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Exhaled nitric oxide in chronic obstructive pulmonary disease: relationship to pulmonary function

K. Ansarin*, J.M. Chatkin*, I.M. Ferreira*, C.A. Gutierrez*, N. Zamel#, K.R. Chapman#

Exhaled nitric oxide in chronic obstructive pulmonary disease: relationship to pulmonary function. K. Ansarin, J.M. Chatkin, I.M. Ferreira, C.A. Gutierrez, N. Zamel, K.R. Chapman. ©ERS Journals Ltd 2001.

ABSTRACT: The following study was undertaken in order to determine how exhaled nitric oxide (eNO) levels in former smokers with chronic obstructive pulmonary disease (COPD) compared to eNO levels in patients with asthma and in healthy nonsmoking volunteers. The study also aimed to determine any relationship between eNO levels in COPD and: 1) conventional measures of lung function; and 2) inhaled corticosteroid (ICS) use.

In former smokers with COPD, nonsmokers with asthma and volunteers, eNO levels, spirometry, lung volumes, carbon monoxide diffusion capacity of the lung ($D_{L,CO}$) and resting oxygen saturation (S_{a,O_2}) were measured.

Median eNO was significantly higher among patients with COPD than among healthy volunteers ($p=0.003$) but lower than among patients with asthma ($p<0.01$). There was no significant difference in eNO levels between COPD patients using ICS and those not using ICS. By contrast, eNO was lower among asthma patients who used ICS (median 32 parts per billion (ppb); 25–75% range 16–54) than among asthma patients who did not (51 ppb; 32–87) ($p=0.034$). Among patients with COPD, eNO was inversely correlated with forced expiratory volume in one second, $D_{L,CO}$ and S_{a,O_2}, and was positively correlated with the residual lung volume/total lung capacity ratio. Among patients with asthma, no significant correlations were found.

Exhaled nitric oxide is increased in patients with chronic obstructive pulmonary disease, an increase that is influenced by structural abnormalities of tobacco-induced lung damage.

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*Asthma Centre of the University Health Network, Toronto, Ontario, Canada and #Dept of Medicine, Division of Respiratory Medicine, University of Toronto, Toronto, Ontario, Canada.

Correspondence: K.R. Chapman, Suite 4-011 ECW, 399 Bathurst Street, Toronto, Ontario, Canada.
Fax: 1 4166033456

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There are few data describing the levels of exhaled nitric oxide (eNO) in patients with chronic obstructive pulmonary disease (COPD), and published reports are conflicting in their conclusions [1–3]. Although most studies suggest that eNO levels are elevated in COPD, the findings are potentially confounded by small sample sizes, the inclusion of current smokers and the inclusion of patients suffering from acute exacerbations. Some studies have sought a relationship between eNO values and conventional measures of pulmonary function, but these have generally been limited to spirometric measurements. None of these studies included patients with asthma, an obstructive lung disease potentially confused with COPD in the clinical setting, as a comparator group.

Therefore, this study describes levels of eNO in former smokers with clinically significant COPD. It sought to compare the levels measured in these COPD patients to the levels measured in nonsmoking patients with asthma and healthy nonsmoking volunteers. It further sought to assess factors such as inhaled corticosteroid (ICS) use that might alter eNO levels in the two disease settings, and to assess a correlation between eNO levels and more conventional measures of lung structure and function.

Methods

Patients

Thirty-two stable former smokers with COPD, 51 stable nonsmoking patients with asthma and 41 healthy nonsmoking adult volunteers were recruited from the ambulatory respiratory clinics of the University Health Network. Current or recent smokers were excluded to remove the confounding effect of tobacco smoke on nitric oxide (NO); tobacco smoke is known to reduce the eNO levels [4]. Smoking status was determined by patient self-report. All participants in the study gave written informed consent. The protocol was reviewed and approved by the Human Subjects Review Committee of the University of Toronto.

All patients or volunteers were seen and assessed by an academically affiliated respiratory physician. For

patients recruited to the COPD group, inclusion criteria were a diagnosis of COPD as defined by the criteria of the American Thoracic Society (ATS) guidelines [5] and a minimum smoking history of 20 pack-yrs, with abstinence for ≥ 12 months. All patients recruited to the asthma group had a diagnosis of asthma as defined by the criteria of the Canadian Thoracic Society [6], including a reported 12% improvement in forced expiratory volume in one second (FEV1) following inhaled bronchodilator, a 20% improvement in FEV1 following oral corticosteroid or a positive response to bronchial challenge with methacholine, histamine or other provocative agent. All patients in the asthma group were either lifetime nonsmokers or were former smokers who had consumed <20 pack-yrs and had been abstinent for ≥ 12 months. Healthy volunteers were either lifetime nonsmokers or had a lifetime consumption of <10 pack-yrs with no consumption of tobacco in the past 12 months. All such normal volunteers completed a standard questionnaire and were free of chronic or recurrent respiratory symptoms with no past history of significant pulmonary disease. Potential study subjects were excluded if they had suffered from a respiratory tract infection within 6 weeks of the study or if they suffered from other significant medical illnesses known to affect eNO measurements. Among patient groups, the use of inhaled or systemic corticosteroids (CS) during the past month was noted and allowed division of the groups into CS using and non-CS using subgroups.

Measurements

All patients performed flow/volume spirometry both before and after four puffs (100 $\mu g \cdot puff^{-1}$) of salbutamol *via* pressurized dry suspension metered-dose inhaler (Ventolin, GlaxoWellcome Canada Inc., Mississauga, Canada). Spirometry was performed according to the standards of the ATS. Lung volumes were measured by body plethysmography. Carbon monoxide diffusion capacity of the lung ($D_{L,CO}$) was measured by the single-breath technique (PK Morgan Ltd, Chatham, Kent, UK). Arterial oxygen saturation (S_{a,O_2}) was measured by pulse oximeter (N-20, Nellcor Puritan Bennett Inc., Pleasanton, CA, USA) while the subject sat quietly for ≥ 10 min and breathed room air.

eNO was measured in all subjects by the chemiluminescence technique (using a Sievers 280 NO Analyzer, Boulder, CO, USA). Two-point calibration was performed daily between 0 parts per billion (ppb) and 11.6 parts per million (ppm), using calibration gases (Praxair, Toronto, Canada). The lowest detection limit for eNO was 1–2 ppb. The measurements were performed at an expiratory flow of 40 $mL \cdot s^{-1}$ while the subject maintained a mouth-pressure of 20 mmHg in order to keep the vellum closed, thereby avoiding nasal NO contamination. The NO level was recorded on a real-time x-y plot by a technique that has been reported in detail previously [7]. All subjects had three recorded NO measurements, at intervals of ≥ 1 min. Measured values of eNO were considered acceptable if plateau values varied $<5\%$. The mean value of the three measurements was recorded as the final eNO level of the individual.

Analysis

Given their non-normal distribution, eNO levels are reported as median values with the 25–75% range shown. Other continuous variables are reported as mean $\pm$ SD. The difference between the means of the three groups and between steroid-using and nonsteroid-using subgroups was tested by Kruskal-Wallis one-way analysis of variance on ranks and Mann-Whitney rank sum test. The correlation between eNO and parameters of pulmonary function was sought using log-eNO to calculate the Pearson product moment correlation coefficient and least squares linear regression. In the case of non-normal parameters, a Spearman rank order correlation was used. Differences were considered significant if p-values were <0.05.

Results

The demographic characteristics of the patients and volunteers are shown in table 1. As expected, the COPD patients were significantly older than patients with asthma or healthy volunteers ($p<0.001$). However, the median duration of symptoms in the asthma group was significantly longer than the corresponding value for patients with COPD ($p<0.01$). Furthermore, by definition, the COPD patients had all been smokers in the past, while only a minority of the patients with asthma had smoked and none of the volunteers had smoked. The mean duration of smoking was, therefore, significantly longer among the COPD patients than the other groups ($p<0.001$). Approximately half of each of the patient groups, asthma and COPD were using inhaled steroids at the time of the study. Mean FEV1 and mean single-breath $D_{L,CO}$ were significantly lower among COPD patients than asthma patients, while residual volume to total lung capacity (RV/TLC) ratio was significantly higher ($p<0.001$, for all).

eNO levels are shown in figure 1 for the various groups. Median eNO levels were significantly higher among patients with COPD than among healthy nonsmoking volunteers ($p=0.003$). However, median eNO levels were significantly lower among patients with COPD than among patients with asthma ($p<0.01$; fig. 1). There was no significant difference in median eNO levels between the subgroup of COPD patients who ICSs (26 ppb; 18–33 ppb) and the subgroup that did not (28 ppb; 22–40). By contrast, eNO levels were significantly lower among asthma patients who used ICSs (32 ppb; 16–54) than among asthma patients who did not (51 ppb; 32–87) ($p=0.034$).

The relationships between log-eNO and pulmonary function variables for patients with COPD or asthma are listed in table 2. Among patients with COPD, eNO was inversely correlated with FEV1, $D_{L,CO}$ and resting S_{a,O_2} and was positively correlated with RV/TLC ratio. Among patients with asthma, no significant correlations were found, although there

Table 1. – Demographic, clinical and pulmonary function characteristics of the groups studied

Characteristics	COPD	Asthma	Healthy control
Age yrs	70.8±1.4	45±2.4	41±2.5
Sex female/male	10/22	38/13	32/9
History of smoking n			
Nonsmoker	0	43	41
Exsmoker	32	8	0
Current smoker	0	0	0
Duration of smoking yrs	42(32.7–49.2)	2.5(2–7)	
Duration of symptoms yrs	7(4–11)	18(5–32)	na
Steroid use n			
CS	16	26	0
Non-CS	16	25	41
ICS dose^{+} ($\mu g \cdot day^{-1}$ of beclomethasone or equivalent)	1 500 (800–1800)	1 200 (900–2000)	na
Postbronchodilator FEV_1 % pred	50.5±3.8	92.2±3.2	nd
$D_{L,CO}$ $mL \cdot min^{-1} \cdot mmHg^{-1}$	15.9±1.4	25.8±1.3	nd
$D_{L,CO}$ % pred	69.4±4.7	94.7±2.5	nd
RV/TLC %	55.7±2.6	37.4±2.01	nd

Data are presented as mean±SD or median (25–75% range) unless stated. COPD: chronic obstructive pulmonary disease; CS: steroid using; Non-CS: steroid naïve; ICS: inhaled corticosteroid; FEV_1: forced expiratory volume in one second; $D_{L,CO}$: carbon monoxide diffusion capacity of the lung; RV/TLC: residual volume to total lung using capacity ratio; na: not applicable; nd: not determined.

was a nonsignificant trend towards a positive correlation with $D_{L,CO}$. These results are shown in figure 2. There was no correlation between eNO and age within or among groups. There was a positive correlation between eNO and duration of smoking for patients with COPD; too few asthma patients had smoked to allow a meaningful assessment of this variable.

Discussion

The present data show that, in former smokers with COPD, levels of eNO are elevated as compared to levels measured in the exhaled air of healthy non-smoking individuals, although not as elevated as is seen in patients with untreated asthma. The present data suggest that the factors responsible for elevated eNO levels in COPD are different from the factors responsible for elevated levels in asthma. No clear relationship was found between ICS use and eNO levels in COPD, although such a relationship is well-established in asthma and was reconfirmed in the present study [8–11]. However, there was a relationship between eNO levels in COPD and several abnormalities of pulmonary function.

Several groups of investigators have attempted to describe eNO values in COPD previously with conflicting results [12]. There are many possible explanations for such inconsistant results. Firstly, the techniques for measuring eNO of lower airway origin are just now being standardized. Secondly, most studies have measured eNO in relatively small numbers of subjects. Thirdly, several studies were confounded by the inclusion of current smokers

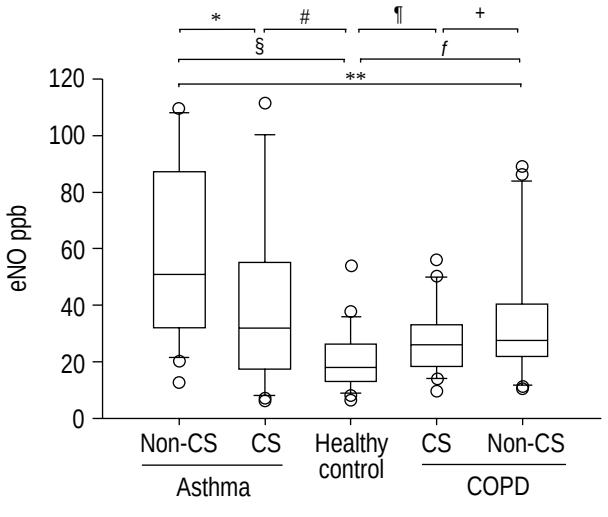

Fig. 1. – Box plots of exhaled nitric oxide (eNO) values in patients with asthma, chronic obstructive pulmonary disease (COPD) and in healthy volunteers. ppb: parts per billion; CS: using corticosteroids; Non-CS: not using corticosteroids. Circles indicate outliers between group comparison p-values shown. *: p=0.034; #: p=0.009; ¶: p=0.027; +: p=0.55; §: p<0.001; f: p=0.008; **: p=0.014.

Table 2. – Correlation between exhaled nitric oxide and pulmonary function variables

Pulmonary function variables	COPD		Asthma		Control	
	R	p	R	p	R	p
Age yrs	0.24	0.14	0.17	0.21	0.18	0.25
Smoking	0.47	0.007				
FEV_1 % pred	0.50	0.004	0.04	0.76		
$D_{L,CO}$ % pred	0.58	0.001	0.32	0.06		
RV/TLC %	0.57	0.003	0.12	0.49		
S_{a,O_2}	0.41	0.04	0.04	0.82		

p: p-value; FEV_1: forced expiratory volume in one second; $D_{L,CO}$: carbon monoxide diffusion capacity of the lung; RV/TLC: residual volume to total lung using capacity ratio; S_{a,O_2}: arterial oxygen saturation; COPD: chronic obstructive pulmonary disease.

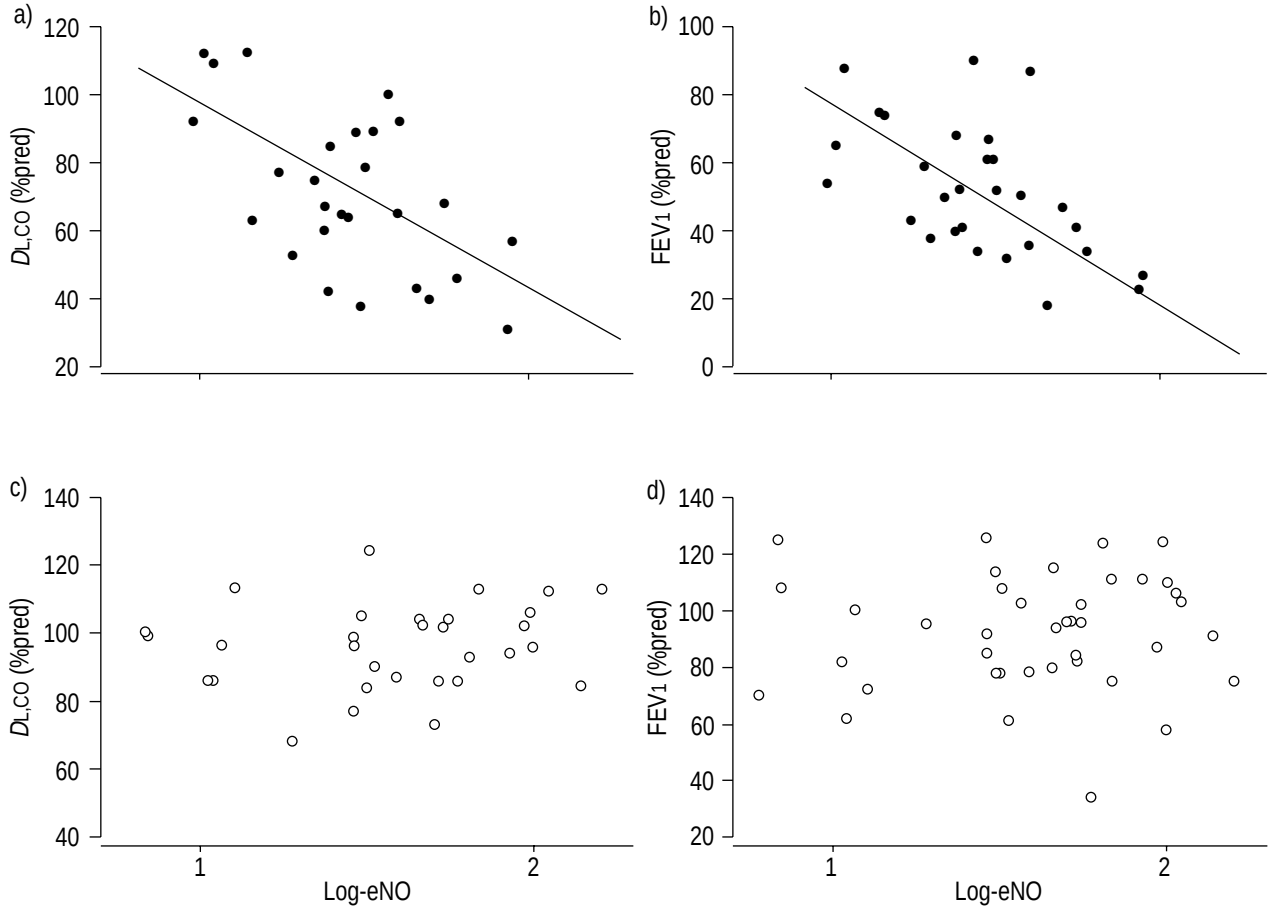

Fig. 2. – Log eNO *versus*; a) carbon monoxide diffusion capacity of the lung ($D_{L,CO}$) in chronic obstructive pulmonary disease (COPD) patients; b) forced expiratory volume in one second (FEV_1) in COPD patients; c) $D_{L,CO}$ in asthma patients; and d) FEV_1 in asthma patients. Lines represent least linear regression (for COPD only): a) r=-0.58, p=0.001; b) r=-0.50, p=0.004.

among their patient or control populations, although smoking is known to reduce eNO values. Finally, no study has examined in detail the pulmonary function of COPD and asthma patients concurrently, in order to allow assessment of how such variables might influence eNO in either of these disorders. Thus, published studies have described both normal and elevated eNO values in COPD and have suggested both positive and negative correlations between eNO and the severity of airflow limitation. No study has examined the relationship between eNO levels in COPD and ICS use. By contrast, the present study population was more homogenous. All patients had COPD secondary to many years of tobacco use and all patients were former smokers who were clinically stable at the time of study. Moreover, healthy nonsmoking individuals and patients with stable asthma were studied contemporaneously, and these two groups acted as comparators. Detailed pulmonary function measurements were undertaken in patient groups.

The authors believe that the present findings suggest a more important influence of lung mechanics than corticosteroid-responsive airway inflammation on eNO values in patients with COPD. Although the elevated eNO levels in untreated asthma decrease markedly with ICS therapy [13], the present study was unable to show a relationship between ICS use and NO levels in patients with COPD. Although median values of eNO levels were numerically lower among COPD patients using inhaled steroid than those who were not, this difference was not statistically significant. The possibility of a Type II error cannot be ruled out and it must also be noted that the present study was observational and cross-sectional and not a randomized, placebo-controlled trial of therapy. Nonetheless, the absence or relative weakness of any correlation between ICS use and eNO values in COPD is in marked contrast to the findings in patients with asthma. Many factors might explain the absence of such a relationship in the present study. First, the patients suffered from severe airflow limitation but were clinically stable. That is, the airway inflammation present during the period of most active lung injury may have been minimal at the time of study. Second, COPD is a heterogeneous clinical syndrome. The former smoker with severe and persistent airflow limitation may be suffering from one or more of several pathophysiological processes resulting in a reduced FEV_1. In some such patients, airway inflammatory mechanisms predominate, while in others, diffuse alveolar destruction predominates.

Third, the airways inflammation of COPD involves different cellular pathways than the airways inflammation of asthma. Neutrophilic inflammation is thought to predominate in COPD and it is plausible that other noninvasive markers in exhaled breath may be better indicators of such inflammatory processes.

There was a strong correlation between eNO values in COPD and measures of lung function abnormality. The authors believe that this relationship suggests an alternative explanation for the elevation of eNO levels in patients with diffuse pulmonary damage secondary to years of tobacco smoking. NO is a highly reactive chemical with a short half-life within the body. At the alveolar level, NO combines rapidly with reduced haemoglobin (400 times faster than carbon monoxide) and is therefore scavenged by pulmonary capillary blood. In the presence of an altered ventilation-perfusion mismatch, this scavenging will take place less efficiently. Indeed, more than a decade ago, BORLAND and HIGGINBOTTAM [14] reported the measurement of diffusion capacity using exogenous NO rather than CO as a tracer gas. If this supposition is correct, the elevations of NO seen in the present study's stable COPD patients are a better reflection of abnormal pulmonary mechanics than any active inflammatory process. Such a finding has major potential implications for the use of NO as a diagnostic tool. Although the authors believe that a ventilation-perfusion mismatch is an important factor in eNO elevation in COPD, the possibility that other, as yet unknown factors related to disease severity may play a role is acknowledged. For example, patients with COPD may have chronic lower airway colonization with micro-organisms even at times of stability. Their presence could contribute to NO synthase stimulation as is thought to occur in bronchiectasis.

The present findings in chronic obstructive pulmonary disease have confirmed and extended earlier observations. The present authors, like others, believe that exhaled nitric oxide levels may prove useful in the diagnosis and monitoring of various lung diseases. However, nitric oxide levels may be perturbed by many factors and cannot be regarded as a simple marker of airways inflammation alone. In the presence of significant airflow limitation or other perturbation of lung function, elevated nitric oxide levels must be interpreted cautiously.

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