated in several studies (Abelson et al, 1990; Moller et al, 1984; Mortemousque, 2007; Rimas et al, 1992).

According to our findings, we can describe a dynamic response profile to specific provocation. In our study, NPT response at the 1st minute was faster than CPT (p=0.005), with 15/21 patients presenting a positive NPT *versus* 6/21 patients with positive CPT. We speculate that this can eventually be explained by the existence of particular characteristics in nasal and ocular mucosa, resulting in differences related to the contact with the allergen and/or the time response of type I hypersensitivity. The NPT progressive response was linear whereas CPT one was exponential, till the 5th minute of response.

On the other hand, CPT response was stronger at the 5th minute when comparing to NPT, achieving borderline significance (p=0.05). This corroborates other results related to the evaluation of patient discomfort of NPT *versus* CPT using a visual-analogue scale, with a higher discomfort being appointed to CPT (Riechelmann et al, 2003). Apparently, these results are different from the study of Malmberg et al, 1978, in which the conjunctiva of 55% of the patients that underwent both NPT and CPT, using sequentially diluted allergen solutions, was less sensitive to allergen challenge than nasal mucosa. However, the intensity of the positive CPT response was not described in this study. Our patients submitted to CPT had higher specific IgE values, but it is unlikely that this could explain the higher intensity symptoms score. The absence of a direct correlation between the degree of allergen sensitization and the severity of clinical symptoms is well known.

As expected by direct allergen exposure, the higher intensity of nasal response was induced by NPT, while CPT was responsible for the higher intensity of ocular response.

At the 5th minute, procedures to collect secretions were performed, and consequently the clinical evaluation of the response to specific provocation tests was disrupted. However, patients were clinically monitored till 4th hour. Interestingly, after the 5th minute, the intensity of the conjunctival response rapidly decreased while a similar intensity of nasal response persisted for a longer period. This data is not shown because the procedures for collection of secretions could alter the dynamic of response.

Even though the allergen was unilaterally inoculated, NPT induced bilateral nasal symptoms in 100% and bilateral ocular symptoms in 47.6%. On the other hand, CPT induced unilateral ocular symptoms in 100% and bilateral nasal symptoms in 95.2%. This is in accordance with previous studies and can be explained by different mechanisms mentioned above (Section 4. NPT as a diagnostic approach in respiratory allergy). An additional explanation for the higher number of patients with nasal symptoms induced by CPT, when comparing with the number of patients in whom NPT induced ocular symptoms, is the direct contact of the inoculated allergen with the nasal mucosa, through its passage via naso-lacrimal duct.

This study describes, for the first time to our knowledge, the clinical patterns of NPT and CPT responses, using a clinical score system. NPT is faster than CPT and has a linear progression, while CPT has an exponential progression and has a stronger response. The induction of both nasal and ocular responses by NPT or CPT, corroborates the systemically response triggered by local allergen application. Although both methodologies can elicit extra-local symptoms, these are safe procedures. Finally, these data support the applicability of CPT in the diagnosis of allergic rhinitis, even in the absence of ocular signs/symptoms, surpassing some NPT limitations (such as nasal polyps or deviated nasal septum) and decreasing specific challenge risk.

6. Conclusion

The specific provocation tests have been widely used in the investigation of pathophysiological mechanisms, immunological and therapeutic aspects of allergic disease, since they mimic the response to allergen exposure, under controlled conditions. It is well known that NPT has limitations, but it has been helpful to a better clarification of the underlying mechanisms of allergic reaction, and also to recognize the systemic framework of allergic disease. The usefulness of NPT is focused in the diagnosis of allergic rhinitis itself, but it has also a relevant role in the diagnosis of allergic respiratory disease. The upper and lower airways do not exist as anatomically and functionally distinct areas. There are important relationships between both the nose and the paranasal sinuses, and asthma. These epidemiological, clinical and immunopathologic concordance between allergic rhinitis and asthma supports the concept of bidirectional "unified airway" respiratory inflammation model. Multiple evidence supports a close interaction and influence of the nose on contiguous and distant organs via neural reflex and systemic inflammatory processes.

In clinical practice, NPT plays a central role in the diagnosis of allergic rhinitis in some circumstances, as described. This is the only method that could establish the correct aetiology of the allergic disease, namely local allergic rhinitis and occupational rhinitis. The specific therapeutic implications emphasize the attempt to reach the most complete diagnostic approach.

The monitoring of the response to NPT is not standardized, but several parameters have been used, for example symptom scores. Our data suggest that the clinical symptom pattern to NPT develops has a dynamic response which is characterized by a linear progression of symptoms intensity till a 5th minute peak. The prevalence of non-nasal symptoms had a great variability in the studies performed by our group. Those symptoms had a lower score comparing to nasal symptoms. In our opinion, the symptom score is a valuable method to monitor the NPT response.

7. Acknowledgements

We would like to acknowledge Dr Borja Bartolomé, Bial Aristegui, I&D Department, Bilbao, Spain; Dr António Martinho & Dr Artur Paiva, PhD, Histocompatibility Center, Coimbra, Portugal.

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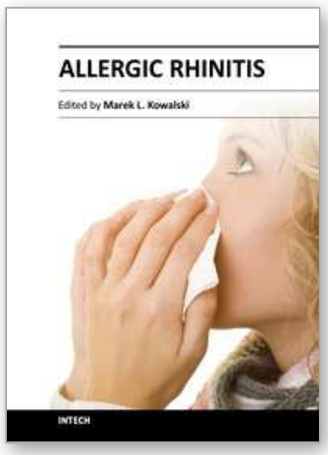

Allergic Rhinitis

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Allergic rhinitis, while troublesome for a patient, may be also a challenge for the physician. That is why physicians must still learn more on the pathophysiology, clinical spectrum and novel diagnostic and therapeutic approaches to the disease. The chapters of this volume address a variety of important topics related to allergic rhinitis. They begin with a description of innovative translational approaches allowing for unification of animal and human models. Contributing authors provide up-to-date reviews of clinical aspects of allergic rhinitis in children, its association with bronchial asthma and other co-morbid conditions. They also discuss the impact of allergic rhinitis on sleep and sports. Together with articles on diagnostic approaches as well as novel treatments, the book offers a comprehensive and stimulating review of the topic. May this book find a wide readership among allergists and other physicians interested in allergic disease, and also among pediatricians, general practitioners and other specialists who increasingly have to deal with this seemingly benign, but sometimes extremely troublesome, disease.

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