

Exhaled nitric oxide in asthmatic children and adolescents after nasal allergen challenge

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Epidemiological data suggest a comorbidity link between nasal and bronchial allergic disease. Exhaled nitric oxide (F_{ENO}) is a sensitive marker of bronchial inflammation and increases after bronchial allergen provocation. We studied F_{ENO} in 19 children and adolescents with allergic asthma and 10 controls before and 2, 6 and 24 h after a single nasal allergen challenge. The correlation between F_{ENO} and other markers of allergic inflammation, such as eosinophils in blood and eosinophil cationic protein (ECP) in serum and nasal lavage was also assessed. F_{ENO} remained unchanged 24 h post-challenge in both steroid and steroid-naïve patients. At 6 h post-challenge, F_{ENO} decreased in both asthmatics and controls. The asthmatic subjects showed a positive correlation between F_{ENO} and blood eosinophils before ($r = 0.71$, $p = 0.001$) and after the challenge, and between F_{ENO} and ECP in nasal lavage ($r = 0.62$, $p = 0.02$) 2 h after the challenge. Mean ECP in nasal lavage increased post-challenge but not significantly. We conclude that a single nasal allergen challenge does not augment bronchial inflammation although F_{ENO} is related to blood eosinophil count and to the nasal inflammatory response. Our data do not support the theory of a direct transmission of the nasal inflammation to the lower airways.

**Christophe Pedroletti¹,
Joachim Lundahl², Kjell Alving³ and
Gunilla Hedlin¹**

Departments of ¹Woman and Child Health, ²Allergy and Immunology Research and ³Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden

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Christophe Pedroletti, Department of Allergy and Lung Disease, Astrid Lindgren Childrens Hospital, Karolinska Hospital, 171 76 Stockholm, Sweden
Tel.: +46 8 517 77570
E-mail: christophe.pedroletti@kus.se

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Large epidemiological studies have explored the relationship between rhinitis and asthma (1, 2), and have led researchers to propose the concept of 'united airways'. There has been a lot of interest in the nose–lung interaction but the mechanisms (3) are, nevertheless, still unclear. Potential pathways of direct nose–lung interaction could be inhalation of inflammatory mediators from the nose to the lung or a (neurogenic) naso-bronchial reflex inducing acute bronchial constriction. However, the mechanism could also be indirect: the result of a systemic disease characterized by increased number of activated eosinophils in blood and tissue.

Nitric oxide (NO) in exhaled air (F_{ENO}) has been used as a marker of airway inflammation. Numerous studies have shown elevated levels of NO and a positive correlation with eosinophilic inflammation in asthma (4–8) and increased levels of F_{ENO} after bronchial allergen provocation (9).

However, it is still unclear whether a nasal allergen challenge induces lower airway

inflammation, which can be measured as an increased F_{ENO} , and whether NO in nasal air (nNO) could be used to assess nasal inflammation resulting from nasal allergen challenge.

The primary objective of our study was to find out whether a single nasal allergen challenge in children allergic to cat would affect bronchial inflammation reflected by F_{ENO} .

Material and methods

Subjects

Nineteen children (mean age 13 yr) with a history of rhinitis, mild-to-moderate asthma, normal lung function (forced expiratory volume in 1 s, FEV_1 , > 70%) and a positive skin prick test to cat were enrolled in the study. Twelve children were treated with inhaled steroids, either fluticasone propionate or budesonide (median dose 100 µg/day). Other anti-inflammatory treatment, including nasal steroids, leukotriene antagonists,

anti-histamines and anti-allergics, was not accepted. None of the asthmatic children kept furred pets at home. Ten non-atopic non-asthmatic children served as controls (mean age 12 yr). All subjects were non-smokers and had no signs of upper respiratory tract infection during the last week prior to investigation. For further subject characteristics see Table 1.

Study design

All children were challenged with increasing doses of cat allergen. $F_{E}NO$, nNO and lung function measurements were performed at baseline and 2, 6 and 24 h post-challenge.

Blood eosinophils, serum eosinophil cationic protein (ECP) (asthmatics and controls) and ECP in nasal lavage (asthmatics only) were also investigated at baseline, 2 and 24 h after the provocation.

NO measurements

We measured $F_{E}NO$ and nNO on-line with the chemiluminescence technique (Aerocrine NO system, Aerocrine AB, Stockholm, Sweden). $F_{E}NO$ measurements were performed according to the recommendations made by the American Thoracic Society (ATS) (10). Measurement of nNO was performed by a similar technique described in

the previous document. Briefly, NO-free air is insufflated through the nasal cavity at a flow rate of 50 ml/s. Simultaneously, the $F_{E}NO$ manoeuvre is performed to close the vellum and prevent oral NO from diffusing into the nasal cavity. Nasal gas is constantly sampled from this circuit, directly into the analyser to also allow nNO measurements on-line. Mean nNO is calculated during a nNO plateau of at least 3 s. A plateau is approved when nNO does not vary more than 10%.

Nasal challenge

Challenge was performed outside the pollen season (September–December) with a water-based extract of cat dander (Aquagen SQ, ALK, Horsholm, Denmark) according to a standardized protocol. A volume of 0.1 ml at 10-fold increasing concentration was instilled in the right nostril. The concentrations ranged from 0 (diluent) to 100,000 SQ units/ml. An acute phase reaction was considered to have occurred when at least one of the following symptoms had occurred: sneezing (≥ 3 times), itchy throat or nose, heavy rhinorrhea, or nasal congestion.

Nasal lavage

Nasal lavage was collected according to a standardized procedure (11). We used a

Table 1. Subject characteristics

Asthmatics								Controls					
Age	Sex	Height	Weight	FEV ₁	$F_{E}NO$	IGCS	Drug	Age	Sex	Height	Weight	FEV ₁	$F_{E}NO$
15	F	160	50	83	20.3	100	F	11	F	161	69	81	13.4
11	F	161	72	94	20.1	100	B	16	F	163	52	98	8.9
12	M	160	56	78	18.1	400	B	11	F	149	60	96	4.1
9	F	131	37	106	3.6	100	B	18	F	166	76	93	2.9
16	M	171	49	85	320.3	500	F	12	M	154	55	113	6.3
12	F	157	50	78	37.2	0		9	M	134	28	96	3.3
15	M	173	69	103	61.8	100	B	11	F	143	39	91	12.2
16	F	166	50	98	59.6	100	B	11	M	145	36	94	8.1
12	F	159	52	114	11.8	100	B	9	M	130	27	116	4.1
12	M	150	38	98	32.8	100	B	12	M	152	34	84	20.6
15	M	160	58	85	106.5	0							
13	M	147	53	89	6.7	0							
13	F	162	51	93	18.3	100	F						
9	M	145	48	82	7.5	0							
14	M	177	57	68	53.0	100	F						
18	M	175	66	116	26.6	0							
10	F	142	44	74	2.9	350	F						
13	M	157	55	86	107.3	0							
13	F	167	47	83	3.4	0							
13		160	51	86	20.3			11		150	45	95	7.2

Age is expressed in years, height in centimetres and weight in kilograms.

FEV₁, forced expiratory volume in 1 s, percentage of predicted; $F_{E}NO$, fraction of exhaled nitric oxide, parts per billion; IGCS, inhaled glucocorticosteroids, micrograms/day. Drugs: F, fluticasone propionate; B, budesonide.

The last row represents the median values.

disposable metered-dose pump filled with isotonic saline solution at room temperature. Excessive mucus was first cleared by one spray of the saline solution immediately followed by a forceful exsufflation through the nostril. Directly after, multiple sprayings were performed until 2–3 ml had passively returned into a plastic cup. The recovered fluid was then centrifuged and the supernatant aspirated and stored at -20°C for further analysis of inflammatory markers.

Measurements of eosinophilic markers in blood and nasal lavage

The eosinophil blood cell count was performed with flow cytometry. ECP concentrations were determined with radioimmunoassay (ECP RIA kit, Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). Nasal ECP (n-ECP) was only measured in asthmatic children. To correct for dilution effects between patients, n-ECP was divided by the concentration of total protein (BCA protein assay, Pierce Chemical Company, Rockford, IL, USA) in nasal lavage fluid.

Statistics

Normal distribution was based on normal probability plots. The two-way ANOVA test was used to evaluate changes in the parameters over time and

differences between groups. One-way ANOVA was used to study changes in n-ECP in asthmatics. Correlations between markers were studied with Pearson's test. $p < 0.05$ was considered significant.

Ethics

The study was approved by the local ethics committee at the Karolinska Hospital and the children and their parents gave their informed consent before inclusion.

Results

All patients showed a significant acute allergic nasal reaction following nasal allergen challenge. None of the controls reacted to the provocation. The results of all parameters under investigation are presented in Table 2.

$F_{\text{E}}\text{NO}$, blood eosinophils, serum ECP (s-ECP) and n-ECP were log-normally distributed. The nNO and spirometry parameters were normally distributed without log-transformation.

The $F_{\text{E}}\text{NO}$ levels were higher in the asthmatic group compared with controls ($p < 0.01$), and decreased significantly 6 h post-challenge in both groups ($p < 0.01$). However, there was no difference in the amplitude of this decrease between the groups. Both patients with and without inhaled steroids showed elevated levels of $F_{\text{E}}\text{NO}$ and there was no difference between

Table 2. Parameters under investigation at baseline, 2, 6 and 24 h after the nasal allergen challenge

	Baseline	2 h	6 h	24 h
FEV ₁ , percentage of predicted				
A (n = 19)	90.2 $\pm$ 3.0	90.7 $\pm$ 3.1	91.0 $\pm$ 2.4	88.9 $\pm$ 2.4
C (n = 10)	96.2 $\pm$ 3.5	95.7 $\pm$ 4.1	96.3 $\pm$ 3.6	94.0 $\pm$ 5.0
MEF ₅₀ , percentage of predicted				
A (n = 19)	81.7 $\pm$ 4.3	83.8 $\pm$ 4.6	82.8 $\pm$ 3.6	77.9 $\pm$ 3.7
C (n = 10)	105.3 $\pm$ 7.0	99.5 $\pm$ 9.5	100.6 $\pm$ 7.4	97.0 $\pm$ 7.2
$F_{\text{E}}\text{NO}$ (ppb)				
A (n = 19)	48.3 $\pm$ 16.8	45.3 $\pm$ 15.0	42.5 $\pm$ 14.3	46.9 $\pm$ 14.4
C (n = 10)	8.4 $\pm$ 1.8	8.2 $\pm$ 1.9	7.0 $\pm$ 1.4	9.6 $\pm$ 2.1
nNO (ppb)				
A (n = 19)	134.8 $\pm$ 13.4	155.1 $\pm$ 15.6	140.3 $\pm$ 14.9	130.2 $\pm$ 15.5
C (n = 10)	130.1 $\pm$ 10.6	129.7 $\pm$ 17.9	122.2 $\pm$ 10.9	122.2 $\pm$ 13.8
n-ECP $\mu\text{g/g}$ protein				
A (n = 14)	90.8 $\pm$ 15.6	120.2 $\pm$ 19.3	Not studied	121.2 $\pm$ 24.4
s-ECP ($\mu\text{g/l}$)				
A (n = 18)	13.3 $\pm$ 2.7	13.2 $\pm$ 2.7	Not studied	16.5 $\pm$ 5.5
C (n = 4)	7.6 $\pm$ 1.1	7.7 $\pm$ 0.8		7.6 $\pm$ 1.0
Eos (%)				
A (n = 18)	9.9 $\pm$ 1.1	8.7 $\pm$ 1.2	Not studied	9.4 $\pm$ 0.9
C (n = 4)	0.7 $\pm$ 0.2	0.7 $\pm$ 0.1		0.7 $\pm$ 0.1

FEV₁, forced expiratory volume after 1 s; MEF₅₀, maximal expiratory flow rate at 50% of the expiration. Lung function parameters are presented as percentage of predicted. $F_{\text{E}}\text{NO}$, fraction of exhaled nitric oxide; n-NO, nasal nitric oxide concentration; n-ECP, eosinophil cationic protein measured in nasal lavage divided by the total concentration of protein; s-ECP, ECP in serum; Eos %, percentage of eosinophils of total white blood cell count.

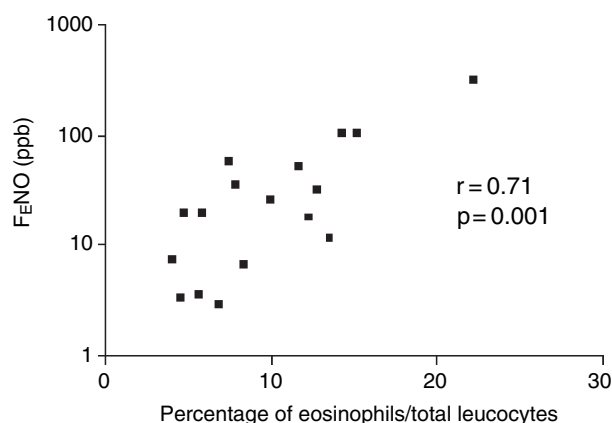


Fig. 1. Correlation between $F_{E}NO$ and blood eosinophil percentage of total white blood cell count at baseline in 18 asthmatic children allergic to cat.

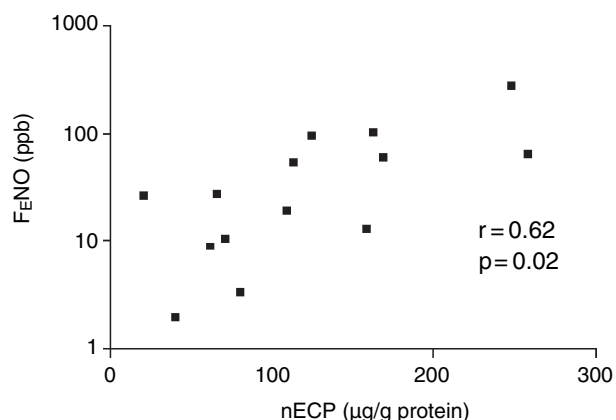


Fig. 2. Correlation between $F_{E}NO$ and nasal eosinophil cationic protein (ECP) 2 h after the provocation ($n = 14$).

these two groups of patients both pre- and post-challenge (not shown).

Nasal NO was similar in asthmatics and healthy subjects. A minor increase in nNO was seen in asthmatics post-challenge but this change was not significant.

Blood eosinophils, but not s-ECP, were significantly higher in the asthmatic group compared with controls ($p < 0.001$) and correlated with $F_{E}NO$ at all time-points (Fig. 1). Serum ECP did not correlate with $F_{E}NO$ or blood eosinophils. Although mean n-ECP, only studied in asthmatics, tended to increase after the nasal provocation, this was not significant. Nasal ECP correlated with $F_{E}NO$ only 2 h post-challenge (Fig. 2).

Lung function [FEV_1 and maximal expiratory flow rate at 50% of the exhalation (MEF_{50})] did not change over time and showed no correlation with inflammatory parameters.

Discussion

We have demonstrated that a single nasal allergen challenge does not increase bronchial inflammation in cat allergic children as measured by $F_{E}NO$. There was a significant correlation between $F_{E}NO$ and blood eosinophils in the asthmatic children both before and after the provocation, as well as a positive correlation between $F_{E}NO$ and n-ECP, but only 2 h after the challenge.

Epidemiological studies have shown that asthma is present in 13–35% among patients with seasonal allergic rhinitis (SAR) and that perennial allergic rhinitis co-exists in about 75% of patients with allergic asthma (1). Not surprisingly, increased levels of $F_{E}NO$ and airway hyper-responsiveness have been demonstrated in patients with SAR (12, 13). These findings have lead to the concept of 'united airways' in which nasal inflammation is supposed to be transmitted to the lung. However, the mechanisms behind this proposed link has not been fully clarified. Possible mechanisms include neurogenic reflexes (14), inhalation of nasally secreted cytokines (15) and increased number of circulating and activated eosinophils targeting the lung as well, or the result of co-exposure of both the upper and lower respiratory tract to airborne allergens.

The $F_{E}NO$ is a sensitive, non-invasive, marker of airway inflammation and is known to increase after bronchial provocation (9, 16). Therefore, we applied this marker to investigate whether a nasal allergic reaction could induce a significant bronchial inflammatory response within 24 h. However, an increase in $F_{E}NO$ within 24 h post-challenge could not be demonstrated. In fact, there was a significant decrease in $F_{E}NO$ 6 h after the challenge in both groups. This could be due to repeated spirometry measurements (17), diurnal variation in $F_{E}NO$ (18) or the effect of the mechanical manipulation lavaging the nose. Recently, Braunstahl et al. have demonstrated that adhesion molecules are up-regulated in the endothelium of bronchial mucosal capillaries 24 h after a nasal allergen challenge (19) resulting in an influx of eosinophils to the mucosal wall. However, Andersson et al. could not find an increase in $F_{E}NO$ up to 8 h after a nasal allergen challenge (20). The results from Braunstahl et al. could, at least partly, be the effect of a mechanical stimulus on the mucosa during the bronchoscopy as biopsies were taken before and 24 h after the challenge. Although $F_{E}NO$ is decreased by anti-inflammatory treatment it is not likely that a low dose of inhaled steroids in some of our patients should have masked the potential

bronchial inflammatory response to a nasal allergen challenge since there was no difference in the bronchial response between steroid-naïve and steroid-treated patients and since both subgroups showed elevated but unchanged levels of $F_{E}NO$ pre- and post-challenge.

However, a single nasal allergen challenge might be too weak to induce significant bronchial inflammation. Similar to Andersson's results (20), nasal status had returned to normal 24 h post-challenge demonstrating the absence of a late response effect. It was recently shown that daily provocations for several days could, in fact, induce bronchial inflammation in mice (21). This has not been studied in humans. Therefore, a transmission of the allergic inflammation might still be possible in allergic patients with rhinitis and asthma who are continuously exposed to allergens.

We found a strong correlation between $F_{E}NO$ and the number of circulating eosinophils, which is in good agreement with previous studies (5, 22). We also found an increase in n-ECP and a significant correlation between $F_{E}NO$ and n-ECP 2 h post-challenge which has not been reported before. As the number of patients was rather small and the n-ECP varied considerably the data should, however, be interpreted with caution.

Higher levels of nNO in patients with allergic rhinitis compared with healthy controls have been reported by some (23, 24) but not all (12, 25, 26) investigators. There might be several reasons for these conflicting results. First, investigators have not always ensured that nasal air samples are taken with the subject's vellum closed, which makes a mixture of orally and nasally $F_{E}NO$ possible. Secondly, and most problematic, most of the NO in the upper airways originates from the paranasal sinuses (27). Consequently, nasal concentrations of NO will depend on NO diffusion from the sinuses through the ostiae to the nasal cavity. The concentrations of NO would therefore be strongly affected by the inflammatory oedema in the nose and sinuses leading to impaired NO diffusion to the nasal cavity. This could be one reason for large variations in nNO. Finally, in this study we do not believe that a larger sample size would have resulted in a significant increase in nNO. The slight increase in nNO seen at 2 and 6 h post-challenge was probably due to heavy nasal obstruction in some patients rather than NO synthase induction, since NO concentrations seems airflow-dependent. At 24 h, mean nNO was actually reduced, although change was not significant. Secondly, there was a very large inter-individual variation

in the reactions to nasal challenge probably due to the limitations of the method. We believe that more studies are needed to evaluate the usefulness of nNO as a marker of nasal inflammation.

We conclude that a single nasal provocation with cat allergen does not seem to augment bronchial inflammation in allergic asthmatic children assessed by $F_{E}NO$. Although the challenge causes a local reaction in the nasal cavity, the impact on systemic disease seems too weak to increase bronchial inflammation. Future studies may reveal whether repeated nasal allergen challenges can induce bronchial inflammation.

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