

Exhaled nitric oxide in seasonal allergic rhinitis: influence of pollen season and therapy

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Summary

Exhaled nitric oxide (eNO) has been proposed as a potential indirect marker of lower airway inflammation in asthma. To investigate the existence of lower airways inflammation in allergic rhinitis eNO measurements were performed in 32 patients with symptomatic and asymptomatic seasonal allergic rhinitis early in and out of pollen seasons and in 80 healthy volunteers. To further define how exhaled NO is modified by therapy, NO levels were detected following 1-month treatment with either inhaled steroids or non-steroids therapy with nedocromil. Exhaled NO (mean $\pm$ SE) was significantly elevated in patients with seasonal allergic rhinitis with and without symptoms (24.2 ± 2.5 and 13.9 ± 2.9 ppb, respectively) as compared to healthy volunteers (4.5 ± 0.3 ppb) both in and out of pollen season (21.2 ± 2.1 and 9.0 ± 1.4 p.p.b., respectively) with a higher increase during the allergen exposure in season. Higher levels of exhaled NO were detected in patients with symptoms, either from the upper or lower airways, and with bronchial hyperreactivity. The increased exhaled NO in symptomatic patients was reduced only by inhaled steroids and not by nedocromil. These findings possibly suggest the existence of lower airway inflammation in both symptomatic and asymptomatic patients with seasonal allergic rhinitis in and out of pollen season. Thus, exhaled NO may be used as a non-invasive index for early detection of lower airway inflammation and for monitoring the optional treatment in patients with seasonal allergic rhinitis.

Keywords: exhaled nitric oxide, rhinitis, pollen season, anti-inflammatory therapy

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Introduction

Epidemiological, pathophysiological and clinical studies have strongly suggested a relationship between rhinitis and asthma. Allergic rhinitis is often correlated with asthma, and probably constitutes a risk factor frequently preceding the development of asthma [1,2]. Previous studies have shown that allergic rhinitis is associated with nasal mucosal inflammation similar to that described in asthma in the lower airways [3–9]. Indeed there is strong evidence of epithelial

mast cell and eosinophil accumulation, T-lymphocyte activation and enhanced cytokine expression in nasal mucosa [3,4]. The same proinflammatory mediators, Th₂ cytokine, chemokines, eotaxin [7] and adhesion molecules [8] appear to be involved in the nasal and bronchial inflammation of patients with rhinitis and asthma. Inflammatory cells assessed by sputum induction are present not only in the airways of patients with asthma but also in the airways of patients with seasonal allergic rhinitis even outside natural exposure [9]. A significant proportion of patients with allergic rhinitis also have bronchial hyperresponsiveness even in the absence of other respiratory symptoms [10,11].

Thus, there is evidence suggesting that lower airway inflammation may be present in allergic rhinitis.

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Nitric oxide in exhaled air has been proposed as a potential indirect marker of inflammation in the lower airways [12,13]. Increased levels of NO in exhaled air have been reported in normal individuals with upper respiratory tract infections [14] and in patients with asthma [15,16], especially those with allergic asthma [17]. Treatment with inhaled steroids, which reduces the airway inflammation, is associated with a reduction in exhaled levels of nitric oxide in asthma in a dose dependent manner [18]. The increase in exhaled NO may reflect increased expression of an inducible form of the enzyme NO synthase (iNOS) in response to inflammatory stimuli such as cytokines [19].

Increased levels of nasal and exhaled NO have also been reported by some investigators in symptomatic allergic rhinitis, which may reflect an inflammation of the lower airways [20–22]. This increase can be modulated by local treatment with nasal steroids [21].

The prime aim of this study was to investigate whether exhaled NO is increased in patients with seasonal allergic rhinitis and how NO concentrations may vary early in allergy and out of pollen seasons. The measurement of exhaled NO was used as an indirect index to detect the existence of lower airways inflammation in patients with seasonal allergic rhinitis at these two seasonal time points, early in season in symptomatic and asymptomatic patients and out of pollen season in asymptomatic subjects. A secondary aim of this study was to define how NO concentration in exhaled air is modified either by therapy with inhaled steroids or by non-steroid inhaled anti-inflammatory therapy such as nedocromil.

Material and methods

Study subjects

We studied 32 subjects with a mean age of 37.0 ± 10.5 years ($x \pm SD$) with history of documented seasonal allergic rhinitis for at least 2 years. At the beginning of the study nine patients were asymptomatic

and came to the clinic for a routine follow-up, whereas 23 patients were symptomatic. From those patients with symptoms 10 patients had only rhinitis symptoms and 13 had additionally asthma-like symptoms such as dry cough or wheezing. Patients' characteristics are shown in Table 1.

All patients were atopic and they had at least a positive skin prick test (> 3 mm wheal) to common seasonal aeroallergens such as grasses, *olea europaea*, *parietaria judica*.

None of the patients had nasal polyposis, sinus disease, perennial rhinitis or other serious illness and none was treated with any nasal or inhaled medication before the study.

A control group of 80 healthy non-smoking volunteers with a mean age of 32 ± 10 years ($x \pm SD$) also participated in the study. These control subjects had no history of any respiratory disease (asthma or rhinitis) or any other illness, they were receiving no medication, and were non-atopic.

None of the patients or healthy volunteers had any history of upper or lower respiratory infection in the month preceding the study. All subjects were non-smokers.

Subjects gave their consent for participation in the study and the study protocol was approved by the local ethics committee of the hospital.

Study design

Patients with seasonal rhinitis were recruited from the outpatient allergy clinic. A detailed history was taken and a clinical assessment was performed in all subjects at the study entry. Rhinitis and asthma-like symptoms (dyspnoea, cough or wheezing) were recorded in the patients file. Atopy was assessed by skin prick tests, using the most common allergens (*Derm. Pteronissinus*, *Derm. Farinae*, Mixed Grasses I and II, *Parietaria* sp., Weeds, Timothy, Flowers, Mixed Trees I and II, *Olive Europaea*, Pine, Cat and Dog Hair, Mixed Moulds, *Aspergillus*, *Alternaria*,

Table 1 Subject characteristics

Patient group	n	Age (yr) (mean $\pm$ SD)	Sex M/F	FEV ₁ (% pred.) (mean $\pm$ SD)	FEV ₁ /FVC	No of patients with BHR positive (PD ₂₀ $\leq$ 2 mg/mL)
Controls	80	32 $\pm$ 10	32/48	105 $\pm$ 15.1	102 $\pm$ 4.3	ND (not done)
Symptomatics with seasonal rhinitis	10	37 $\pm$ 13	4/6	103 $\pm$ 13	99 $\pm$ 8	3/10
Symptomatics with seasonal rhinitis and asthma	13	35 $\pm$ 10	5/8	102 $\pm$ 17	101 $\pm$ 9	10/13
Asymptomatics	9	42 $\pm$ 8	1/8	114 $\pm$ 8	103 $\pm$ 5.8	3/9

Table 2 Inhaled treatment in different patient groups

Patient group	Total No.	No. of patients with positive BHR (PD ₂₀ < 2 mg/mL)	Therapy with inhaled steroids**	Therapy with inhaled nedocromil**
Symptomatics with rhinitis	10	3	6 (2)*	4 (1)*
Symptomatics with asthma and rhinitis	13	10	11 (9)*	2 (1)*
Asymptomatics	9	3	3 (1)*	6 (2)*

*Number in brackets is the number of patients with a positive bronchial hyperreactivity test in each treatment group.

**All patients were also treated with nasal steroids.

Candida, Positive Histamine control, Negative Saline control) (HAL allergenen Lab B.V. Harlem).

At the first visit, at the start of the pollen season, all subjects (patients and controls) underwent measurements of nitric oxide (NO) in nasal and orally exhaled air. Additionally, a baseline spirometry and a methacholine challenge test for assessment of bronchial hyperreactivity was performed on the same day after the NO measurements. Patients were not allowed to consume any caffeine 2 hours prior to the NO measurements nor use aerosol bronchodilators for 8 hours before any challenge.

At the first visit patients were advised to take prophylactic treatment for at least 1 month. The treatment was selected randomly by the treating physician and consisted either of inhaled steroids or nedocromil, an inhaled non-steroid anti-inflammatory therapy. Twenty patients were treated with inhaled steroids (budesonide 400 µg daily or fluticasone propionate 250 µg daily), whereas 12 patients were treated with nedocromil sodium (8–12 mg daily). The treatment given in the three different patients groups is shown in Table 2. In all patients nasal steroids were administered as prophylactic treatment (budesonide or fluticasone 50 µg daily) for 1 month.

A second visit to the department was made by all patients with seasonal rhinitis 1 month following prophylactic therapy either with inhaled steroids or with nedocromil. A clinical assessment based on clinical symptoms and examination and nasal and exhaled NO measurements were also performed at this visit.

A third visit was made by the patients 4–7 months later, in a period of the year that was out of pollen season, and a clinical assessment as well as nasal and exhaled NO measurements were repeated. A bronchial hyperreactivity test with methacholine was not performed at this time point in a sufficient number of patients as some of them did not accept the repetition of the procedure.

The clinical assessment based on clinical symptoms and examination was performed and recorded in patients' files by the same physician for all visits.

Pulmonary functions and bronchial hyperreactivity tests

A water sealed spirometer (Biomedin, Milan, Italy) was used for measuring FEV₁ and FVC. The best of three manoeuvres was expressed as a percentage of the predicted value.

Bronchial hyperreactivity was assessed by a methacholine provocation test. The methacholine solution was nebulized with a hand-held nebulizer (Dosimeter MB3, MEFAR Bovezzo, Italy). The methacholine concentration increased from 0.01 mg/mL to 2 mg/mL. The challenge test was continued up to the dose of methacholine that caused a 20% drop of the baseline FEV₁ value or up to the point where the maximum dose was inhaled. The PD₂₀ was calculated automatically by interpolation of the logarithmic dose response curve as the cumulative dose causing a 20% fall in FEV₁. Following the methacholine challenge subjects were given two puffs of salbutamol (200 mg) by a metered dose inhaler.

Nitric oxide measurements

The measurement of NO concentration in the expired and nasal air in this study was performed in accordance with the recommendations of the European Respiratory Society (ERS) Task Force Report on standardization of equipment specification and respiratory manoeuvres [23]. Exhaled NO was measured by a chemiluminescence analyser (model LR2000, Logan Research, Rochester, UK) sensitive to NO from 1 to 5000 p.p.b., which was designed for on line recording of exhaled NO concentration. The analyser was calibrated daily with NO/N₂ calibration gas containing 116 p.p.b. In addition to NO this analyser measures carbon dioxide concentration, pressure and volume in real time. The subjects exhaled slowly from total lung capacity over 30–40 s through a mouthpiece against a mild resistance to exclude nasal contamination, as has been shown previously, probably due to elevation of the soft palate against the oropharynx. All subjects maintained an exhalation flow

rate of 250 mL/s. NO was sampled from a side arm attached to the mouthpiece. The mean value was taken from the point corresponding to the plateau of end-exhaled CO₂ reading (5–6% CO₂) as representative of an alveolar sample. The results of the analysis were computed and graphically displayed on plots of NO and CO₂ concentrations, pressure and flow rate against time.

Nasal NO was measured with a Teflon tube inserted into one of the nostrils, whilst the subject held his/her breath (i.e. with no active exhalation), after having inspired to total lung capacity. This allowed analysis of the local NO concentration with free flow of ambient air from one nostril to the other and subsequent direction into the analyser. The NO level was measured when a plateau level was achieved and in the absence of a CO₂ signal, indicating satisfactory breath holding. Three readings each of exhaled and nasal air were taken, with the results being expressed as the mean value.

Statistical analysis

Exhaled NO values were expressed as mean $\pm$ SE. Analysis of variance for NO values classified by groups using Scheffe test and two-ways analysis of variance for repeated measures in between different treatment groups was used. Spearman correlation was used between exhaled NO values and bronchial hyperreactivity. A P -value < 0.05 was considered significant.

Results

Spirometry and methacholine provocation test

All patients had FEV₁ higher than 80% of predicted values (min. 80% – max. 142%) and a mean of FEV₁/FVC 100.8 ± 8.0 (min. 84% – max. 118%).

Bronchial hyperresponsiveness was tested in 29 patients with seasonal rhinitis (three patients refused to be tested) at the first visit and 16 patients had a positive methacholine test (55%) (cumulative PD₂₀ < 2 mg/mL). Details of patients regarding existence of symptoms, lung functions and bronchial hyperreactivity are shown in Table 1.

Exhaled NO and nasal NO levels in and out of pollen season

The exhaled NO levels in patients with seasonal allergic rhinitis were nearly five-fold greater than in controls (mean $\pm$ SE 21.2 ± 2.13 ppb vs. 4.5 ± 0.3 ppb, $P < 0.001$) when measured at the start of pollen season. When measured out of season the levels of exhaled NO in these patients with seasonal allergic rhinitis showed

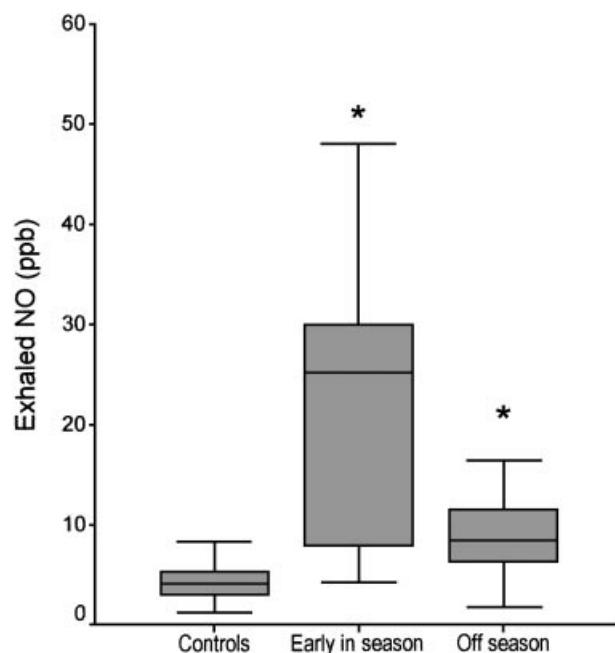


Fig. 1. Exhaled NO levels in control subjects and in patients with seasonal allergic rhinitis early in season and out of pollen season. (Boxes present the media, the 25th and the 75th percentile of per cent.) Asterisk denotes significant differences in between groups: * $P < 0.001$.

statistically significant ($P < 0.005$) lower values (9.0 ± 1.4 ppb), compared to the levels found early in season. However, these levels were still significantly increased ($P < 0.01$) as compared to the NO values in controls. (Fig. 1)

Nasal NO levels were not significantly different when measured early in the season (884 ± 127 ppb) and out of season (673 ± 122 ppb) and did not differ from control values (707 ± 32 ppb).

Significantly increased levels of exhaled NO were detected in both symptomatic (24 ± 2.5 ppb) and asymptomatic patients (13.9 ± 2.9 ppb) compared to controls, with a much higher increase in patients with symptoms ($P < 0.001$) (Fig. 2). Between symptomatic patients exhaled NO measurements were not different when compared to those who had only rhinitis symptoms (24.2 ± 4 ppb) and those who had additionally asthma-like symptoms (23.9 ± 3.0 ppb) (Fig. 2).

The hyperresponsive patients with seasonal rhinitis had two-fold greater values (26.4 ± 2.5 ppb) than the non-hyperresponsive subjects (14.0 ± 2.8 ppb) ($P = 0.003$). A statistical significant correlation was found between levels of exhaled NO and the slope of methacholine provocation curve (with Spearman correlation coefficient $r = 0.432$, $P = 0.019$) (Fig. 4) with no correlation with PD₂₀ ($r = 0.268$, $P = 0.333$).

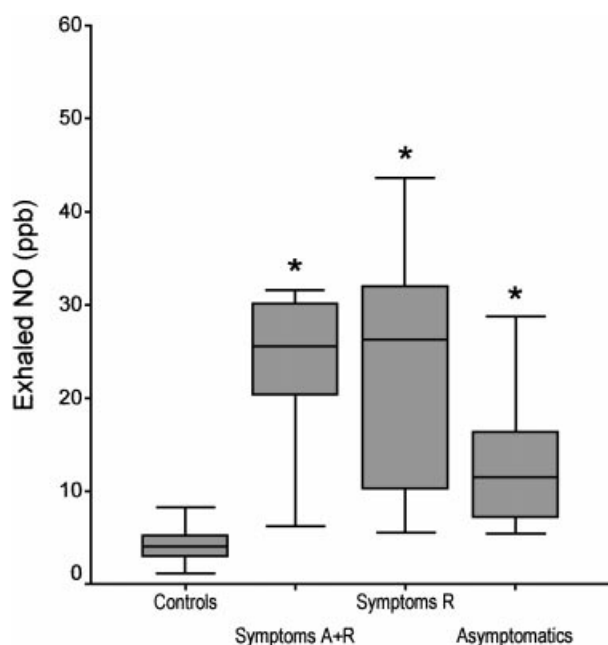


Fig. 2. Exhaled NO levels measured early in season (a) in control subjects, (b) in patients with asthma-like symptoms and rhinitis (A + R), (c) in patients with symptoms of rhinitis (R), (d) in asymptomatic patients. (Boxes present the media, the 25th and the 75th percentile of per cent.) Asterisk denotes significant compared with controls: $P < 0.001$.

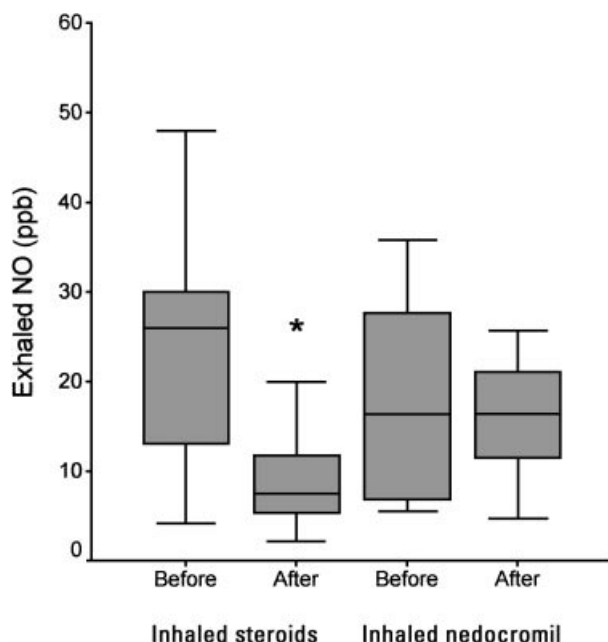


Fig. 3. Exhaled NO levels in patients with seasonal allergic rhinitis before and after inhaled therapy with steroids and nedocromil. (Boxes present the media, the 25th and the 75th percentile of per cent.) Asterisk denotes significant differences compared with the exhaled NO values before therapy with steroids: $P < 0.001$.

Exhaled NO and nasal NO levels before and after treatment

Prophylactic treatment for 1 month with nasal and inhaled steroids prevented all asymptomatic patients with seasonal allergic rhinitis developing rhinitis or asthma-like symptoms (dry cough or wheezing) and significantly improved the clinical symptoms in symptomatic patients. The clinical improvement was assessed by clinical examination and symptoms referred by the patient and evaluated by the same physician. In contrast, treatment with nasal steroids and nedocromil prevented patients from clinical symptoms of rhinitis but did not reduce the occurrence of dry cough or wheezing in season.

In addition, treatment with inhaled steroids significantly reduced the levels of exhaled NO (from 23.7 ± 2.7 to 9.4 ± 1.4 ppb, $P < 0.001$) while therapy with nedocromil did not cause any significant change in the values of exhaled NO measurements (from 17.8 ± 3.5 to 15.6 ± 2.1 ppb). (Fig. 3). Exhaled NO values before and after each treatment in different patient groups according to the existence of symptoms are shown in Table 3.

No significant change in nasal NO was observed following treatment either with the combination of nasal and inhaled steroids (from 859 ± 196 to 649 ± 73 ppb) or the combination of nasal steroid and inhaled nedocromil (from 923 ± 133 to 900 ± 187 ppb).

Discussion

In this study increased levels of exhaled NO were found in subjects with seasonal allergic rhinitis as compared to normal control subjects when measurements were performed early in the pollen season. Lower concentrations of exhaled NO were found in seasonal sensitized patients out of the pollen season, but these NO levels were still higher compared to the NO levels found in normal subjects.

If the elevated exhaled NO is associated with lower airway inflammation [12] our results show that there might be a chronic inflammation in the lower airways in patients with seasonal allergic rhinitis not only during the time of high allergen exposure in the pollen season but also out of the pollen season.

According to our findings exposure of the sensitized patients to aeroallergens during season aggravates lower airways inflammation and potentially leads to clinical symptoms, although a persistent milder lower airways inflammation is present in patients with seasonal rhinitis even when they are asymptomatic and out of the pollen season.

Our results are in accordance with those of a previous study by Martin *et al.* [20] who reported increased exhaled NO levels in symptomatic patients with seasonal rhinitis when measurements were performed during the pollen

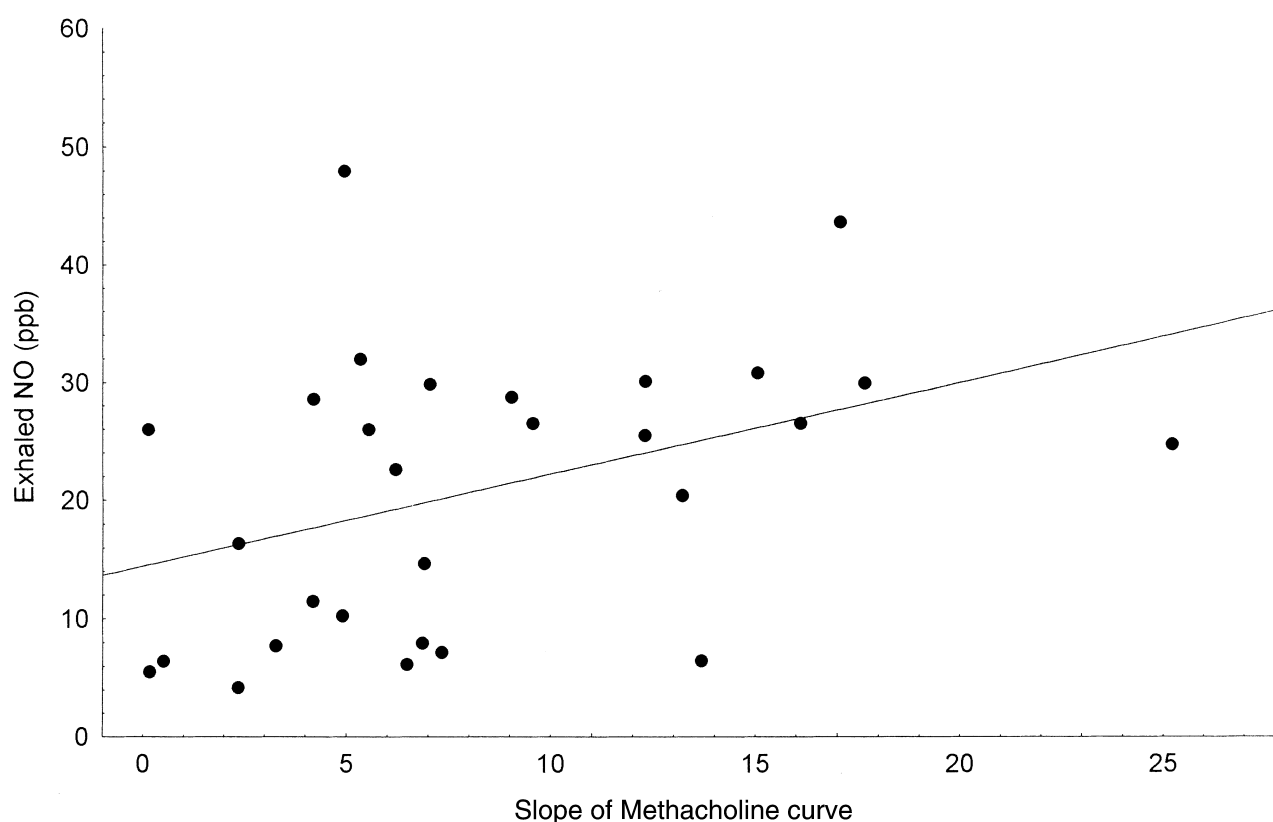


Fig. 4. Relationship of exhaled NO levels early in season and the slope of Methacholine test in patients with seasonal rhinitis. (Spearman correlation coefficient $r = 0.432$, $P = 0.0192$.)

season. In our study measurements were performed early in season in symptomatic and asymptomatic patients with seasonal allergic rhinitis. Furthermore when exhaled NO measurements were repeated out of the pollen season we found significantly elevated levels of exhaled NO in those patients with seasonal allergic rhinitis as was reported in the study by Henriksen *et al.* [22].

Our results are in contrast to those reported by Kharitonov *et al.* [21] who did not detect any difference in exhaled NO concentration between patients with seasonal allergic rhinitis without clinical asthma and control subjects. The increase of exhaled NO in the present study cannot be explained by other factors known to influence the concentration of exhaled NO. Current

Table 3 Exhaled NO before and after therapy in patient groups

Patient group	No	Therapy with inhaled steroids**		No.	Therapy with inhaled Nedocromil**	
		eNO before therapy (mean $\pm$ SE)	eNO after therapy (mean $\pm$ SE)		eNO before therapy (mean $\pm$ SE)	eNO after therapy (mean $\pm$ SE)
Symptomatics with rhinitis	6	27 $\pm$ 4.3	*7.9 $\pm$ 1.3	4	20 $\pm$ 8.1	12 $\pm$ 4.1
Symptomatics with asthma and rhinitis	11	25.6 $\pm$ 3.6	*11.1 $\pm$ 2.2	2	14.4 $\pm$ 8.2	13.3 $\pm$ 7.5
Asymptomatics	3	10.5 $\pm$ 2.7	6.7 $\pm$ 1.9	6	15.6 $\pm$ 4.1	17.6 $\pm$ 2.6

* $P < 0.05$ (ANOVA analysis). **All patients were also treated with nasal steroids.

smoking, acute upper or lower respiratory tract infection were absent in both the control and allergic rhinitis groups. Contamination of the exhaled air by NO coming from the nasal cavity is unlikely, since in the method used for the measurement of NO the soft palate is elevated when expiration is performed against a resistance [23] and the exhaled NO concentration is what is mainly derived from the lower respiratory tract [13].

In our study NO measurements were performed early in season in a group of sensitized but asymptomatic patients before symptoms occurred and in a group of symptomatic patients suffering either from rhinitis symptoms alone or having additional asthma-like symptoms such as dry cough or wheezing. The increased levels of exhaled NO found early in the season in asymptomatic patients with a history of seasonal allergic rhinitis suggest the presence of lower airways inflammation even in the absence of symptoms. The higher concentration of exhaled NO in those patients with symptoms may reflect the higher degree of lower airways inflammation.

It is remarkable that in the present study a similar increase in exhaled NO levels was detected in symptomatic patients with seasonal rhinitis irrespective of the presence of asthma-like symptoms. Sub-clinical inflammation has also been reported in patients affected by allergic rhinitis, with evidence of eosinophil accumulation and enhanced collagen deposition [6]. This finding suggests that lower airways inflammation is present in patients with rhinitis even without clinical asthma and indicates that there is a common pathophysiological link between these two different clinical entities. This in turn might support the view that allergic rhinitis and allergic asthma belong to one common disease of common airways [24]. The identification of elevated NO levels in patients with allergic rhinitis suggests that NO measurements is a sensitive method of monitoring lower airway inflammation in these patients.

The existence of airway inflammation in the lower airways in patients with seasonal rhinitis may be linked to a greater risk of development of asthma later in life. The data from the present study support this speculation by showing that patients with seasonal rhinitis who are hyperresponsive to methacholine have significantly higher exhaled NO levels than the non-hyperresponsive patients. It is known that allergen sensitization and bronchial hyperresponsiveness are associated with a greater risk of developing asthma. Thus, our data, like those by Henriksen *et al.* [22], lead to the conclusion that exhaled NO, in addition to being a non-invasive marker of airway inflammation in asthma [12,15,16], might also be a potential prognostic marker for identifying those with allergic rhinitis who may be at risk of developing asthma.

Furthermore, the early detection of airways inflammation in rhinitis, by the measurement of exhaled NO, may be

useful in prevention of the natural progress of the disease to asthma by modulating therapy.

We have shown in this study that inhaled steroids significantly decreased the levels of exhaled NO in symptomatic patients in contrast to inhaled nedocromil. Inhaled glucocorticoids have been shown to inhibit iNOS expression *in vitro* [25–27] and treatment with glucocorticoids has been shown to decrease exhaled NO in patients with stable asthma [18,28] or during an acute exacerbation [29].

In our study 1-month concomitant treatment with nasal and inhaled steroids was related in symptomatic patients to clinical improvement of symptoms, both of the upper and lower airways, and to a decrease of exhaled NO levels, suggesting an effect on the degree of lower airways inflammation. Treatment with inhaled nedocromil, a non-steroid anti-inflammatory agent did not prevent the occurrence of symptoms such as dry cough or wheezing and did not influence at all the levels of exhaled NO. As exhaled NO is considered a marker of lower airways inflammation our results suggest that treatment with inhaled steroids is required to control lower airways inflammation in patients with rhinitis. A more prolonged prophylactic treatment of patients with seasonal rhinitis with inhaled steroids may further reduce this lower airway inflammation and possibly may control the progress of the disease. This hypothesis should be addressed in future prospective studies.

Surprisingly, nasal NO concentration was not statistically different in and out of season in patients with seasonal rhinitis and the minor NO increase seen in patients when compared with normal subjects was not significant. Similar findings are also reported by some other investigators; [22] however, the absence of a significant increase in nasal NO in season in patients with allergic rhinitis is in contrast to some previous reports [20,21]. Our group of patients included both symptomatic and asymptomatic subjects. In symptomatic patients in season the absence of increased nasal NO might be due to nasal congestion and the reduced passage of NO-rich air. Acute nasal congestion induced by nasal challenge reduces nasal NO in atopic subjects, whereas nasal NO levels have been shown to correlate negatively with symptom scores [21]. Different severity of nasal symptoms might explain the discrepancy of results between studies as the same technique of nasal NO detection was used.

In conclusion, the results of this study have shown that exhaled nitric oxide is significantly elevated in patients with seasonal allergic rhinitis with and without symptoms both in and out of the pollen season with a more significant increase during the higher allergen exposure in season. Higher levels of exhaled NO are detected in patients with symptoms, either from the upper or lower airways, and with bronchial hyperreactivity. These increased exhaled NO

levels can be modulated only by inhaled steroids given as anti-inflammatory treatment.

Thus, it is tempting to propose that exhaled NO may be used as a non-invasive index for early detection of airway inflammation in patients with allergic rhinitis and for monitoring the optional treatment in order to prevent the natural progression of the disease and further minimize the risk of these patients developing asthma.

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