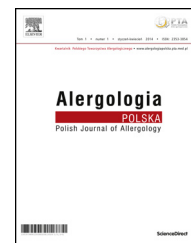


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Original research article/Artykuł oryginalny

Local reaction of the nasal mucosa to an environmental factor, the allergen

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ARTICLE INFO

Article history:

Received: 18.09.2015

Accepted: 07.01.2016

Available online: 21.01.2016

Keywords:

- Environmental
- Allergen
- Allergy
- Nasal mucosa
- Nasal allergen provocation test
- Early and late phase reaction

ABSTRACT

The **Purpose** of the study was to assess the inflammatory response of the nasal mucosa based on nitric oxide concentration in exhaled breath and the level of inflammatory markers tryptase/eosinophil cation protein (ECP) in the nasal lavage fluid obtained via a nasal lavage collection device of our design. **Material:** The study group included 60 subjects: 30 patients diagnosed with an allergy to common environmental allergens (dust mites/grasses) and 30 healthy controls. The **Method** used in the study was NAPT, monitored via measuring nasal nitric oxide (nNO), and tryptase/ECP in nasal lavage fluid. **Results:** The early phase of the allergic reaction involved a drop in nNO with a simultaneous increase in nasal lavage tryptase levels (2.23 µg/L in the allergic rhinitis subgroup), which significantly differentiated between the allergic rhinitis and healthy groups. The late phase of reaction showed a significant increase in nNO and nasal lavage ECP levels (three-fold higher in the allergic rhinitis group than in healthy individuals). **Conclusion:** NAPT affects the nasal mucosa locally by initiating a series of symptoms characteristic for the early and late phases of the allergic reaction resulting from inflammatory marker secretion.

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Introduction

Recent years have seen a growing incidence of allergies, defined as pathological, specific immune system response to allergens. Currently, allergy – in its various forms – is becoming an epidemic and is considered to be the disease of the 21st century [1]. The 2006–2008 Epidemiology of Allergic Diseases in Poland (ECAP) study in 18 617

respondents showed that nearly 40% of the general population of Poland (of 36 million) are affected by an allergic condition, including allergic rhinitis (AR) (>8 mil; 25%), diagnosed bronchial asthma (>1.5 mil; 5%), symptoms of asthma (>4 mil; 12%), atopic dermatitis (>1.5 mil; 4%) [2, 3]. Given the scale of the problem, we need to understand the exact pathophysiology of the reaction in order to introduce effective medical treatment or tertiary prophylaxis, to counteract adverse effects, and minimize the risk of

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<http://dx.doi.org/10.1016/j.alergo.2016.01.001>

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cross-reactivity. Nasal antigen provocation testing (NAPT) seems to be the optimal tool to assess the local nasal mucosal reaction defined as “the set of manifestations caused by IgE-mediated inflammatory reaction of the nasal mucosa to an allergen” [4]. Despite the fact that NAPT only partially imitates natural exposure to an allergen (single allergen dose), the test is a valuable source of information on the condition of those examined, and in case of large discrepancies in the history, skin-prick tests, or sIgE tests it is often decisive in qualifying patients for immunotherapy.

Experimental procedures

The aim of this study was to assess the inflammatory reaction within the nasal mucosa measured via nitric oxide in condensed air exhaled from the nose (nNO) and the level of markers; tryptase/ECP in nasal lavage fluid obtained with a nasal lavage collection device of our own design, which minimized the loss of sample material for an enzyme-linked immunosorbent assay (ELISA).

The study group comprised 60 subjects: 30 patients diagnosed with allergy to common environmental allergens: house dust mites *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, grass pollens (14 females and 16 males; the mean age in the seasonal allergic rhinitis (SAR) group was 29.39 ± 6.001 , in the perennial allergic rhinitis (PAR) group – 24.25 ± 3.415) and 30 healthy controls (HC; mean age 30.63 ± 6.037) with no allergic symptoms.

Study inclusion criteria

- social history confirming an allergy to a given allergen (at least 3 years of symptoms, without medical treatment or specific immunotherapy)
- positive skin-prick test results
- computed tomography results of the paranasal and frontal sinuses excluding inflammation
- preserved patency in the osseous part of the nose

Study exclusion criteria

- a period of no less than 6 weeks from air-borne allergen season preventing the “sensitizing” phenomenon prior to NAPT
- nasal deformities, primary occlusion of the nares, septal perforation, significant septal deviation,
- nasal polyps,
- atrophic rhinitis,
- a less than 6-week interval from the end of symptoms of allergic rhinitis; the patients with SAR were examined in the period from March to May,
- vaccinations (according to immunization schedule, with vaccine formulations) administered within a week prior to testing,
- a less than 8-week interval from a nasal surgical procedure (especially corrective surgery on the inferior turbinate),

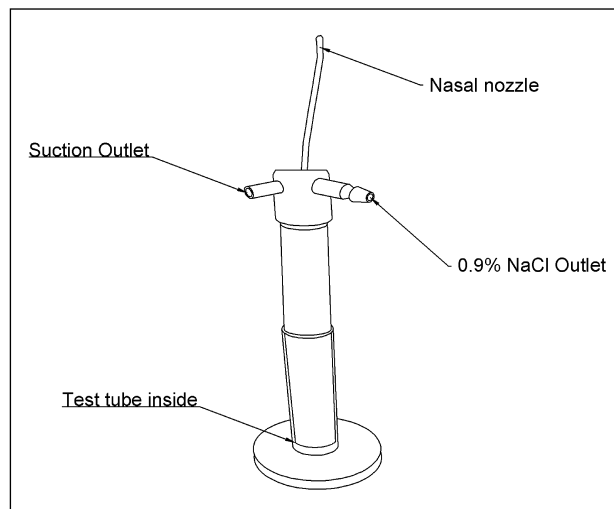


Fig. 1 – Nasal lavage collection device prototype (source on file)

- acute upper respiratory tract infection within 2–4 weeks before the study
- paranasal and frontal sinusitis
- bronchial asthma.

The test method used in the study was NAPT with a standardized allergen dose of 0.2 mL (5000 SBU/mL, Allergopharma) administered nasally with an atomizer. As usual, NAPT-evoked symptoms were assessed via a visual analog scale (VAS) (measured at 5, 10, 15, and 20 min, and at 4 h) and objective degree of inflammation (measured prior to, as well as 30 min and 4 h after): nNO, tryptase/ECP. Measurements of nNO (Hypair FeNO, Medisoft) in the exhaled air were conducted based on on-line nNO concentration analysis, with flow rates of 0.01–1 L/s and approximate pressure of 50 cm H₂O. Tryptase/ECP levels were assessed in the nasal lavage fluid obtained with a collection device made of stainless steel designed by Samoliński (Fig. 1). The device has a stand, which allows the patient to be seated comfortably, two outlets: one for saline influx into nasal cavities, the other connected to a suction tube, for efflux of the lavage fluid at a low pressure of 0.01–0.02 Pa into a test tube placed within the device stand. The tubing for saline influx into the nasal cavity was secured at the bottom with a rubber stopper to minimize loss of the collected material. The collected nasal lavage fluid was centrifuged (at the rate of 1000 rpm) and tested with ELISA (UniCAP, Pharmacia, Sweden, sensitivity threshold 1.0 µg/L). The study was conducted as part of a promotional grant by the Ministry of Science and Higher Education (N N402 520839) and was approved by the Ethical Committee of the Medical University of Warsaw (KB/79/2009). The Student t statistic and the Pearson's coefficient were calculated to determine the correlation between variables. Levene's test was used to estimate the homogeneity of variance. P value of <0.05 was considered to be statistically significant.

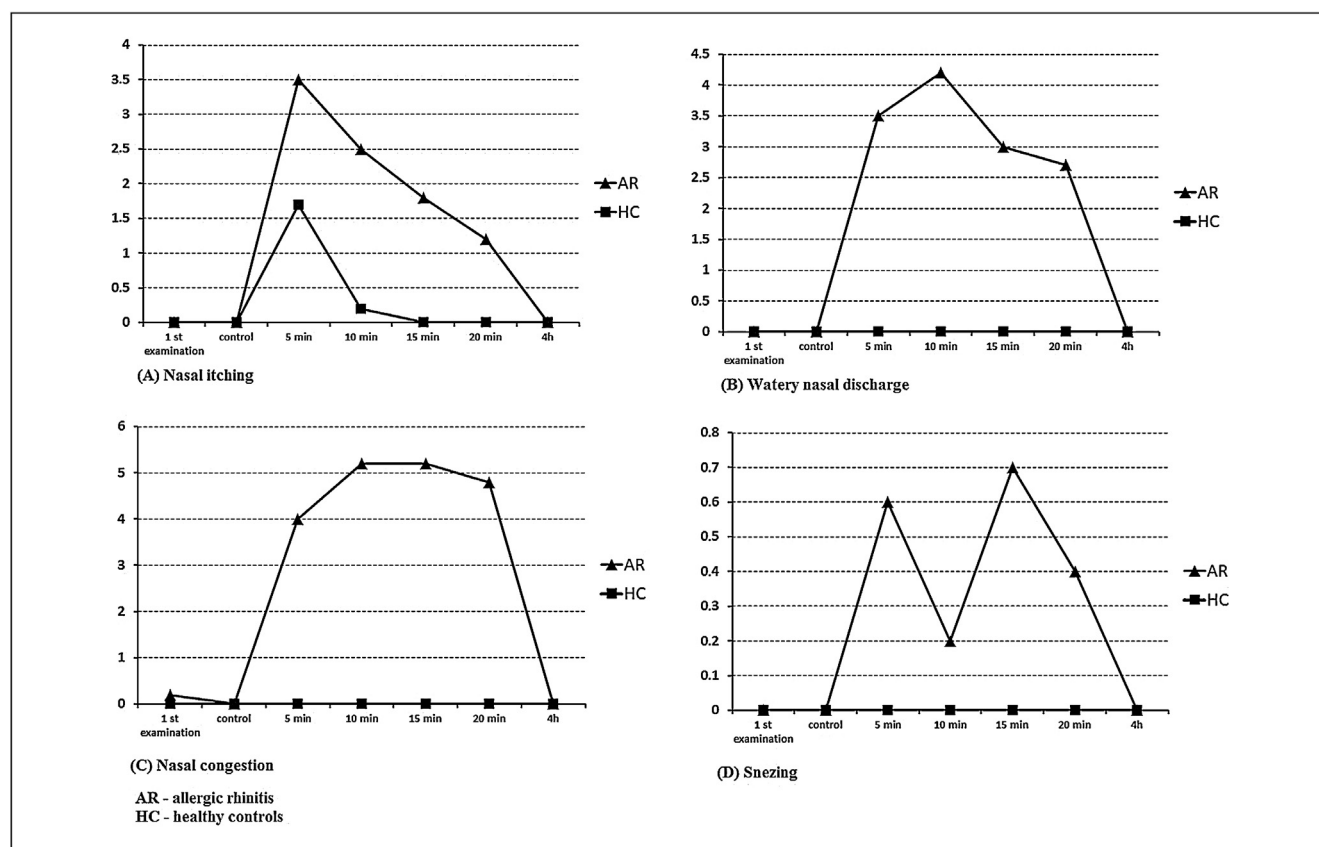


Fig. 2 – Subjective symptom severity measured with a visual analog scale (VAS) in the AR group vs. HC ($P < 0.05$)

Results

Allergen contact with the nasal mucosa initiated a response typical for the early phase allergic reaction in the group allergic to environmental allergens. The recorded manifestations such as nasal itching, sneezing, watery discharge and congestion showed significant differences at every stage of the test; nasal itching: from allergen challenge to minute 5 ($P = 0.006$), minute 10 to minute 15 ($P = 0.034$) and minute 20 ($P = 0.018$), sneezing rate at minute 5 ($P < 0.05$), nasal

discharge at minute 10 ($P < 0.05$) and nasal congestion at minutes 10, 15, and 20 of the test ($P < 0.05$) (Fig. 2). We would like to emphasize that a considerably higher intensity of symptoms was recorded in the PAR group. Conversely, the late phase of the allergic reaction was characterized by nasal and extranasal symptom resolution in the entire study population. Healthy control group showed no nasal or extranasal symptoms in any phases of the study.

During severe nasal symptoms in the early phase of the allergic reaction, we recorded a significant decrease in nNO (by a mean of 22.6%, $P < 0.05$) and its increase in the late

Table I – Inflammatory markers measured in nasal allergen provocation testing (NAPT) in the group diagnosed with allergic rhinitis vs. healthy controls

Markers of inflammation		Mean		Standard deviation		Statistical significance
		AR	HC	AR	HC	
nNO	Before NAPT	1137.03 ppb	628.30 ppb	480.120	363.950	$P < 0.05$
	After NAPT (minute 25)	879.53 ppb	604.63 ppb	474.542	315.069	$P = 0.011$
	After NAPT (hour 4)	1150.50 ppb	655.83 ppb	496.428	387.782	$P < 0.05$
Tryptase	Before NAPT	171.06 $< 1 \mu\text{g/l}$	151.20 $< 1 \mu\text{g/l}$	39.40	29.52	$P = 0.675$
	After NAPT (minute 30)	387.63–2.23 $\mu\text{g/l}$	152.90 $< 1 \mu\text{g/l}$	346.00	29.23	$P < 0.05$
	After NAPT (hour 4)	132.23 $< 1 \mu\text{g/l}$	153.56 $< 1 \mu\text{g/l}$	43.16	32.59	$P = 0.13$
ECP	Before NAPT	269.16 $< 2 \text{ mcg/L}$	163.93 $< 2 \text{ mcg/L}$	270.97	131.93	$P = 0.95$
	After NAPT (minute 30)	199.53 $< 1 \text{ mcg/L}$	162.66 $< 1 \text{ mcg/L}$	184.56	123.41	$P = 0.87$
	After NAPT (hour 4)	945.83–6.11 mcg/L	166.93 $< 2 \text{ mcg/L}$	155.98	136.64	$P < 0.05$

AR – allergic rhinitis; HC – healthy controls; nNO – nasal nitric oxide.

Table II – Correlation between nasal and extranasal symptoms measured via VAS and nasal nitric oxide (nNO) in air concentrate/tryptase and eosinophilic cation protein (ECP)

Markers of inflammation	Total VAS score Pearson's correlation	Statistical significant
nNO before NAPT	0.569**	P = 0.000
nNO minute 35	0.464**	P = 0.000
nNO hour 4	0.529**	P = 0.000
ECP before NAPT	0.267*	P = 0.040
ECP 4 h after NAPT	0.174	P = 0.184
Tryptase before NAPT	0.157	P = 0.231
Tryptase 30 min after NAPT	0.280*	P = 0.030
* Weak correlation.		
** Strong correlation.		

Table III – Markers of inflammation measured via nasal allergen provocation testing (NAPT) in the group diagnosed with seasonal vs. perennial allergic rhinitis

Markers of inflammation		Mean		Standard deviation		Statistical significance
		SAR	PAR	SAR	PAR	
nNO	Before NAPT	1059.44 ppb	1253.42 ppb	427.79	547.95	P = 0.286
	After NAPT (minute 25)	847.33 ppb	927.83 ppb	411.77	536.49	P = 0.657
	After NAPT (hour 4)	1125.39 ppb	1188.17 ppb	488.57	527.46	P = 0.741
Tryptase	Before NAPT	176.88 < 1.00 µg/L	162.33 < 1.00 µg/L	46.04	26.05	P = 0.330
	After NAPT (minute 30)	409.55–2.39 µg/L	354.75–2.16 µg/L	435.947	141.22	P = 0.678
	After NAPT (hour 4)	139.00 < 1.00 µg/L	122.08 < 1.00 µg/L	48.01	34.11	
ECP	Before NAPT	196.61–2.00 µg/L	378.00–2.25 µg/L	141.68	375.52	P = 0.330
	After NAPT (minute 30)	172.33 < 1.00 µg/L	240.33–2.01 µg/L	165.90	210.34	P = 0.06
	After NAPT (hour 4)	596.55–3.41 µg/L	1469.75–8.0 µg/L	689.21	232.39	P = 0.020
SAR – seasonal allergic rhinitis; PAR – perennial allergic rhinitis; nNO – nasal nitric oxide.						

phase comparable to that achieved prior to nasal allergen administration (Table I). The recorded changes in time showed a high correlation with the sum of symptoms measured via VAS. (Table II). Observed changes in nNO concentration were consistent with the significant changes in the levels of inflammatory markers in the nasal lavage fluid. The early phase of the allergic reaction showed higher levels of tryptase, whereas the late phase was characterized by an increase in ECP (three-fold higher than that recorded prior to NAPT, in the allergic rhinitis group) in the nasal lavage fluid. While the two study groups SAR and PAR did not differ significantly in terms of tryptase levels in the lavage fluid, the PAR group demonstrated significantly higher ECP levels ($P = 0.02$) (Table III).

Discussion of results

The nasal mucosa is considered to play a key role in initiating the inflammatory process that spreads to other organs, including the bronchi. The nasal mucosa reaction to the administered allergen is immediate and concerns mostly the activation of cells (mast cells, macrophages) that are enveloped in immunoglobulins, which in effect leads to secretion of reaction mediators, i.e. tryptase, histamine, CysLT, and PGD₂ [4, 5]. The release of tryptase as a result of cell activation is concurrent with the release of histamine; however, the tryptase diffusion to other tissues is much

slower than that of histamine and consequently, reaches the highest serum levels between 60 and 120 min after the anaphylactic reaction [6]. Due to its pathophysiology in as a local “anaphylactic reaction” in the nasal mucosa, NAPT can undoubtedly be a source of valuable information on the degree of sensitivity to a pathogen/allergen. Moreover, elevated tryptase levels can be found in bronchoalveolar lavage and nasal lavage fluids following provocation with a specific allergen [7, 8] in the groups with allergic rhinitis, bronchial asthma or atopic dermatitis. Moreover, tryptase level assessments are also useful in determining risk groups in patients undergoing desensitization to bee/wasp venom [7]. This analysis showed an increase in tryptase levels, especially in the SAR group. Nonetheless, these findings were not statistically significant. It was not until local application of the allergen that the AR and HC groups differed in terms of recorded changes in the early phase of the allergic reaction. Górski et al. observed a similar change with nearly three-fold increase in tryptase levels from 1.2 ± 0.9 to 3.3 ± 0.6 at minute 30 after NAPT [9]. Moreover, Khreisat et al. demonstrated elevated tryptase levels up to $14.5 \mu\text{g/L}$ ($P < 0.005$) following local allergen administration [10].

Changes in the nasal mucosa initiated a series of characteristic manifestations of the early phase of the allergic reaction, which were, consecutively, nasal itching, sneezing, watery nasal discharge, and nasal congestion. The highest intensity of nasal symptoms was recorded at

minutes 15 and 20 of the test (52.5% in the SAR group and 53.9% PAR group). The observed *extranasal* manifestations, such as lacrimation, pharyngeal itching, cough, or dyspnea were very mild.

Parallel to increasing edema of the nasal mucosa (measured via VAS) a significant drop in nNO concentration was observed in the early phase of the allergic reaction. This change in the early phase of the allergic reaction is likely to be associated with increasing nasal congestion at the site of contact with the allergen, which occluded paranasal sinus ostia – the site of nNO production. The recorded change at the level of 22.6% was comparable to that observed by Korn et al., who estimated a 19.2% decrease in nNO [11]. Similar findings were observed by: Boot et al., who reported a drop in nNO by 644 ppb [12], or Maniscalco et al. from 458.4 ppb to 338.6 ppb versus the pre-NAPT value [13]. Serrano et al. measured nNO in a group of 30 patients with grass pollen allergy at minute 15, and hours 2 and 24 following NAPT. They achieved significantly lower nNO levels in the early phase of the allergic reaction from 1497 ppb pre-NAPT to 754 ppb, 1261 ppb, and 1466 ppb at 15 min, 2 h and 24 h after NAPT, respectively (from 1137.03 ppb $P < 0.05$ to 879.53 ppb $P = 0.011$ following allergen administration). Due to its high bioactivity, nitric oxide plays a unique role in physiological and pathological phenomena [14]. It is a marker of inflammation found in the exhaled air and its concentration is several times higher in patients with bronchial asthma or allergic rhinitis. Due to the high sensitivity and specificity in diagnosing bronchial asthma this parameter has been more and more commonly used as a marker to monitor anti-inflammatory treatment [15, 16].

The late phase of the allergic reaction, measured 4 h after allergen application onto the nasal mucosa, was characterized by resolution of all manifestations measured in the early phase and a drop in tryptase levels in nasal lavage fluid. Lopez et al. reported similar findings with a drop in tryptase levels observed 1, 6, and 24 h after nasal allergen challenge [17]. The recorded increase in nNO levels (by 30.8% versus the value measured at 20 min of the test) in comparison to that measured prior to nasal allergen administration was associated with resolution of nasal congestion recorded in the early phase of the allergic reaction. These findings were consistent with those by Boot et al., Valero et al., and Korn et al. who reported an increase in nNO at 7, 8, and 24 h after NAPT, respectively [11, 12, 18].

The parameter that could be reliably used to assess the late phase of the allergic reaction was ECP elevation. ECP (secreted by eosinophils) is a parameter indicating an inflammatory infiltrate associated with eosinophilia. A number of investigators consider ECP to be a marker of allergy severity. Serum ECP levels were shown to increase after allergen exposure in subjects with bronchial asthma even before any symptoms develop and decrease after allergen elimination [19]. Our analysis showed a statistically significant, three-fold rise in ECP levels in the PAR group, which explains the chronic nature of the inflammation irrespective of seasonal symptom exacerbations. A number of investigators emphasized the unique importance of ECP levels in assessing the extent of the body's reaction to pathological effect of the allergen. Pałczyński et al. achieved

a manyfold increase in ECP levels from 3.85 $\mu\text{g/L}$ to 32.17 $\mu\text{g/L}$ in a group of patients with a history of allergy; this change was statistically significant [20]. The findings were consistent with those by Lopez et al., who reported significantly higher ECP levels in 65% of the study population ($P < 0.05$) [17], and Bernardini et al., who achieved significantly higher ECP levels in nasal lavage ($P < 0.05$) at hour 24 of the study [21]. Conversely, Rondón et al. recorded in 30% of subjects significantly elevated levels of the inflammatory marker decidedly sooner, i.e. between hours 1 and 6 of the study; this was statistically significant ($P < 0.05$) [22].

Conclusions

In the early and late phases of the allergic reaction, NAPT with the use of nNO concentration and inflammatory reaction marker measurements offers valuable data on the respiratory system response to nasal allergen challenge. The PAR group demonstrated considerably more severe symptoms, which explains the chronic character of this condition.

Authors' contributions/Wkład autorów

EK-F – study design, data collection and interpretation, statistical analysis, acceptance of final manuscript version, funds collection. AJS – acceptance of final manuscript version, literature search, funds collection. BS – acceptance of final manuscript version, literature search.

Conflict of interest/Konflikt interesu

None declared.

Financial support/Finansowanie

This study was supported by Promotional grant by the Ministry of Science and Higher Education (N N402 520839) in Poland.

Ethics/Etyka

The work described in this article have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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