

## ORIGINAL ARTICLE

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# The Safety of Nasal Allergen Challenge Test Assessed in Lower Airways

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## ABSTRACT

The aim of the study was to assess the safety of nasal allergen challenge, and the use of certain parameters applied in assessing the condition of the respiratory system.

We enrolled 30 patients diagnosed with allergy to common environmental allergens and 30 healthy controls. The safety of nasal challenge tests with an allergen was assessed by measuring the concentration of exhaled nitric oxide from the lower respiratory tract (eNO) and forced expiratory volume in one second (FEV1) in a spirometry test.

In the early phase of the allergic reaction, extra-nasal symptoms were observed, namely cough and breathlessness. These measured symptoms using the VAS scale, were far more frequent in the patients diagnosed with perennial allergic rhinitis. The eNO and FEV1 values in the spirometry test did not show any statistically significant changes. No correlation was observed between the breathlessness and cough complaints to the eNO concentration levels (cough:  $r=0.019$ ,  $p=0.921$ ; breathlessness:  $r=-0.088$ ,  $p=0.644$ ) nor the FEV1 level (cough:  $r=0.002$ ,  $p=0.983$ ; breathlessness:  $r=-0.072$ ,  $p=0.751$ ) in the spirometry test.

In the early phase of the allergic reaction, nasal allergen challenge do not cause any significant changes in the lower airways, as measured with the use of certain parameters applied in assessing the function of the lower airways' function.

**Keywords:** Nitric oxide; Nasal allergen challenge; Safety assessment; Spirometry

## INTRODUCTION

The immediate reaction of the nasal mucous membrane to the exposed allergen is mainly included the stimulation of cells (mastocytes and macrophages),

which are encased by immunoglobulins and caused the secretion of reaction mediators (i.e. tryptase, histamine, CysLT and PGD<sub>2</sub>).<sup>1,2,3,4</sup> In turn, these changes stimulate the receptors of the sensory nerves and of the blood vessels locally in the region of the nasal mucous

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membrane. In addition to above reactions, the mastocytes secrete chemotactic factors and Platelet activating factors (PAFs) contributing to the escalation of inflammation.<sup>5,6,7</sup> The immediate reaction phase normally continues for 20-30 minutes and may be followed by the later phase of the allergic reaction, which begins at approximately 4 hours after administering the allergen.<sup>1</sup>

Nasal allergen challenge (NAC) is easy to perform, highly specific and safe.<sup>8</sup> As a result, NACs are applied for rhinoallergy diagnosis purposes due to their sensitivity, as well as specificity. Although NACs partially imitate natural exposure to an allergen (a one-off allergen dose), they are a valuable source of information on the health of the patients subjected to the tests. This is the only method which enables, in an outpatient setting, the assessment of the co-existing risk of the occurrence of the response of the lower respiratory tract to the application of an allergen in the area of the head of the inferior nasal concha. Blair and Settignano, have proved that the symptoms of allergic rhinitis are more frequent in patients with bronchial asthma (28%-78%) than in those without asthma symptoms (5%-20%).<sup>9,10</sup>

In the event of considerable differences between the patient's medical history, the results of skin prick tests or blood tests (sIgE), NACs often serve as conclusive evidence to support the decision to refer a particular patient for immunotherapy.<sup>1</sup> According to a report by the Committee for Upper Airways Allergies, an NAC may be defined as a method for "restoring the response of the upper Airways to natural exposure to allergens or irritants and doing research into the pathophysiology of the upper airways by testing potential biochemical mediators."<sup>1</sup>

### MATERIALS AND METHODS

The aim of the study was to assess the safety of NPTs, with the use of certain parameters applied in assessing the condition of the respiratory system due to the physiological links between the upper airways and the lower airways.

The sample in this study included a homogeneous group of 60 subjects: 30 patients (14 females, 16 males; mean height 172.90 cm±8.767, mean weight 72.83 kg±13.570, mean age 27.33 years±5.665) allergic to dust mites (*Dermatophagoides pteronyssinus*) and

grasses, and 30 healthy controls (HC) (13 females, 17 males; mean height 176.03 cm±8.588, mean weight 74.10 kg±13.583, mean age 30.63 years±6.037) not showing any allergic symptoms. Subjects were qualified for the study based on guidelines by the Polish Consensus on Provocation Testing (Table 1). In order to increase effectiveness of NAC, the study population was selected based on the dominant allergen (dust mites or grass pollens) identified via skin prick test.

The method applied in the study was the NAC using a standardized biological (SB) 5000 unit per milliliter allergen dose (Allergopharma) of 0.2 ml, administered by means of a calibrated atomizer into both nostrils in room temperature. The subjects' nasal complaints were assessed based on (a) an analysis of the absorption curve for infrared radiation produced through optical rhinometry, and (b) by means of a Visual analogue scale (VAS) including: nasal itching, sneezing, nasal obstruction, watery secretion at 5, 10, 15 and 20 minute following the test (Figure 1).

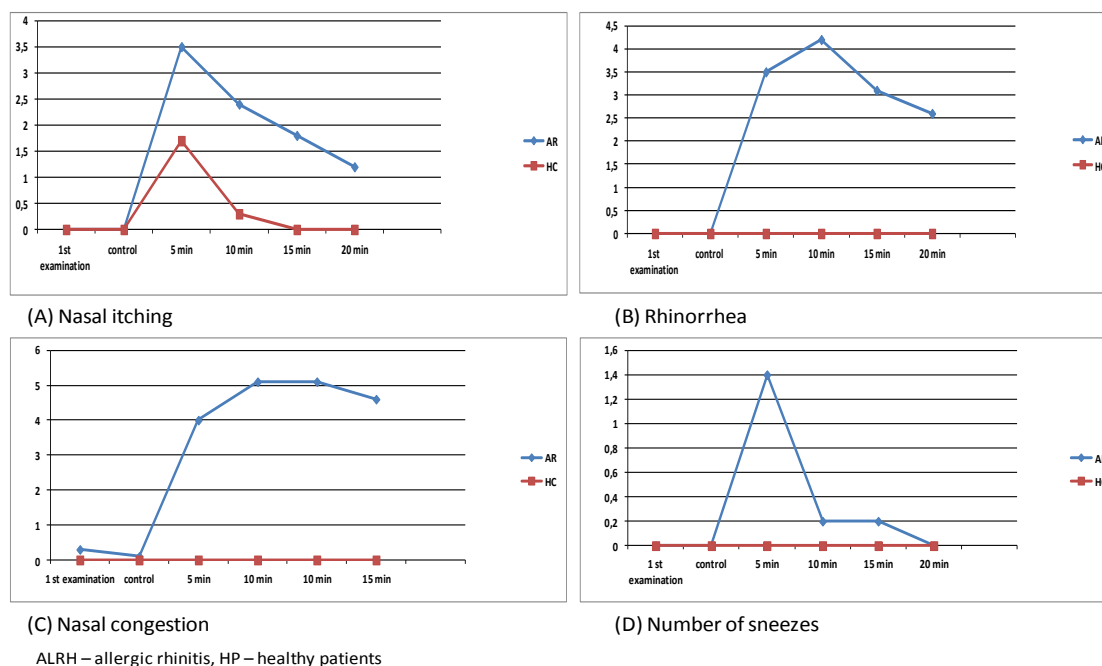
The accompanying reactivity of the lower airways was verified using (a) the Forced Expiratory Volume in the first second (FEV1) value, as measured in a spirometry test, and (b) the concentration of nitrogen oxide in the air exhaled (eNO) from the lower airways, as measured before the test and at the 45<sup>th</sup> minute of the NPT. As the spirometry test was a stressful test, it was performed after measuring the eNO level twice: before the nasal challenge test and at the 45<sup>th</sup> minute following the administration of the allergen. The result of the spirometry test and the eNO measurement (using an online method) was a curve obtained in three repeatable, correct measurements in accordance with the guidelines issued by American Thoracic Society (ATS) and European Respiratory Society (ERS).<sup>11</sup>

### Ethics

The study was approved by the Bioethics Committee at the Medical University of Warsaw (KB/79/2009). The strength of the relation and correlation between the different variables were determined by calculating a t-student statistic and the Pearson linear correlation coefficient. In estimating the homogeneity of the average values, the Levene's test was used. Statistically significant results were defined for  $p < 0.05$ .

**Table 1. The criteria for the inclusion and exclusion of patients in the study**

The criteria for the inclusion	<ul style="list-style-type: none"> <li>- a history of an allergy to a particular allergen (at least 3 years of perceived symptoms, without pharmacotherapy and/or specific immunotherapy);</li> <li>- positive results of skin prick tests;</li> <li>- A CT of the frontal nasal sinuses indicating no inflammation;</li> <li>- nasal patency in the bone part maintained;</li> </ul>
The criteria for the exclusion	<ul style="list-style-type: none"> <li>- a period not shorter than 6 weeks from the airborne allergen season before the NAC , preventing the sensitisation effect;</li> <li>- nasal deformation, choanal atresia, nasal septum perforation, a considerable curvature of the nasal septum;</li> <li>- nasal polyps;</li> <li>- atrophic rhinitis;</li> <li>- fewer than 6 weeks from the disappearance of the symptoms of allergic rhinitis (the patients with seasonal rhinitis were examined from March to May);</li> <li>- vaccination (as per the vaccination calendar, with immunisation preparations) within a week before the test;</li> <li>- fewer than 8 weeks from nasal surgery (especially surgical corrections of the lower nasal concha);</li> <li>- severe infection of the upper airways within two to four weeks before the test;</li> <li>- inflammation of the frontal and nasal sinuses;</li> <li>- bronchial asthma;</li> <li>- hypertension and/or other cardiovascular conditions;</li> <li>- pregnancy and lactation;</li> <li>- active and/or passive smoking;</li> <li>- increased physical exercise before the test.</li> </ul>



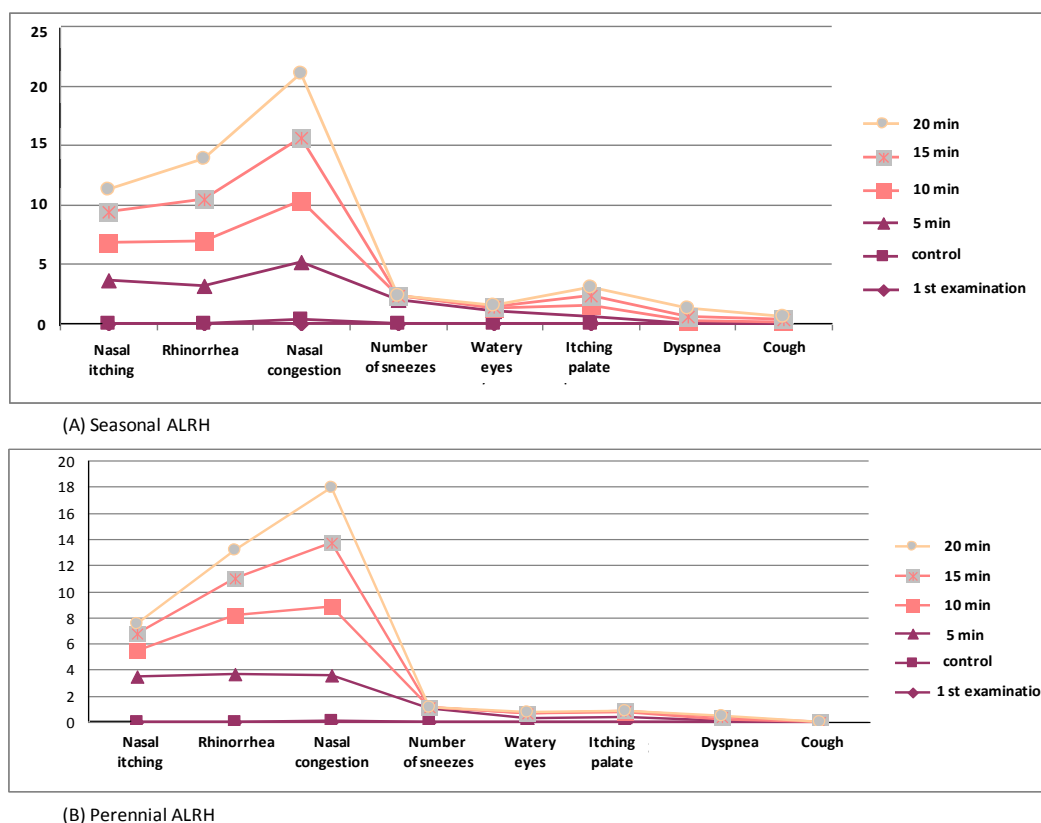
**Figure 1. Curves showing mean nasal symptoms (VAS) during NAC**

**RESULTS**

The safety of the NAC was assessed by measuring the concentration of nitrogen oxide in the air exhaled from the lower airways, as well as the FEV1 value in a spirometry test. A drop in the FEV1 value by 20% in relation to the initial value was considered as a positive response (i.e. the reactivity of the lower airways to the allergen administered through the nose).<sup>13</sup> Significant differences were found in the perceived nasal complaints (VAS), including nasal itching, nasal secretion, nasal obstruction, the number of sneezes in the subjects between the 5<sup>th</sup> and 20<sup>th</sup> minutes of the test ( $p<0.05$ ) in both the ALRH group and the HC group. Nasal itching was the most frequent complaint. The changes over time showed significant differences: between the condition after allergen administration and the 5<sup>th</sup> minutes of the test ( $p=0.006$ ), the 10<sup>th</sup> minutes of the test, the 15<sup>th</sup> minutes of the test ( $p=0.034$ ) and at the 20<sup>th</sup> minutes of the test ( $p=0.018$ ). In addition, an increased number

of sneezes at the 5<sup>th</sup> minutes of the test ( $p=0.05$ ), nasal secretion at the 10<sup>th</sup> minutes ( $p<0.05$ ) and nasal obstruction at the 10<sup>th</sup>, 15<sup>th</sup> and 20<sup>th</sup> minutes of the test ( $p<0.05$ ) were observed in the ALRH group but not the HC group. The number of sneezes recorded at the 5<sup>th</sup> minutes of the test was not significantly different in the early phase of the allergic reaction ( $p=0.85$ ). Extra-nasal complaints such as cough and breathlessness were definitely more severe in the perennial ALRH than seasonal ALRH group (Figure 2).

In the ALRH group, significant differences were observed in the start time and duration of increased nasal cavity obstruction, as well as the infrared radiation absorption rate in the early phase of the allergic reaction when compared to the HC group ( $p<0.05$ ) (Table 2). The average start time of the reaction in the nasal mucous membrane was recorded on the optical rhinometry curve at the 3.15 minute mark of the test, while the end time of the infrared radiation absorption was recorded at 28.15 minutes.



**Figure 2. Curves showing average changes in nasal and extra-nasal symptoms (VAS) in the subjects with ALRH (seasonal ALRH and chronic ALRH) in NACs**

The average value of the infrared radiation absorption rate in the ALRH group was 0.431 OD (for  $p < 0.05$ ). The study groups (seasonal vs. perennial rhinitis) showed no significant differences in terms of reaction time and absorption of infrared light. A strong correlation was observed between the perceived complaints measured using VAS scale and optical rhinometry, from the 5<sup>th</sup> to the 20<sup>th</sup> minute of the test (nasal itching:  $r = 0.13$   $p = 0.016$ ; water nasal secretion:  $r = 0.493$   $p < 0.005$ ; nasal obstruction:  $r = 0.333$   $p = 0.009$ ). With respect to nasal symptoms, significant changes were recorded between the ALRH groups included in the study. The perceived complaints of breathlessness and increased cough at the 15<sup>th</sup> minute of the test ( $p = 0.044$ ) and the 20<sup>th</sup> minute of the test ( $p = 0.040$ ) were definitely more severe in the perennial ALRH group. The FEV1 values were significantly lower in the ALRH group than in the HC group (Table 3).

In the ALRH groups (seasonal ALRH and perennial ALRH), statistically significant differences were found in the FEV1 values. With the bronchial reactivity threshold of 20% (FEV1), the administration of the allergen did not show any significant differences between the groups subjected to the tests. The eNO levels in the ALRH group were higher than those in the control group, but the differences were not statistically significant and the levels were normal (from 25ppB). Higher eNO concentration levels were observed in the group of patients with perennial ALRH. This was probably the result of inflammation, resulting in the priming effect. The Pearson correlation analysis did not reveal any correlation between the perceived complaints and the measured parameters (eNO/cough  $r = 0.019$ ,  $p = 0.921$ , eNO/breathlessness  $r = -0.088$ ,  $p = 0.644$ , FEV1/cough  $r = 0.002$ ,  $p = 0.983$  FEV1/breathlessness  $r = -0.072$ ,  $p = 0.751$ ).

**Table 2. Duration and intensity of allergic reaction measured via optical rhinometry**

HP/ALRH	Mean Value		Standard Deviation		Statistical Significance
	ALRH n=30	HC n=30	ALRH	HC	
ΔE	0.431	0.010	0.482	0.001	$p < 0.05^*$
	28.15	0.00	4.913	0.000	$p < 0.05^*$
<b>A The HP/ALRH study population</b>					
Seasonal/Perennial ALRH	Mean Value		Standard Deviation		Statistical Significance
	Seasonal ALRH n=18	Perennial ALRH n=12	Seasonal ALRH	Perennial ALRH	
ΔE	0.467	0.378	0.569	0.329	$p = 0.651$
T	29.06	28.25	5.047	4.883	$p = 0.855$

**B The Seasonal/Perennial ALRH study population**

ALRH-allergic rhinitis; HC-healthy control; Seasonal ALRH-seasonal allergic rhinitis; Perennial ALRH-perennial allergic rhinitis

**Table 3. eNO levels and FEV1 (% decrease in forced expiratory volume in 1 second) in the ALRH groups versus the control group**

HP/ALRH	Mean Value		Standard Deviation		Statistical Significance
	ALRH n=30	HC n=30	ALRH	HC	
<b>eNO ppB</b>					
before the NAC	22.17	18.97	9.89	6.32	$p = 0.141$
after the NAC	22.50	17.53	12.36	7.09	$p = 0.061^*$
<b>FEV1</b>					
before the NAC	93.73	99.63	8.27	10.89	$p = 0.022^*$
after the NAC	94.28	100.27	9.52	10.14	$p = 0.023^*$
<b>% decrease in FEV1</b>					
after the NAC	0.030	0.008	0.191	0.057	$p = 0.293$
<b>A The HP/ALRH study population</b>					

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**Table 3. Continue**

Seasonal/Perennial ALRH	Mean Value		Standard Deviation		Statistical Significance
	Seasonal ALRH n=18	Perennial ALRH n=12	Seasonal ALRH	Perennial ALRH	
<b>eNO ppB</b>					
before the NAC	21.67	22.92	8.40	12.16	$p=0.217$
after the NAC	21.39	24.17	9.55	16.03	$p=0.474$
<b>FEV1</b>					
before the NAC	93.73	99.63	8.27	10.89	$p=0.022^*$
after the NAC	94.28	100.27	9.52	10.14	$p=0.023^*$
<b>% decrease in FEV1</b>					
after the NAC	-0.001	0.076	0.062	0.294	$p=0.282$

### **B The Seasonal/Perennial ALRH study population**

ALRH-allergic rhinitis; HC-healthy control; Seasonal ALRH-seasonal allergic rhinitis; Perennial ALRH-perennial allergic rhinitis

## DISCUSSION

The NAC is considered as a relatively safe method for studying a patient's allergic reaction to airborne allergens.<sup>8,12,13</sup> This has been confirmed by this analysis on the risk of reactivity of the lower airways in the early phase of the allergic reaction. It is estimated that the co-existing complications in the lower airways (in response to the administration of an allergen through the nose) may be linked with allergen penetration into the larynx or the bronchial tree, and are a result of technical mistakes related to the administration of the allergen onto the surface of the nasal cavity mucous membrane (e.g. while the patient was exhaling the air). Bronchial hyper-responsiveness in nasal provocation tests (NPT) is observed in one-third of all the patients subjected to such tests and who showing no symptoms of bronchial asthma.<sup>14</sup> Decreases in the FEV1 value are far more frequent in the case of patients with co-existing bronchial asthma. This was proven by Yan and colleagues, for example in NPTs with histamine, they observed a significant decrease in the FEV1 value in the later phase of the test, without signs of the non-specific factor entering the bronchia, and with the use of a radioactive substance<sup>15</sup> In a study involving a group of 18 patients with ARLH and 18 healthy subjects, Kirmaz et al. showed in two tests (i.e. a non-specific test with metacholine), a clearly higher responsiveness of the nasal cavity mucous membrane ( $p=0.049$ ) in the ARLH group, and in a test with European olive (*Olea europaea*) extract, no responsive

ness-FEV1 ( $p=0.04$ ).<sup>16</sup> Similarly, Corren et al measured bronchial responsiveness to NPTs every hour for a period of 24 hours, but did not prove any change in the FEV1 over time, and his non-specific test with metacholine showed an increase in bronchial responsiveness to NPT at the 30<sup>th</sup> minutes ( $p=0.011$ ) and 4.5 hours ( $p=0.0009$ ).<sup>17</sup>

Baki et al.<sup>18</sup> subjected a group of children aged 6-17 years to an NAC with Dermatophagoides pteronyssins, measuring saturation (SaO<sub>2</sub>) and the FEV1 value, but found no significant changes over time. Similarly Tuskan et. al. in a study involving a group of 32 patients with allergic asthma of medium intensity, (who use a therapy with low doses of steroids: 200g 1g of fluticasone or 400 1g of budesonide) vs a control group (9 healthy non-smokers and 9 subjects with no signs of bronchial asthma but with an allergy to house dust mite) confirmed a high degree of safety of a nasal allergen challenge (NAC). As regards the assessment of the functional capacity he found there were no recorded changes of the FEV1 and PEF parameters in a spirometry test in the late phase of allergic reaction measured in all groups of subjects. The level of eosinophil cationic protein (ECP) measured in the nasal lavage fluid and in the blood as well as eosinophilic leukocytosis in the cytological smear in the 4th, 12th and 24th hour after a nasal allergen challenge was clearly diverse in the group of subjects with the diagnosed asthma of medium intensity, which points to the particular usefulness of the above mentioned techniques of study in a nasal allergen challenge.<sup>8</sup>

In an NPT with aspirin (L-ASA), Milewski et al<sup>19</sup> proved no responsiveness in the form of bronchial constriction, and even in the group of subjects where the FEV1 before the NPT was lower than 70% of the normal value, the NCT could be applied in patients with unstable bronchial asthma. Alonso-Llamazares et al subjected a group of subjects with bronchial asthma to an NAC with L-ASA, but found no significant changes; increased bronchial responsiveness (20%) and no subjective complaints.<sup>20</sup> In addition to the FEV1 value, which is used to verify bronchial responsiveness to NAC), another very sensitive measure of inflammation is the concentration of eNO in the exhaled air. Kharitonov et al proved a clear correlation between the level of eNO and exposure to external allergens.<sup>21</sup> In the author's own study of a small group of subjects, a slight increase in the eNO level was recorded as soon as the early phase of the allergic reaction began, especially in patients with perennial ALRH. Numerous studies confirm the sensitivity of eNO to NCTs. Korn et al found a significant increase in eNo at the 4<sup>th</sup>, 8<sup>th</sup> and 24<sup>th</sup> hours after nasal administration of an allergen.<sup>22</sup> Gratziou et al proved an upward trend in eNO concentration levels in a group of patients with seasonal ALRH and during the pollen season.<sup>23</sup> Lopuhaä et al observed an upward trend in eNO concentration levels as soon as one hour after the administration of an allergen onto the mucous membrane of the nasal cavity.<sup>24</sup>

There are many mechanisms responsible for the above processes and they have not yet been fully researched. One of the mechanisms links a co-existing risk of the response of the lower airways to nasal administration of an allergen, or a non-specific substance with the sensitization of the bronchial epithelium to environmental factors (including outdoor and indoor allergens), especially among subjects with perennial ALRH. In contrast, Bardin et al carried out an experiment in which patients were administered radioisotope fluid into their maxillary sinuses. In this study, he proved no penetration into the bronchial tree within 24 hours.<sup>25</sup> Another probable mechanism is related to the IgE synthesized immunoglobulin where the allergen is administered, which was confirmed by studies carried out by Beeh et al. They proved that an NAC leads to an increase in the level of IL 5, which is transported to the pulmonary blood system resulting in an increased eNO concentration.<sup>26</sup>

## ACKNOWLEDGEMENTS

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## REFERENCES

1. Bousquet J, Khalataev N, Alvaro A, Denburg J, Fokkens W, Togias A, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008. *Allergy* 2008; (63 Suppl 86):8-160.
2. Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson N Jr, Meyers DA, Norman PS et al.: Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 1983; 128:597-602.
3. Lebel B, Bousquet J, Morel A, Chanal I, Godard P, Michel FB.: Correlation between symptoms and the threshold for release of mediators in nasal secretions during nasal challenge with grass-pollen grains. *J Allergy Clin Immunol* 1988; 82: 869-77.
4. Busse WW, Sedgwick JB. Eosinophil eicosanoid relations in allergic inflammation of the airways. *Adv. Prostaglandin Thromboxane Leukot Res* 1994; 22: 241-49.
5. Ying S, Durham SR, Corrigan CJ, Hamid Q, Kay AB. Phenotype of cells expressing mRNA for TH2-type (interleukin 4 and interleukin 5) and TH1-type (interleukin 2 and interferon gamma) cytokines in bronchoalveolar lavage and bronchial biopsies from atopic asthmatic and normal control subjects. *Am J Respir Cell Mol Biol* 1995; 12:477-87.
6. Shaw RJ, Fitzharris P, Cromwell O, Wardlaw AJ, Kay AB. Allergen-induced release of sulphidopeptide leukotrienes (SRS-A) and LTB4 in allergic rhinitis. *Allergy* 1985; 40:1-6.
7. Ciprandi G, Pronzato C, Ricca V, Passalacqua G, Bagnasco M, Canonica GW. Allergen-specific challenge induces intercellular adhesion molecule 1 (ICAM-1 or CD54) on nasal epithelial cells in allergic subjects. Relationships with early and late inflammatory phenomena. *Am J Respir Crit Care Med* 1994; 150:1653-9.
8. Tuskan TC, Gemicioglu B, Ikitimur H, Yilmaz N, Tuskan K, Ferhan O, et al. The value and safety of specific nasal provocation in the diagnosis of allergic rhinitis in mild persistent asthma under inhaled steroid therapy. *Asian Pac J Allergy Immunol* 2010; 28:115-21.

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9. Blair H. Natural history of childhood asthma: 20-year follow-up. *Arch Dis Child* 1997; 52(8):613-9.
10. Settipane GA. Allergic rhinitis-update. *Otolaryngology Head Neck Surg* 1986; 94(4):470-5.
11. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. *Am J Respir Crit Care Med* 2005; 171(8):912-30.
12. Andersson M, Greiff L, Svensson C, Persson C. Various methods for testing nasal responses in vivo: a critical review. *Acta Otolaryngol* 1995; 115(6):705-13.
13. Baki A, Ucar B. Diagnostic value of nasal provocation test with *Dermatophagoides pteronyssinus* in childhood asthma. *Allergy* 1995; 50(9):751-4.
14. Downie SR, Andersson M, Rimmer J, Leuppi JD, Xuan W, Akerlund A, et al. Association between nasal and bronchial symptoms In subjects with persistent allergic rhinitis. *Allergy* 2004; 59:320-6.
15. Yan K, Salome C. The response of the airways to nasal stimulation in asthmatic with rhinitis. *Eur J Resp Dis* 1983; 128(Pt 1):105-9.
16. Kirmaz C, Degirmenci P B, Tunali D, Yuksel H. Lower respiratory tract complications during nasal provocation: nonspecific stimulant or specific allergen? *Ann Allergy Asthma Immunol* 2007; 98(6):524-32.
17. Corren J, Adinoff AD, Irvin CG. Changes in bronchial responsiveness following nasal provocation with allergen. *J Allergy Clin Immunol* 1992; 89(2):611-8.
18. Baki A, Ucar B. Diagnostic value of nasal provocation test with *Dermatophagoides pteronyssinus* in childhood asthma. *Allergy* 1995; 50(9):751-4.
19. Milewski M, Mastalerz L, Nizankowska E, Szczeklik A. Nasal provocation test with lysine-aspirin for diagnosis of aspirin-sensitive asthma. *J Allergy Clin Immunol* 1998; 101(5):581-6.
20. Alonso-Llamazares A, Martinez-Cóccera C, Domínguez-Ortega J, Robledo-Echarren T, Cimarra-Alvarez M, Mesa del Castillo M. Nasal provocation test (NPT) with aspirin: a sensitive and safe method to diagnose aspirin-induced asthma (AIA). *Allergy* 2002; 57(7):632-5.
21. Kharitonov SA, Rajakulasingam K, O'Connor B, Durham SR, Barnes PJ. Nasal nitric oxide is increased in patients with asthma and allergic rhinitis and may be modulated by nasal glucocorticoids. *J Allergy Clin Immunol* 1997; 99(1 pt 1):58-64.
22. Korn S, Beier J, Heilmann Ch, Kornmann O, Buhl R, Beeh M K. Discrepant nasal and bronchial nitric oxide kinetics during early and late phase allergic reactions. *Respir Med* 2005; 99(12):1595-9.
23. Gratziau C, Lignos M, Dassiou M, Ruossos C. Influence of atopy on exhaled nitric oxide in patients with stable asthma and rhinitis. *Eur Respir J* 1999; 14(4):897-901.
24. Lopuhaä CE, Koopmas JG, Jansen HM, Van Der Zee SJ. Similar levels of nitric oxide in non-asthmatic and rhinitis and asthma after bronchial allergen challenge. *Allergy* 2003; 58(4):300-5.
25. Bardin PG, Van Heerden BB, Joubert JR. Absence of pulmonary aspiration of sinus contents in patients with asthma and sinusitis. *J Allergy Clin Immunol* 1990; 86(1):82-8.
26. Beeh KM, Beier J, Kornmann O, Meier C, Taeumer T, Buhl R. A single nasal allergen challenge increases induced sputum inflammatory markers In non-asthmatic subjects with seasonal allergic rhinitis: correlation with plasma interleukin-5. *Clin Exp Allergy* 2003; 33(4):475-82.