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Exhaled Nitric Oxide, Bronchial Hyperresponsiveness and spirometric Parameters in Patients with Allergic Rhinitis during Pollen Season

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ABSTRACT

Allergic rhinitis and asthma share common epidemiological features and inflammatory processes. The aim of the present study was to document the influence of natural allergen exposure in exhaled NO (eNO) and in spirometric parameters of patients with seasonal allergic rhinitis(SAR) and to investigate the differences among subjects with positive versus negative bronchial provocation to metacholine(BP_{Mch}).

Twenty-six non-smoking patients (13F/13M; mean age 28.4ys) with a documented history of SAR, 15 healthy, non-atopic(6F/9M; mean age 37.1ys) and 6 non-symptomatic atopic subjects (3F/3M; mean age 36.5ys) were studied. At the first visit during pollen season each subject filled symptom-score card, underwent eNO and nasal NO (nNO) measurements and spirometry. BP_{Mch} was performed within the next 10 days. At the second visit out of pollen season, all measurements but BPMch were repeated. Control subjects underwent eNO and nNO measurements.

eNO was significantly increased during pollen season in BP_{Mch} positive vs BP_{Mch} negative(46.22±32.60 vs 17.81±12.67, p=0.014) and vs non-atopic controls(11.40±5.84, p<0.001) as well as atopic controls(13.56±5.34, p=0.001). No difference was detected out of pollen season in both patients' groups. nNO values were increased only in BPMch(+) group compared to both control groups in pollen season (vs non-atopics p=0.002, vs atopics p=0.002) and only vs non-atopics out of season, p=0.004. Regression analysis has shown that the difference in FEF_{25-75} values (off season-in season) is a predictor of positive BP_{Mch} .

eNO is markedly increased in BP_{Mch} patients with allergic rhinitis while mid-expiratory flow may represent an early marker of lower airway involvement in respiratory allergy.

Keywords: Allergic rhinitis; Asthma; Bronchial hyperresponsiveness; Exhaled nitric oxide; Spirometry.

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INTRODUCTION

Allergic rhinitis is a common IgE-mediated inflammatory condition of the upper airways which is often associated with several co-morbidities that include allergic conjuctivitis, sinusitis, otitis media and asthma.[1] Epidemiological studies consistently report that 60 to 85% of patients with asthma have concomitant allergic rhinitis, whereas up to 40% of patients with allergic rhinitis have clinically demonstrable asthma.[2] Furthermore, several studies have shown that asthma and rhinitis share common inflammatory processes although the exact pathophyciological interactions between upper and lower airways still remain unclear.[3]

Asthma is characterized by a reversible airflow obstruction and forced expiratory volume in the first second (FEV_1) is considered the gold standard to evaluate bronchial obstruction[4]. Recent evidence support the importance of small airways' involvement in the pathogenesis of asthma[5]. So, the forced expiratory flow at the 25% and 75% of the pulmonary volume ($FEF_{25–75}$) as an indirect marker of small airways assessment might be more sensitive to obstruction in small airways than FEV_1. Besides, it has been demonstrated that both FEV1 and mainly $FEF_{25–75}$ are impaired in patients with allergic rhinitis without symptoms from the lower airways[6]. The $FEF_{25–75}$ has been proposed as a reliable marker of early bronchial impairment in allergic rhinitis.[7]

Bronchial hyperresponsiveness (BHR) a distinct feature of pathophysiology in asthma, has been shown to reach a great prevalence among patients with allergic rhinitis; affecting almost 50% of seasonal AR sufferers and 70% of those with perrenial symptoms.[8] The occurence of non-specific BHR in a proportion of non-asthmatic individuals with AR seems to reflect subclinical inflammatory processes in the lower airways. In such patients increased eosinophilic counts and high levels of eosinophilic cationic protein (ECP) have been demostrated at the peak of their pollen season.[9] AR has shown to be a predisposing factor for later developing asthma and the transition from rhinitis only to the developement of clinical asthma is probably a gradual one, with BHR representing an intermediate step.[10]

Nitric oxide, a free radical gas that diffuses freely from its site of production has multiple biological and pathophysiologic functions. As exhaled nitric oxide (eNO) is increased in asthmatic patients and its levels is well correlated with the presence and level of inflammation while it decreases with glucocorticoid treatment[11]. Because the biomarker is non invasively and easily collected, it has the potential to be used not only as a diagnostic tool but also as a management tool for assessing severity, monitoring response to therapy, and gaining control of asthma symptoms. Moreover, numerous studies have shown increased levels of eNO in patients with AR without asthma[12,13] suggesting that this finding could represent an early marker of an underlying ongoing inflammation in the lower respiratory tract.

Seasonal AR (SAR) represents the prevalent type of the disease in Mediterranean countries and although the new clinical terminology according to ARIA guidelines[14] may be more useful in clinical practise, the intermittent allergen exposure during pollen season combined with the minimal persistent inflammation[15] that remains set important research issues.

The aim of the present study was to document the influence of natural allergen exposure on eNO as well as on spirometric parameters of patients with SAR and furthermore to investigate the possible differences among subjects with positive vs negative BHR as reflected by bronchial provocation to metacholine (BP_{Mch}). The possible relationship of the above mentionted parameters in patients with SAR without asthma was the secondary goal of this study.

PATIENTS AND METHODS

Patients Characteristics

Twenty-six non-smoking patients (13 females and 13 males; mean age 28.4 years, age range 16-47 years) with a documented history of SAR at least the last two consecutive years were recruited from the Allergy Unit of "Attikon" University Hospital. The diagnosis of SAR was based on the typical clinical symptoms and the documentation of sensitization with skin prick tests (SPTs), to at least one major inhalant allergen in Greece *(Grasses sp, Parietaria spp or Olea europea)*. Asthma was definitely absent in study participants based on symptoms as well as of the absence of abnormal variability in pulmonary function by spirometry, reversibility and PEF monitoring.

Exclusion criteria for patients recruitment were: a-sensitization in perrenial allergens also, b- seasonal symptoms non consistent with the pollen season of the

offending allergens, c- asthma or past history of antiasthmatic medication during the last two years, d- symptoms or signs of upper or lower airway infection during the last 30 days before inclusion to the study as well as use of nasal or oral corticosteroids or antihistamines during this time period and e- pregnancy.

As control groups, 15 healthy, non-atopic (6 females and 9 males; mean age 37.1 years, age range 27-56 years) and 6 non-symptomatic atopic subjects (3 females and 3 males; mean age 36.5 years, age range 31-43 years) were studied. All control subjects were non-smokers without respiratory tract infection within 30 days prior to the investigation. The Ethics Committee of "Attikon" University Hospital approved the study and written informed consent was obtained from each patient and control subject. Patient anonymity was preserved.

Study Design

Upon entry of patients into the study (December 2007- January 2008, out of pollen season) a detailed history was taken and physical examination and SPTs in inhalant allergens were performed. In all study participants total serum IgE concentration in IU/ml, (UniCAP system, Phadia, Uppsala, Sweden) and measurement of eospinophils in peripheral blood (cells per count) were carried out.

Subjects attended the Allergy Clinic on two occasions, 5-7 months apart. At the first visit (March-May 2008, in pollen season) each subject filled symptom score card, underwent eNO and nasal NO (nNO) measurements and spirometry. BPMch was performed within the next 10 days. At the second visit (November-December 2007, out of pollen season) except BPMch the same investigation was carried out.

Control subjects underwent eNO and nNO measurements at the time period of the first visit.

Symptom Scores

The patients gave an overall assessment of their rhinitis symptoms in both visits. Symptoms of rhinorrhoea, nasal blockage, sneezing, nasal itching, sense of postnasal drip/pharynx irritation and eye streaming/itching were rated on o four-point scale (0= no symptoms, 1= mild, 2= moderate and 3= severe). The sum of the above mentioned symptom scores ranging from 0 to 18, was included in study analysis.

Skin Prick Tests

Atopy was assessed in all subjects (patients and controls) by skin prick tests, using a battery of 22 common inhalant allergens: Dermatophagoides Pteronyssinus, Dermatophagoides Farinae, Phleum pr, Cynodon D, Lolium per, Secale cer, Parietaria off, Artemisia vul, Taraxacum Vulg, Plantago lan, Olea Europea, Platanus, Betula, Quercus, Fagus, Poplar, Cat dander, Dog epithelium, Cladosporum, Penicillium, Alternaria Alt, Aspergillus mix; positive (histamine hydrochloride 10 mg/dl) and negative control (normal saline) were also used (Stallergenes, Paris, France). A wheal diameter>3 mm was considered as criterion of positive skin prick test. For methodological reasons patients were classified as mososensized (sensitization in Parietaria, Olea or grasses) and polysensitized (more than one of the above allergens). Positive SPTs in more than one species of grasses was considered as monosensitization due to the well-documented extended cross-reactivity.

Spirometry and Bronchial Provocation Tests

A MasterScope spirometer (Erich Jaeger, Wurzburg, Germany) was used for flow volume spirometry. The best of three maneuvers was expressed as a percentage of the predicted value.

Bronchial hyperreactivity was assessed by a methacholine provocation test (BP_{Mch}) that took place in Asthma and Allergy Center of the Pulmonary and Critical Care Clinic of University of Athens at Evgenidion Hospital. The methacholine solution (Lopharma, Milan, Italy) was nebulized with a hand-held nebulizer (Dosimeter MB3, MEFAR Bovezzo, Italy). The challenge test was continued with increasing doubling doses up to the dose of methacholine that caused a 20% drop of the baseline FEV_1 value or up to the point where the maximum dose (800 mg) was inhaled. The PDlog20 was calculated automatically by extrapolation of the logarithmic dose response-curve as the cumulative dose causing a 20% fall in FEV_1. In case of FEV_1 decline >10% after the initial dose of 0.9% NaCl the BP_{Mch} test was considered positive and Pdlog20 value was arbitrately set at <0.01 mg. Patients with positive test even at the maximum dose were considered as BP_{Mch} positive.

NO Measurements

Exhaled and nNO were measured with a fast response chemiluminescence analyzer (Analyser CDL

88 sp, ECO MEDICS, Duernten, Sweden) by the online single breath technique, according to the ATS and ERS recommendations[16]. The analyser had a sampling rate of 200 ml/min and a detection rate of 0.1 to 5000 parts per billion (ppb).

Briefly, during eNO measurement procedure, the subjects were asked to perform a single slow exhalation starting from total lung capacity through a mouthpiece and a one-way non-rebreathing valve against a resistance of 16 cm H_2O under a visual biofeedback, to maintain a 50 ml/sec steady flow. Subjects were at rest, sitting down, having refrained from eating and exercise for at least 1 hour, and breathed filtered NO-free air (Denox 88, ECO MEDICS, Duernten, Sweden) without a nose-clip before the single exhalation maneuver.

Ambient air NO was recorded before and after each test. The analyzer was calibrated daily with a zero signal and certified NO/N_2 calibration gases. eNO concentration was measured at the plateau ($\geq$ 2 sec during an exhalation $\geq$ 6 sec) of the end-exhaled reading and expressed as ppb.

The nNO was measured via the same equipment with the best validatated method of aspiration at constant flow rate (3 L/minute) from one naris with gas entrained via the other naris. Slow oral exhalation against a resistance of 16 cm H_2O closed the velum reliably thus, preventing leak of nasal NO via the posterior velopharyngeal aperture.

Statistical Analysis

Data of quantitative (continuous) variables are presented as mean and standard deviation (SD). Normality of distribution was examined with Kolmogorov-Smirnov and Shapiro–Wilk's test. In cases that normal distribution was assessed paired or independent t-test was used for differences of means. In case of a non-normal distributed characteristic, a Mann-Whitney test was carried out.

Chi-square test was used to compare differenses in categorical variables (sex, type of sensitization etc). Crude and adjusted correlations of total serum IgE were investigated with Pearson and Spearman's rho test coefficient. The existence of possible relations between parameters was assessed with chi-square test, linear and logistic regression.

A two tailed p-value less than 0.05 was considered to indicate statistical significance. All analyses were performed with the Statistical Package for Social Science (SPSS, Chicago, IL, USA), version 13.0.

RESULTS

In the whole Study Population

The duration of SAR symptoms in study population had a mean value 9.8 years (range 2-25, SD 5.85). The mean IgE was 489.38 IU/ml (range 17.7-4416.0, SD 912.08) and the eosinophils in peripheral blood 331.28 per mm^3 (range 65-760, SD 209.86). The 11 out of 26 (42.3%) patients were monosensitized and 18/26 (69.23%) had positive BP_{Mch}.

The eNO, nNO measurements and the spirometric values of all study subjects are presented in Table 1a, while the NO values of non atopic controls are shown in Table 1b and of atopic in Table 1c respectively. Both eNO and nNO have shown statistically significant increased values during pollen season while no differences were detected in spirometric parameters.

eNO of patients in comparison with non-atopic controls was significantly higher during pollen season (p=0.001); in contrary no diferrence was detected at the 2^{nd} measurement out of pollen season (p=0.517). Reffering to atopic controls, the SAR group showed increased eNO values during pollen season that almost reached statistical significance (p=0.051) while out of pollen season no difference was shown (p=0.981).

nNO measurements in and out of pollen season showed no difference with both control groups (in season versus (vs) non-atopics p=0.086, out of season vs non-atopics p=0.248, in season vs atopics p=0.106, out of season vs atopics p=0.32).

According to Bronchial Hypperesponsiveness

Nine out of 18 BP_{Mch} positive patients [BP_{Mch} (+)] were males (50%) while the proportion in BP_{Mch} negative patients [BP_{Mch} (-)] was 5/8 (62.5%), p=0.43. Reffering to pattern of sensitization 7/18 (38.9%) of BP_{Mch} (+) and 3/8 (37.5%) of BP_{Mch} (-) were monosensitized (p=0.64).

According to BP_{Mch} result 18 patients were positive (mean age 27.72±8.90 ys, symptom onset 10.67±6.25 ys) while 8 patients were negative (mean age 28.88±8.18ys, symptom onset 7.63±4.72ys). The total IgE showed a value of 324.57±332.78 IU/ml in BP_{Mch} positive and 1041.30±1897.96 IU/ml in BP_{Mch} negative patients. The number of eosinophils/mm^3 in peripheral blood was 357.27±212.30 and 201.33±168.70, respectively. The PDlog20 in Mch positive patients showed a mean value of 249.03±295.29 mg.

Table 1. eNO and nNO values and spirometric parameters in study population; 1a: patients, 1b: non-atopic controls, 1c:atopic controls

Table 1a.

Mean ± SD	Measurement		Statistic	p-value
	In season n=26	Out of season n=26		
eNO	35.86±30.70	16.05±11.97	z = 3.62	**<0.001**
nNO	1387.46±965.29	1015.06±643.39	t = 2.69	**0.012**
FEV1 (%predicted)	97.64±9.65	96.18±11.22	t = 0.94	0.355
FEF_{25-75} (%predicted)	89.19±18.94	87.80±17.64	t = 0.505	0.618
FEV1/FVC	84.42±5.114	84.19±4.26	t = 0.28	0.781
Nasal symptom score	7.54±3.72	0.92±2.61	z = 4.14	**<0.001**

Table 1b.

9 males / 6 females	Minimum (Min)	Maximum (Max)	mean	SD
eNO in pollen season	3.90	24,50	11.40	5,83671
nNO in pollen season	499.40	1110,60	774.95	179,55847

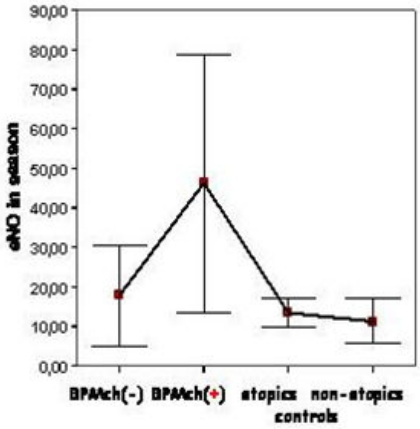
Table 1c.

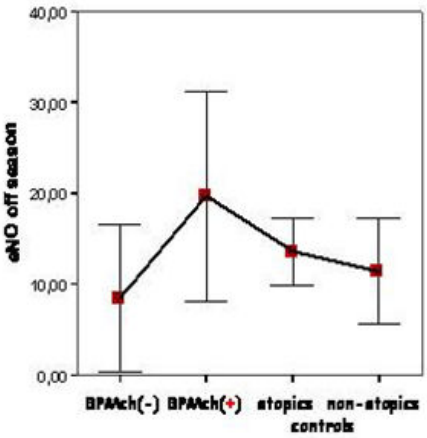
3 males / 3 females	Minimum (Min)	Maximum (Max)	mean	SD
eNO in pollen season	8.70	18.30	13.56	5.33661
nNO in pollen season	381.20	1110.00	742.83	279.95044

The eNO and nNO measurements and the spirometric values of BP_{Mch} (+) and BP_{Mch} (-) are presented in Table 2. The values of eNO during and off pollen season in patients' groups in comparison with controls are presented in Figure 1; BP_{Mch} (+) showed significantly higher values in pollen season vs both control groups and this difference remained significant only vs non-atopics out of pollen season. In contrary, no differences were detected in BP_{Mch} (-) group.

Table 2. eNO and nNO values and spirometric parameters according to bronchial hypperesponsiveness

Mean ± SD	BPMch		Statistic	p-value
	Negative	Positive		
eNO in pollen season	17.81±12.67	46.22±32.60	z = 2.45	**0.014**
eNO off season	8.45±8.02	19.73±11.59	z = 2.59	**0.010**
nNO in pollen season	807.27±471.57	1703.55±1025.02	z = 2.39	**0.017**
nNO off season	811.36±594,28	1155.74±631.89	t = 1.30	0.20
symptom score in season	7.12±4.55	7.94±3.40	t = 1.28	0.13
symptom score off season	0.25±0.71	1.17±3.09	z = 0.57	0.72
FEV1(% predicted) in pollen season	100.62±7.07	96.68±10.70	t = 0.95	0.35
FEV1(% predicted) off pollen season	97.62±6.39	96,49±12.49	z = 0.89	0.37
FEF_{25-75} (% pred) in pollen season	100.62±22.78	85.35±14.47	t = 2.04	0.053
FEF_{25-75} (% pred) off pollen season	91.37±17.44	87.11±16.99	t = 0.58	0.56
FEV1/FVC in pollen season	86.87±5.46	83.41±4.83	z = 1.61	0.107
FEV1/FVC off pollen season	84.87±3.18	84.16±4.48	t = 0.41	0.68
FEF_{25-75} (% pred) Difference off –in pollen season	-9.25±15.72	2.41±12.24	t = 2.03	0.054





Patients vs controls	eNo in season	eNo off season
BPAMch(-) vs non-atopics	0.209	0.32
BPAMch(-) vs atopics	0.39	0.175
BPAMch(+) vs non-atopics	**<0.001**	**0.013**
BPAMch(+) vs atopics	**0.001**	0.061

Figure 1. eNO in and off season in BP_{Mch}(+) and BP_{Mch} (-) patients' groups compared with controls

nNO values were increased only in BP_{Mch} (+) group compared to both control groups in pollen season (vs non-atopics p=0.002, vs atopics p=0.002) and only vs non- atopics out of season, p=0.004.

Correlation Analysis

Total serum IgE values did not correlate with eNO and nNO values in both time points. Similarly, no correlation was detected between symptom scores with eNO, nNO and spirometric values.

The BP_{Mch}(+) did not correlate with the pattern of sensitization (polysensitized or monosensitized); in contrary regression analysis has shown that the difference in FEF_{25-75} values (off season- in season) represents significant predictor of positive BP_{Mch} result. Finally, in BP_{Mch}(+) group PDlog20 did not correlate, although a trend was observed, with eNO values as shown in Figure 2.

DISCUSSION

The results of the present study assessed in subjects with SAR, as sole clinical manifestation of respiratory allergy, that the natural exposure to the allergens during pollen season induces elevated eNO levels that return almost to normal values after the cessation of exposure. These findings were confirmed in comparision with both non-atopic and atopic control subjects. Furthermore, our data clearly demonstrate that elevated eNO concentration is strongly associated with the presense of bronchial hypperesponsiveness in this population; BP_{Mch} (+) represents an important factor that determines the increased NO levels detected in exhaled air of subjects with SAR. Moreover, an important finding of this study was that FEF_{25-75} parameter is lower during pollen season in SAR patients with bronchial hypperesponsiveness, suggesting that it's decrease compared with out of pollen season values could represent an early sensitive

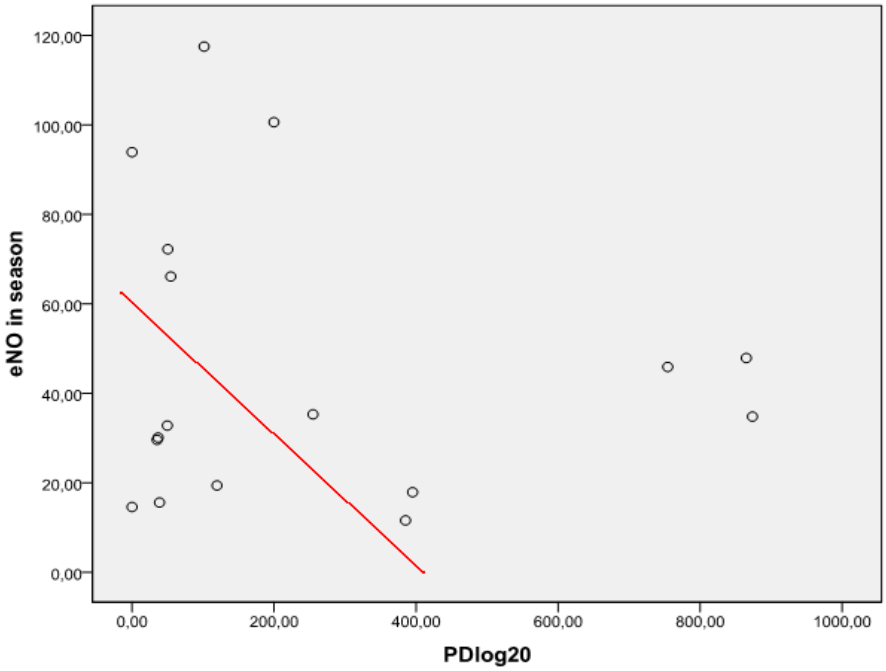


Figure 2. Correlation of eNO in season with PDlog20 in BP_{Mch}(+) patients

marker of lower airways' infllammation in patients with nasal symptoms only.

Referring to eNO, the results of the present study are in aggreement with those of our previous works[17,18] that have shown increased eNO levels in SAR patients during pollen season and with data from other researchers in pediatric[19,20] and adult population.[21] In the present study, no difference was detected in eNO levels between non atopic and atopic controls consistently to another previous study of our group.[17] In contrary, other studies report increased eNO in healthy, non smokers atopics compared to non-atopics with similar characteristics[22] while Prasad et al have shown that healthy atopic children showed higher eNO levels than healthy non-atopic as well as the non-atopic children with asthma.[23] Olin et al provides an interesting explanation for these rather confusing results showing that only atopic subjects that are exposed to the offending allergen show increased eNO levels while their values are within the normal range out of pollen season[24]. However, in our study this does not seem to be the case, as we have performed the eNO measurements in the controls during pollen season. Another remarkable finding is that in the whole study population the increased eNO levels turned to normal values out of pollen season; in contrary Prieto et al. report that eNO concentrations decreased from 63.1 ppb during the pollen season to 30.2 ppb out of season but were still significantly increased when compared with healthy controls (12.8 ppb, p<0.001),[25]. Our findings suggest that atopic status is not the most important determitant of enhanced NO production as others believe; however, the small sample size does not allow us to reach definite conclusions.

A primary aim of the study was to determine differences in eNO concentrations between subjects with SAR with increased responsiveness to metacholine and subjects with nornal responsiveness. The results of our study demonstrate that the presence of increased responsiveness to direct stimulus is associated with increased concentrations of eNO. Studies dealing with eNO correlation with bronchial responsiveness refer in vast majority in subjects with allergic asthma; the conclusions vary as some researchers report positive correlation[26,27] while others failed to find such relation in children with mild intermittent asthma[28] and in adults with mild-moderate asthma.[29] A small study in fourteen subjects with SAR reported increased concentration of eNO in subjects with seasonal rhinitis and increased responseviness to metacholine and adenosine-5-monophosphate,[25] while

the same group in a population of thirty-eight adults with SAR, found increased eNO levels during pollen season in subjects with as well as without bronchial hyperresponsiveness.[30] Although a correlation trend was observed betwween eNO and Pdlog20 in BP_{Mch}(+) this finding did not reach statistical significance; the same was reported in a previous study in allergic rhinitis[30] while a well-designed study in fifty-three asthmatics adults reported signicant correlation of these two parameters.[31] A pssible interpretation could be that the correlation of eNO with PDlog20 cannot be observed in the phenotype of mild respiratory allergy – allergic rhinitis only and/or mild asthma- and is more prominent in more severe forms of asthma.

An important finding in the present study is the demostration of lower mid-expiratory values in spirometry of SAR patients with bronchial hyperresponsiveness during pollen season. Our data are in full accordance with those reported by Ciprandi et al. that found lower FEF_{25-75} values in 65/121 (53.7%) of patients with persistent allergic rhinitis.[32] According to some authors, FEF_{25-75} that reflects predominantly small airway caliber might represent an early indicator of reduced lung function in patients with allergic rhinitis.[33]

Referring to eNO correlation with total serum IgE and the pattern of sensitization, we failed to prove such a relation. In contrary Mete et al. report that the number of positive SPTs in subjects with allergic rhinitis correllates with the severity of bronchial hyperresponsiveness.[34] In accordance other researchers have found that in pediatric population with allergic rhinitis or asthma, eNO concentration correlates with the number of positive SPTs and total serum IgE concentration.[26] The small sample size of our study and the categorization of our population in poly and monosensitized are possible reasons for our contradictory results.

Nasal NO concentration was increased during pollen season only in patients with bronchial hypperesponsiveness, while in the whole population no difference was detected in comparison with both control groups. Data from previous studies are also inconclusive: Kharitonov et al. reported increased nNO concentration in untreated AR patients[35] while Moody *et al.* found no difference compared with controls in subjects with perrenial allergic rhinitis.[36]

In conclusion, the results of this study confirm the elevated eNO levels during pollen season in SAR patients, but furthermore show a clear correlation between bronchial hypperesponsiveness and eNO and suggest that mid-expiratory flow may represent an early marker of lower airway involvement in respiratory allergy. Our findings strongly support the use of these parameters in clinical practise for the close monitoring of patients with allergic rhinitis. Our study, in concordance with other recent studies,[37] imply that lower airway inflammation may exist in patients with allergic rhinitis even in the absence of lower airways' symptoms.

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All co-authors have no conflict of interest to declare.

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