Exhaled nitric oxide parameters and functional capacity in chronic obstructive pulmonary disease

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Abstract
The extended exhaled nitric oxide (eNO) parameters, including peripheral or alveolar eNO, are investigational biomarkers in COPD. In this study, the hypothesis was tested that elevated peripheral eNO correlates with decreased functional capacity and lower global health status. Twenty-seven subjects with the Global Initiative for Chronic Obstructive Lung Disease stage 3 and 4 COPD were enrolled. Functional capacity and health status were tested using the 6 min walk test and St George's Respiratory Questionnaire (SGRQ) respectively. eNO parameters were estimated using multiple exhalation flow rates and were corrected for axial diffusion. The extended NO measurements were $FE_{NO}0.05$ 14.2 ppb (range 5.1–23.2), C_{ANO} 4.6 ppb (2.2–6.9), D_{awNO} 8.8 ml s^{-1} (4.8–12.9), C_{awNO} 83.2 ppb (29.9–128.7) and J'_{awNO} 405 pl s^{-1} (111–731). The distance traveled in the 6 min walk test was correlated with peripheral nitric oxide ($r = -0.59$, $p = 0.03$). SGRQ symptom score was correlated with maximum airway NO flux ($r = -0.73$, $p = 0.01$). SGRQ total score was correlated with maximum airway NO flux ($r = -0.56$, $p = 0.05$). In this study of subjects with severe COPD, peripheral nitric oxide correlated with functional capacity while large airway NO parameters correlated with symptom scores.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the United States and is a significant cause of morbidity and mortality. The disease often manifests clinically as productive cough and worsening dyspnea [1]. In addition, it has several systemic consequences such as peripheral muscle weakness and nutritional abnormalities, often affecting performance status. Pulmonary function measurement is the most important indicator of respiratory impairment in COPD [2, 3], yet it is a weak predictor of disability and performance [4–6].

Inflammatory marker analysis is gaining interest in airway diseases such as COPD [7–9] but has not been used in assessing performance status. Measurement of exhaled nitric oxide (eNO) is a non-invasive method of assessing airway inflammation. In patients with COPD, the peripheral airway (bronchioles) is the predominant site of obstruction and inflammation [10–12], and the peripheral nitric oxide levels may be more predictive of the disease course and control.

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Recently, methods have been described for partitioning exhaled NO measurements into alveolar and large airway components [13–16]. This is performed by measuring NO from breath sampled at various exhalation flow rates. These methods yield four extended NO measurement parameters—the steady-state alveolar concentration or peripheral NO (C_{ANO}, ppb), airway compartment diffusing capacity (D_{awNO}, ppb), the steady-state concentration in the gas phase of the airway compartment (C_{awNO}, ppb), and the maximum flux of NO through the airway wall (J'_{awNO}, pl s^{-1}). Two recent studies [17, 18] found that C_{ANO} was elevated in COPD patients as compared to healthy controls and that C_{ANO} was unaffected by smoking and inhaled corticosteroids, suggesting that C_{ANO} may be a robust inflammatory marker in COPD. The use of the extended NO parameters as an index of symptoms and functional capacity has not been previously reported. In this study, the hypothesis was tested that elevated C_{ANO} correlates with worse functional capacity and lower global health status.

2. Methods

2.1. Study design

A cross-sectional study was carried out to evaluate the correlation between the exhaled NO levels and the functional and health status and lung function in subjects with COPD.

2.2. Subjects

Subjects were recruited from the Pulmonary Clinic at the Michael E. Debakey Veterans Affairs Medical Center (MED-VAMC), Baylor College of Medicine, Houston, TX. Inclusion criteria were (a) age equal to or greater than 45 with (b) post-bronchodilator forced expiratory volume in 1 s ($FEV_{1.0}$)/forced vital capacity (FVC) ratio less than 70%; $FEV_{1.0}$ less than 70% of predicted, (c) history of cigarette smoking of at least 20 pack years and (d) stable clinical course (symptoms and medications) for 8 weeks. All subjects were Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages 3 and 4. Subjects with chronic respiratory problems other than COPD and any other chronic medical conditions were excluded. All patients refrained from using inhaled short acting bronchodilators 6 h before the study measurements. The protocol was approved by the Institutional Review Boards of Baylor College of Medicine and the MED-VAMC. All subjects read and signed written informed consent prior to study entry.

2.3. Lung function

Measurements of $FEV_{1.0}$ and $FEV_{1.0}$/FVC were made with a dry spirometer (SpiroVision-3+, Futuremed, Inc.) which met American Thoracic Society standards. Each subject performed at least three consistent and reproducible forced expiratory maneuvers according to guidelines published by American Thoracic Society/European Respiratory Society [19].

2.4. Exhaled NO measurements

Exhaled NO levels were measured between 9:30 and 10:30 am. Current smokers were asked to refrain from smoking 2 h prior to the clinic visit. NO measurements consisted of three exhalations at each flow, which were sufficient to obtain reproducible NO results [20]. Exhaled breath was collected offline using a custom device [21]. The subject inhaled through an NO filter (model N7500-2, North Inc.) and out through a flow transducer (model 4021, TSI, Inc.). Mouth pressure was monitored with a pressure transducer (model 800, Autrotran, Inc.). In accordance with the American Thoracic Society (ATS) recommendations, a minimum of 8 cm H_2O mouth pressure was maintained to prevent NO contamination from the nasal cavity. Since the NO level in breath reaches a plateau level during a single exhalation at constant flow [22], the first portion of the exhaled breath was discarded to ambient air and the second portion, the plateau region, was collected in a 1 l Tedlar bag. The patients were instructed to inhale to total lung capacity. During exhalation, a 3-way valve diverted exhaled air into a Tedlar bag for the remainder of the exhalation. Exhalation was performed at six flow rates (ml s^{-1}): 8.3, 33.3, 50, 100, 150, 250. The exhalation time before valve diversion to the Tedlar bag varied depending on the flow rate: for 8.3 ml s^{-1}, breath was collected from 15 to 19 s during exhalation; for 33.3 ml s^{-1}, 12–15 s; for 50 ml s^{-1}, 11–14 s; for 100 ml s^{-1}, 9–12 s; 150 ml s^{-1}, 7–11 s and for 250 ml s^{-1}, 4–5 s. Flow rates were maintained within 5% of the target flow rate by the subjects using feedback from the flow transducer output in a Labview interface on a laptop computer. Samples collected with flow rates outside of ±5% of the target flow rate were discarded. Three single-breath collections were collected into a Tedlar bag (model 232–01, SKC, Inc.). The NO exchange parameters were calculated using a two-compartment model of the lung [14]. In this technique, the elimination rate of NO was measured at multiple flow rates. C_{ANO} and J'_{awNO} were determined as the slope and intercept of the resulting linear relationship. Next, in a technique described by Silkoff *et al* [15], two low flow rates were used to estimate D_{awNO} and C_{awNO} by using the slope (D_{awNO}) and intercept (J'_{awNO}/D_{awNO}) of a plot of the NO elimination rate versus the plateau NO. The NO exchange parameters were corrected for axial diffusion of NO for the flow rate using the method of Condorelli *et al* [16] by multiplying large airway NO flux by 1.7. In addition, the initial uncorrected large airway flux was divided by 0.53 l/s and subtracted from the initial uncorrected small airway/alveolar NO.

Exhaled NO was measured by a mid-infrared laser-based analyzer [21, 23]. The Tedlar bags were measured within 2 h of breath collection. Preliminary results indicate that the NO level is stable in the Tedlar bags for this time period [23].

2.5. Functional and health status

The functional status was assessed using the 6 min walk test and was conducted according to a standardized protocol [24]. Subjects performed the 6 min walk test after exhaled NO and $FEV_{1.0}$ measurements. Dyspnea, as measured with

the modified Borg dyspnea scale, oxygen saturation, and pulse rate were assessed at the start and end of the 6 min walk test. Patients also completed St George's Respiratory Questionnaire (SGRQ), a disease-specific quality of life index with established validity, responsiveness and interpretability [25].

3. Statistical analysis

Statistical tests were performed using SPSS 16.0 (SPSS Inc., Chicago, IL). Data were expressed as median $\pm$ standard deviation (SD) or geometric mean (95% confidence interval). Data were tested for normality using the Shapiro–Wilk normality test using $p < 0.05$ as the threshold for normality. The difference in C_{ANO} using the two-compartment model of the lung and the model incorporating axial diffusion of NO was calculated using a paired *t*-test. The extended NO parameters using the model incorporating axial diffusion were used for the following analyses. The relationship between the exhaled NO parameters and the distance traveled on the 6 min walk test, SGRQ activity, impact, symptom and total scores, and percent predicted $FEV_{1.0}$ were determined using the Spearman rank order correlation coefficient since extended NO parameters are not normally distributed. Age, weight, and smoking status were included in the model. The Mann–Whitney test was used to compare nonparametric data. Differences were considered significant at a value of $p < 0.05$.

4. Results

4.1. Subject characteristics

A total of 28 subjects were enrolled in the study. Subjects had a mean age of 70 years and a mean $FEV_{1.0}$ of 1.23 l. The SGRQ domain scores were symptom score mean 68 (range 40–100), activity score 78 (55–100), impact 33 (10–100) score 72 (59–94), and total score 41 (12–61). No patient was taking an oral corticosteroid. Study subject characteristics are summarized in table 1.

4.2. eNO and clinical measures

The nitric oxide parameters have a skewed distribution and are summarized in table 2. C_{ANO} for the two-compartment model was 4.9 ppb $\pm$ 1.5 SD and for the model incorporating axial diffusion was 3.1 ppb $\pm$ 1.1 ($p = 0.005$). J'_{awNO} for the two-compartment model was 398 pl s^{-1} $\pm$ 251 and for the model incorporating axial diffusion was 592 $\pm$ 301 ($p = 0.04$). Using the model incorporating axial diffusion, a correlation was found between the distance traveled in the 6 min walk test and C_{ANO} ($r = -0.59$, $p = 0.03$), and between the distance traveled and $FE_{NO}0.05$ ($r = -0.41$, $p < 0.02$). Figure 1 shows the scatter plot of C_{ANO} by the distance traveled in the 6 min walk test. A correlation was found between the SGRQ symptom score and $FE_{NO}0.05$ ($r = -0.44$, $p < 0.02$), between the SGRQ symptom score and J'_{awNO} ($r = -0.73$, $p = 0.01$) shown in figure 2, and between the SGRQ total score and J'_{awNO} ($r = -0.56$, $p = 0.01$) in figure 3. A comparison of the correlation coefficiency is summarized in table 3.
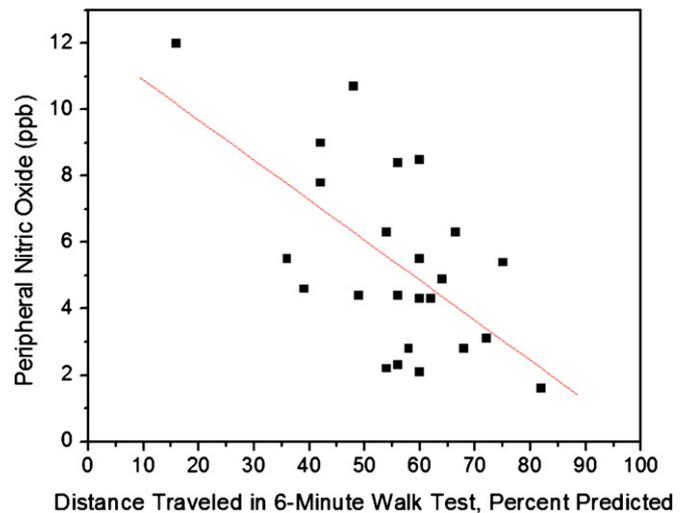

Figure 1. Scatter plot of alveolar, or peripheral, nitric oxide (C_{ANO}) versus the distance traveled in the 6 min walk test. The Spearman correlation coefficient was used to test the correlation ($r = -0.59$, $p = 0.03$).

Table 1. Patient characteristics.

Gender (M/F)	22/6
Age (yr)	70 (48–83)
Height (m)	1.8 (1.5–1.9)
Weight (kg)	84 (56–113)
Current/ex-smokers	10/18
Pack-yrs ex-smokers	46 (20–95)
Pack-yrs current smokers	69 (25–133)
$FEV_{1.0}$, (l)	1.23 (0.7–3.2)
$FEV_{1.0}$% pred	34 (21–70)
ICS	16
SGRQ symptoms	68 (40–100)
SGRQ activity	78 (55–100)
SGRQ impact	33 (0–100)
SGRQ total	72 (59–94)
6 min WT% pred	41 (12–61)

Data are presented as mean (range) or *n*. *M*: male, *F*: female; $FEV_{1.0}$: forced expiratory volume in 1 s; % pred: percent predicted; ICS: inhaled corticosteroids; SGRQ: St George's Respiratory Questionnaire; 6 min WT% pred: percent predicted 6 min walk test.

Table 2. Extended exhaled NO parameters.

$FE_{NO}0.05$ (ppb)	14.2 (5.1–23.2)
C_{ANO} (ppb)	4.6 (2.2–6.9); $r = 0.97 \pm 0.2$ (0.92–0.99)
D_{awNO} (ml s^{-1})	8.8 (4.8–12.9)
C_{awNO} (ppb)	83.2 (29.9–128.7)
J'_{awNO} (pl s^{-1})	405 (111–731); $r = 0.95 \pm 0.3$ (0.90–0.99)

Data are presented as geometric mean (95% confidence interval). $FE_{NO}0.05$: fractional exhaled NO at 50 ml s^{-1} exhalation flow; C_{ANO}: alveolar nitric oxide concentration; D_{awNO}: airway diffusing capacity for nitric oxide; C_{awNO}: airway wall nitric oxide concentration; J'_{awNO}: maximum total airway nitric oxide flux.

Table 3. Comparison of correlation coefficiency (r) values between extended NO parameters and clinical parameters.

	$FE_{NO}0.050$	C_{ANO}	D_{awNO}	J^{*}_{awNO}	$\%FEV_1$	6MWT	SGRQ symp	SGRQ activity	SGRQ impact	SGRQ total
$FE_{NO}0.050$	–	$r=0.041$ $p=0.13$	$r=0.42$ $p=0.52$	$r=0.52$ $p=0.09$	$r=-0.71$ $p=0.01$	$r=-0.41$ $p=0.02$	$r=0.44$ $p=0.01$	$r=0.45$ $p=0.56$	$r=0.39$ $p=0.91$	$r=0.49$ $p=0.23$
C_{ANO}		–	$r=0.41$ $p=0.39$	$r=0.41$ $p=0.19$	$r=-0.61$ $p=0.04$	$r=-0.59$ $p=0.03$	$r=0.49$ $p=0.07$	$r=0.51$ $p=0.12$	$r=0.43$ $p=0.31$	$r=0.56$ $p=0.19$
D_{awNO}			–	$r=0.39$ $p=0.65$	$r=0.52$ $p=0.23$	$r=0.38$ $p=0.56$	$r=0.39$ $p=0.41$	$r=0.49$ $p=0.42$	$r=0.32$ $p=0.71$	$r=0.65$ $p=0.08$
J^{*}_{awNO}				–	$r=-0.69$ $p=0.05$	$r=0.51$ $p=0.18$	$r=-0.73$ $p=0.01$	$r=-0.51$ $p=0.21$	$r=-0.54$ $p=0.14$	$r=-0.56$ $p=0.01$
$\%FEV_1$					–	$r=0.41$ $p=0.32$	$r=0.45$ $p=0.21$	$r=0.61$ $p=0.45$	$r=0.53$ $p=0.71$	$r=0.55$ $p=0.05$
6MWT						–	$r=0.62$ $p=0.13$	$r=0.72$ $p=0.02$	$r=0.52$ $p=0.32$	$r=0.68$ $p=0.04$
SGRQ symp							–	$r=0.42$ $p=0.52$	$r=0.39$ $p=0.61$	$r=0.61$ $p=0.13$
SGRQ activity								–	$r=0.42$ $p=0.23$	$r=0.51$ $p=.12$
SGRQ impact									—	$r=0.54$ $p=0.23$

Extended NO parameters with axial diffusion were used for this analysis. C_{awNO} is not included in the analysis since it is mathematically related to the other NO parameters. $FE_{NO}0.05$: fractional exhaled NO at 50 ml s^{-1} exhalation flow; C_{ANO}: alveolar nitric oxide concentration; D_{awNO}: airway diffusing capacity for nitric oxide; C_{awNO}: airway wall nitric oxide concentration; J'_{awNO}: maximum total airway nitric oxide flux; FEV_1: percent predicted forced expiratory volume in 1 s; 6MWT: percent predicted 6 min walk test; SGRQ: St George's Respiratory Questionnaire; Symp: symptom score.
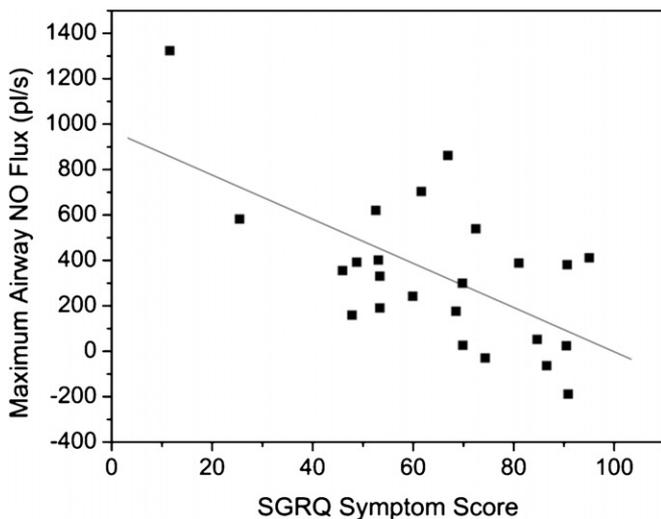

Figure 2. Scatter plot of maximum airway NO flux (J'_{awNO}) in pl s^{-1} versus St George's Respiratory Questionnaire symptom score. A higher score indicates worse symptoms. The Spearman correlation coefficient was used to test the correlation ($r=-0.73$, $p=0.01$).
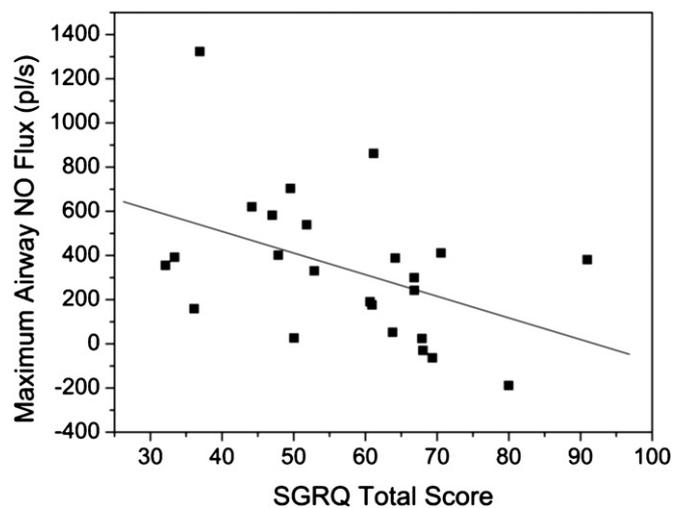

Figure 3. Scatter plot of maximum airway NO flux (J'_{awNO}) in pl s^{-1} versus St George's Respiratory Questionnaire total score. A higher score indicates worse quality of life. The Spearman correlation coefficient was used to test the correlation ($r=-0.56$, $p=0.01$).

5. Discussion

In this study, elevated C_{ANO} and $FE_{NO}0.05$ were associated with poorer functional status, as assessed by shorter distance traveled in the 6 min walk test. Elevated C_{ANO} and $FE_{NO}0.05$ were associated with poorer lung function, as assessed by the percent predicted $FEV_{1.0}$. In addition, elevated $FE_{NO}0.05$ and J'_{awNO} correlated with worse global health status, as assessed by higher SGRQ scores. C_{ANO} was relatively high but was within the range of a previous study of 15 subjects with

GOLD 3–4 COPD, C_{ANO} was 3.4 ± 0.1 ppb, J'_{awNO}: 609.4 ± 71 pl s^{-1} and D_{awNO}: 15 ± 1.2 pl (ppb s)$^{-1}$ [18].

Of note, the associations between the extended NO measurements, the 6 min walk test, and the SGRQ were present after correction for axial backdiffusion. Recent studies have shown that associations made with the two-compartment model were later found to be spurious when no association was found after correction for axial diffusion [26, 27]. Gelb *et al* studied ex-smoking patients with stable COPD and age-matched controls and found that, after correction for

NO axial backdiffusion, there was no difference in C_{ANO} in normal subjects versus patients with COPD [27]. The present study contradicts the findings of the two previous studies and warrants further investigation.

In this study, extended NO measurements were investigated as biomarkers of the severity of COPD. In most inflammatory lung diseases, the clinical utility of exhaled NO has been shown to be its association with eosinophilic inflammation and its prediction of steroid responsiveness in airway disease. Rutgers *et al* showed that NO metabolism was not increased in 16 patients with stable COPD [28]. There was a close association between exhaled NO levels and sputum eosinophils. In a study of 60 ex-smokers with severe COPD, Kunisaki *et al* showed that single measurement eNO was more closely associated with $FEV_{1.0}$ responses to 4 weeks of inhaled corticosteroids than are standard markers of systemic inflammation, serum CRP, IL-6, and IL-8 [29]. To investigate single measurement exhaled NO as a predictor of short-term response to oral corticosteroid in COPD, Dummer *et al* found that eNO predicted increase in $FEV_{1.0}$ but was only a weak predictor of response to corticosteroid [30]. In the present study, the association between peripheral nitric oxide and functional capacity may be explained by inflammation present in the peripheral airway that is not eosinophil based. As this is a cross-sectional study, the use of extended NO measurements in predicting clinical outcomes, such as response to corticosteroids, could not be assessed. A longitudinal study is required and could be an area for future study.

Health status instruments assist clinicians and researchers in understanding the global impact of a disease or condition on health status, to establish a profile of dysfunction in a clinical population or to investigate the relationship between health status and prognosis. These tools are valuable in clinical trials but often are too time consuming for community practice. A single biomarker to assess functional capacity, health status, peripheral airway inflammation, and response to treatment could eliminate lengthy tests and augment brief questionnaires.

In this study, worsening global health status and increasing subjective symptoms were associated with increasing airway NO flux. These data suggest that nitric oxide parameters may provide information regarding performance status and global health status. $FE_{\mathrm{NO}}0.05$ is not an independent biomarker when extended NO parameters are used. However, it is interesting to note that $FE_{\mathrm{NO}}0.05$ alone correlated with functional capacity and SGRQ symptom score.

Reliable biomarkers are needed in COPD to evaluate new classes of drugs targeting peripheral inflammation [31, 32]. Three previous studies [17,18, 26, 27, 33] investigated the recently introduced NO exchange parameters in COPD. In two studies, C_{ANO} was elevated in COPD patients as compared to healthy controls. Brindicci *et al* [18] studied 47 COPD patients of different severity according to the GOLD. They found that COPD severity was correlated with increased C_{ANO} regardless of the patient's smoking habit or current treatment. In addition, inhaled corticosteroids only mildly affected J'_{awNO} and did not affect C_{ANO} or D_{awNO}. The inflammation assessed by eNO may be unique from the steroid-responsive inflammation in COPD, and the eNO inflammation may be pharmacologically treated using different drugs, such as iNOS inhibitors or NOS donors [34]. C_{ANO} is reproducible, free of diurnal variation, and unaffected by smoking, bronchodilator or inhaled corticosteroids. Further, the findings in this study suggest that C_{ANO} is a marker of low functional status, an outcome used in pharmacologic clinical trials.

6. Conclusion

In this set of patients with GOLD stage 3 and 4 COPD, peripheral nitric oxide correlated with functional status and large airway NO parameters correlated with health status. A longitudinal study is needed to determine the predictability of clinical outcomes and effects of treatment targeted at peripheral inflammation as the next steps in validating the NO parameters in COPD.

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