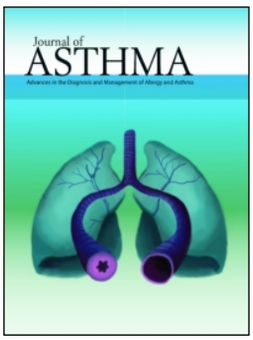

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Running head: Suitable protocol for alveolar NO measurement

A suitable protocol for measuring alveolar nitric oxide in asthma with differing severity to assess peripheral airways inflammation

A feasible protocol for measuring alveolar nitric oxide in patients with asthma of differing severity

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Abstract

Objective: Extended nitric oxide (NO) analysis offers the partitioned monitoring of inflammation in central and peripheral airways. Different mathematical models are used to

estimate pulmonary NO dynamics in asthma with variable results and limitations. We aimed to establish a protocol for extended NO analysis in patients with differing asthma severity.

Methods: Forty patients with stable asthma and twenty-five matched control subjects were recruited. Exhaled NO was measured at constant flow rates between 10 and 300 mL/s. Twelve controls performed NO measurements weekly for four weeks.

Results: The proportions of patients with technically acceptable measurements at 10-30-50-100-150-200-250-300 mL/s exhalation flow rates were 8-58-100-98-98-95-90-80%, respectively. Alveolar NO (C_{ANO}) and total flux of NO in the conducting airways (J_{awNO}) were calculated with the linear method from NO values measured at 100-150-200-250 mL/s exhalation flows. The mean intra-subject bias for J_{awNO} and C_{ANO} in controls was 0.16 nL/s and 0.85 ppb, respectively. Both J_{awNO} (1.31 /0.83-2.97/ vs. 0.70 /0.54-0.87/ nL/s, $p<0.001$) and C_{ANO} (4.08 /2.63-7.16/ vs. 2.42 /1.83-2.89/ ppb, $p<0.001$) were increased in patients with asthma compared to controls. In patients. C_{ANO} correlated with RV/TLC ($r=0.58$, $p<0.001$), $FEF_{25-75\%}$ ($p=0.02$, $r=-0.36$) and $D_{L,CO}$ ($r=-0.46$, $p=0.004$). J_{awNO} was not related to lung function parameters.

Conclusions: Calculation of alveolar NO concentration with the linear method from values obtained at medium flow rates (100-250 mL/s) is feasible even in asthmatic patients with severe airflow limitation and may provide information on small airways dysfunction in asthma.

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extended nitric oxide analysis; exhaled biomarker; airway inflammation; severe asthma; disease monitoring

Introduction

Bronchial asthma is a heterogeneous disease characterized by accumulation of inflammatory cells in the airways, hypertrophy and hyperplasia of airway smooth muscle layer, increased mucus production, and airway wall remodelling (1). Inflammatory changes take place simultaneously in central and distal airways and in the alveoli (2-4). The assessment of airway inflammation at these different localizations can aid better understanding of disease pathomechanism and facilitate the development of more targeted therapies.

Airway inflammation can be studied non-invasively by measuring the exhaled nitric oxide (NO) concentration. The two-compartment model allows the evaluation of NO dynamics in the large central or bronchial and in more distal airways (small airways and the alveolar/acinar region) (5, 6). For this purpose, measurements are performed at multiple expiratory flow rates (10-500 mL/s) and mathematical models are applied (6). The recent technical standard task force report of the European Respiratory Society recommends the use of NO plateau values measured at least at three different exhalation flows and proposes several mathematical equations to calculate bronchial and alveolar NO parameters (7). However, there are currently no standardized method that can reliably be applied to asthmatic patients with varying airflow limitation, smoking status and disease severity.

Therefore, in this study we aimed to establish a feasible method for the partitioned measurement of exhaled NO in asthma. Patients and control subjects carried out expiratory manoeuvres at a broad range of flows. We established a protocol for the calculation of central bronchial and peripheral airway NO parameters with low week-to-week variation. Furthermore, we compared central and peripheral airway inflammation between patients and control subjects and correlated NO variables to clinical parameters in asthma.

Materials and Methods

Subjects

Patients were recruited at the Outpatient Clinic of Department of Pulmonology at Semmelweis University, Budapest, Hungary. They complained of symptoms consistent with the asthma diagnosis, they showed positivity for at least one airway allergen at skin prick testing or serum specific IgE testing. Patients presented documented airflow limitation, and they had airway hyperresponsiveness or were positive for bronchodilator reversibility during prior testing (1). A change in asthma therapy was not required in 4 weeks prior to the recruitment. Main exclusion criteria were other chronic respiratory diseases, asthma exacerbation in the previous 4 weeks, and signs of acute respiratory infections in the 2 weeks before recruitment. Healthy control subjects were recruited among employees working at the Department. Main exclusion criteria for controls were allergic airway disease or chronic respiratory disease in history, systemic steroid or antibiotic treatment in the previous 4 weeks, and signs of acute respiratory infections in the previous 2 weeks. Patient and control subjects were considered ex-smokers if they had stopped smoking at least 6 months before inclusion. The study was approved by the ethics committee, and all procedures were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Measurements were performed between 10 a.m. and 3 p.m. from 1 December 2015 until 30 November 2017.

Study design

Clinical study: Twenty-five control subjects and 40 patients with asthma were recruited. Medical history was noted, blood leukocyte count and CRP concentration were measured, exhaled nitric oxide concentration was recorded, and lung function tests were performed. Exhaled nitric oxide measurements were performed before lung function tests in all cases. Furthermore, patients filled out the Asthma Control Test (ACT) (8).

Repeatability study: Exhaled NO measurements were repeated at a weekly basis for 4 weeks in twelve control subjects, who also participated in the clinical study.

Nitric oxide measurements at multiple constant exhalation flow rates

Subjects were asked not to use inhaled medication, refrain from eating, drinking and smoking 2 hours prior to measurement. Exhaled NO concentration was measured during a manoeuvre starting from total lung capacity at the expiratory flows of 10-30-50-100-150-200-250-300 mL/s with a chemiluminescent analyser (Sievers Nitric Oxide Analyzer i280, GE Analytical Instruments, Boulder, Co, USA). Instrument calibration was performed daily according to the manufacturer's instructions. The background NO concentration was < 5 ppb. Restrictors, as provided and calibrated by the manufacturer, were applied to generate the required expiratory flows and ensure the closure of the velum during expiration. Manoeuvres at different flows with a duration of ≥ 20 s (10 mL/s exhalations flow), ≥ 10 s (30 mL/s), ≥ 6 s (50 and 100 mL/s) and ≥ 5 s (150, 200, 250 and 300 mL/s) were considered sufficient. Subjects received

visual feedback of the expiratory flow during the entire manoeuvre. Plateau values of NO recordings were identified manually. Recordings corresponding to the initial expiratory volume of 150 mL air (i.e. anatomic dead space) were disregarded. We considered a recording technically acceptable and valid if the plateau NO concentration was in a 3-second window with minimal sloping (9) where the actual exhalation flow was ± 10% of the target rate in compliance with the recommendations for $FENO_{50}$ (fractional exhaled nitric oxide concentration at 50 mL/s exhalation flow) analysis. The mean values of two NO recordings with < 10 % difference were used for further calculations.

Calculation of C_{ANO} and J_{awNO}

Data were analysed based on the two-compartment model using the linear method of Tsoukias et al. (5-7), which estimates acinar/alveolar NO (C_{ANO}) as the measure of the distal airways and total flux of NO in the conducting airway compartment (J_{awNO}) as a marker of central airways.

Other variables

Leukocyte count (Sysmex XE-2100, Sysmex Corporation, Kobe, Japan) and serum CRP concentration (Beckman Coulter AU680, Beckman Coulter Inc., Indianapolis, IN, USA) were determined from venous blood samples (asthma: N=38, control N=25). Measurements for spirometry, body plethysmography and diffusion capacity were performed according to current guidelines (PDT-111, Piston, Budapest, Hungary) (10-12). Two patients with asthma could not perform the manoeuvre for diffusion capacity measurement. An ACT score ≤ 19 referred to uncontrolled asthma (8).

Statistical analysis

Demographic data were compared with unpaired t-test and expressed as mean ± standard deviation, categorical variables were compared with the Fisher's exact test. Inhaled corticosteroid (ICS) doses, blood eosinophil percentage, CRP value, $FENO_{50}$, J_{awNO} and C_{ANO} data did not show a normal distribution (D'Agostino-Pearson normality test), therefore these variables were analysed with non-parametric tests (Mann-Whitney, Kruskal-Wallis with Dunn's post-hoc and Spearman tests) and expressed as median /interquartile range/. Measurement repeatability was assessed using the Bland-Altman plot (13). $P<0.05$ was considered significant (GraphPad Prism 5.0, GraphPad Software, San Diego, USA). Multiple regression analysis with smoking habits as covariates were used to assess relationship between C_{ANO} and lung function measures (Statistica 13.2, StatSoft, Tulsa, USA).

The sample size of the clinical study was calculated to reach a statistical power (1-β) of 0.80 and effect size of 0.75 with respect to the asymptotic relative efficiency of non-parametric tests. This effect size was based on the variability of J_{awNO} and C_{ANO} data in the repeatability study.

Results

Subject characteristics

The main clinical characteristics of patients and control subjects are shown in Table 1. Patients were treated at treatment steps of GINA 1 (steroid-naïve, n=7), GINA 3-4 (moderate-severe, n=16) and GINA 5 (severe on anti-IgE therapy, n=17) (1). Forty percent of patients had uncontrolled asthma according to the ACT scores.

Measurements of exhaled NO at different constant exhalation flow rates

Subjects performed exhalation manoeuvres at various constant flows (10-300 mL/s) both in the repeatability and clinical studies (number of measurements/flow in controls subjects: 61, in patients with asthma: 40; Table 2). Only a fraction of patients could perform a manoeuvre with a technically acceptable recording at very low flow rates (<50 mL/s), while the majority of manoeuvres were technically correct at higher flows (>50 mL/s). Therefore, for the extended NO analysis only the linear model could be applied (7), and NO values obtained at 100, 150, 200 and 250 mL/s expiratory flow rates were used as inputs for the calculation of J_{awNO} and C_{ANO} (asthma: r=0.98 ± 0.03, control: r=0.98 ± 0.02). As four patients could not perform the manoeuvre at 250 mL/s exhalation flow, values at 300 mL/s were included in the model in three cases. Using this strategy, 92.5% of all calculations in asthma were executed on data at 4 flow points (2.5% at 3 flow rates, 5% at 2 flow rates). Data obtained at 4 flow rates were used to calculate J_{awNO} and C_{ANO} in each control volunteer. All subjects could perform valid manoeuvres for $FENO_{50}$. Exhaled NO concentrations were elevated in asthma at all flow rates between 50 and 250 mL/s ($p<0.001$, Figure 1).

Intra-subject repeatability of J_{awNO} and C_{ANO}

Weekly J_{awNO} values in control subjects were 0.73 /0.59-0.73/, 0.51 /0.41-0.67/, 0.58 /0.50-0.73/ and 0.50 /0.39-0.68/ nL/s. The Bland-Altman analysis for the lowest and highest individual values showed a mean difference of 0.16 nL/s (95% limits of agreement: -0.56-0.89 nL/s; Figure 2a). C_{ANO} values at the weekly measurements were 2.64 /2.25-3.08/, 3.19 /2.30-4.22/, 1.75 /1.17-3.12/ and 2.27 /1.89-2.86/ ppb. The Bland-Altman graph for the

lowest and highest individual values demonstrated a mean bias of 0.85 ppb (95% limits of agreement: -3.67-5.36 ppb; Figure 2b).

Increased J_{awNO} and C_{ANO} in patients with asthma

J_{awNO} was increased in patients with asthma compared to control subjects (1.31 /0.83-2.97/ nL/s vs. 0.70 /0.54-0.87/ nL/s, p<0.001; Figure 3a). In asthma, J_{awNO} showed a strong positive correlation with $FENO_{50}$ (p<0.001, r=0.94; Figure 3b) and blood eosinophil percentage (p=0.001, r=0.50), but not with lung function parameters, leukocyte count, CRP or age (p>0.05).

Alveolar NO concentration was higher in patients than in controls (4.08 /2.63-7.16/ ppb vs. 2.42 /1.83-2.89/ ppb, p<0.001; Figure 4a). In asthma, C_{ANO} concentration positively correlated with blood eosinophil percentage (p=0.002, r=0.50), CRP concentration (p=0.001, r=0.50), age (p=0.003, r=0.46), RV/TLC (p<0.001, r=0.58; Figure 4c), and airway resistance (p=0.02, r=0.38). It inversely correlated with FEV_1 % predicted (p=0.03, r=-0.34), $FEF_{25-75\%}$ % reference (p=0.02, r=-0.36; Figure 4b), and diffusion capacity of the lung for carbon monoxide (p=0.004, r=-0.46; Figure 4d). We found a significant positive correlation between C_{ANO} and J_{awNO} (p=0.005, r=0.44), and between C_{ANO} and $FENO_{50}$ (p<0.001, r=0.60).

The association of C_{ANO} to RV/TLC and $D_{L,CO}$ remained significant in patients with asthma when using multiple regression analysis with smoking status (beta=0.48, p=0.003 and beta=-0.45, p=0.005) or packyears (beta=0.46, p=0.007 and beta=-0.43, p=0.007) as a covariate. However, the relationship between C_{ANO} and $FEF_{25-75\%}$ % reference became statistically insignificant in a model controlled for smoking status (p=0.09) or packyears (p=0.14).

The effect of smoking status on NO parameters

We also analysed non-smoking and ex-/current smoking control subjects and patients in separate subgroups. There was an increase in J_{awNO} in patients compared to controls with relevant smoking status (control vs. asthma in non-smokers: 0.68 /0.61-0.78/ nL/s vs. 1.22 /0.79-2.52/ nL/s, p=0.001 and in smokers: 0.75 /0.26-1.00/ nL/s vs. 1.64 /0.95-0.3.67/ nL/s. p=0.009; Figure 5a). Likewise, C_{ANO} was higher in asthma than in control subgroups (control vs. asthma in non-smokers: 2.48 /1.83-3.04/ ppb vs. 3.75 /2.46-6.35/ ppb, p=0.04 and in smokers: 2.04 /1.82-2.66/ nL/s vs. 5.70 /3.86-13.81/ ppb, p=0.001; Figure 5b). We found no difference in J_{awNO} and C_{ANO} between non-smokers and smokers within the control (p=0.99 and p=0.99) and asthmatic (p=0.99 and p=0.23) groups.

Discussion

The inflammation and dysfunction of small airways are related to important clinical aspects of asthma such as airway hyper-responsiveness (14) or exacerbation risk (15), therefore targeted anti-inflammatory treatment which can mitigate small airways inflammation can convey clinical benefit. However, the non-invasive assessment of distal lung inflammation is an unmet need in clinical practice. Models using exhaled NO concentration measured at different flow rates allow the partitioned assessment of airway inflammation in central and distal airways. The European Respiratory Society technical standard document provides details for mathematical modelling of pulmonary NO dynamics and highlights the need for further studies (16). In this study, we presented a feasible protocol for alveolar NO measurement and showed that inflammation and dysfunction of small airways are related in asthma.

In our study, patients performed exhalation manoeuvres at constant flow rates between 10 and 300 mL/s, but we could measure exhaled NO concentrations at low flow rates only in a

minority of patients with asthma. Similarly, Gelb et al. also noted that measurements were not reproducible at low exhalation flows (≤ 50 mL/s) in asthmatics with FEV_1 < 80% predicted (17), representing a significant number of treated patients in clinical settings. In addition, we also observed some failure in manoeuvre performance at 300 mL/s exhalation flow, which questions the feasibility of applying very high flow rates in this population. Up to 30% of patients with severe asthma had to be excluded from previous studies due to negative C_{ANO} values, suggesting inadequate models and the requirement of additional flow rates (18, 19). However, we established a linear model based on exhaled NO values at four flow points (100, 150, 200 and 250 mL/s), which could be successfully and reliably applied to measure alveolar NO concentrations in patients with differing asthma severity and smoking history.

We confirm previous findings that alveolar NO concentration is increased in a mixed group of asthmatic patients with mild-to-severe disease compared to matched control subjects (17). Other studies also showed that peripheral airway inflammation, as reflected by C_{ANO}, is elevated in clinically important phenotypes of asthma: in patients with refractory asthma on high ICS dose compared to mild-to-moderate asthma (20), in patients with steroid-dependent severe asthma compared to severe asthmatics on high dose ICS (19) and in subjects with nocturnal symptoms (21). Several authors observed that alveolar NO concentration could not be modified by initiating ICS therapy or increasing its dose (22, 23). While a decrease in C_{ANO} was reported after oral steroid therapy in some studies (17, 20), interestingly, others found no treatment effect (23). This suggests that despite ongoing anti-inflammatory treatment, increased inflammation in peripheral airways is a distinct disease characteristic in certain asthma phenotypes, which is also steroid-resistant in some cases. Hence, the extended NO analysis might facilitate the identification and better understanding of asthma subgroups, and it can also aid monitoring of novel anti-inflammatory therapies.

We analysed the relationship between alveolar NO concentration and physiological measures of distal lung dysfunction. We reported a moderate correlation between *C*ANO and RV/TLC, which is a known marker of air trapping and hyperinflation in severe and non-severe asthma (24). This finding extends the results of a previous study that showed a similar, but stronger correlation between the two parameters in severe asthma (18). In our study, there was a weak correlation between *C*ANO and $FEF_{25-75\%}$, which was not present when the analysis was controlled for smoking. This lung function parameter is debated to truly reflect peripheral airways dysfunction, partly due to its high measurement variability (25). Likewise, one study described a correlation with similar strength in mild-to-moderate asthma (26), but others found no relationship between alveolar NO concentration and $FEF_{25-75\%}$ in mild-to-severe asthmatics (19). Interestingly, in our cohort *C*ANO moderately correlated to pulmonary diffusion capacity, as previously observed in alveolitis (27). Besides the upregulation of the inducible NO synthase in alveolar epithelial cells, the decreased diffusion of NO might be another mechanism leading to increased *C*ANO in asthma, nonetheless, it must be noted that pulmonary diffusion capacity was within the normal range in patients. Despite of the exploratory nature of these results, they imply that increased *C*ANO can reflect distal airways dysfunction in asthma.

It was previously shown that alveolar NO concentration is strongly associated with eosinophil percentage in bronchoalveolar lavage fluid in mild asthmatics (20). However, the weak-to-moderate correlation between *C*ANO and blood eosinophil percentage in our study suggests that inflammation in peripheral airways is not closely related to systemic eosinophilic inflammation, as already shown for $FENO_{50}$ (28).

We also reported a positive correlation between age and *C*ANO, which was also observed in another cohort of asthmatic patients (29). It is known that *C*ANO increases with age in healthy subjects, which could be explained by the reduced pulmonary NO diffusion resulting from

decreased capillary blood volume at an older age (30). It can be speculated that this mechanism might also be present in older patients with asthma.

We found a close correlation between $FENO_{50}$ and J_{awNO} in asthma highlighting that these parameters assess inflammation at similar sites within the airways, and the additional information gained by the calculation of J_{awNO} might be limited as also suggested by others (31).

The weekly repeatability of C_{ANO} and J_{awNO} in control subjects was assessed in a one-month period, relevant to clinical settings for asthma follow-ups. The intra-subject repeatability of these parameters was somewhat better in a previous study using a day-to-day setup (19), nevertheless, the observed difference between controls and patients exceeded the mean intra-subject bias.

Some authors correct alveolar NO for trumpet model and axial NO back-diffusion (32, 33). However, these formulae disregard the effect of central airways constriction on axial back-diffusion, which potentially result in overcorrection (34, 35). According to the recent technical standard document the use of correction factors for axial back diffusion is not recommended (7). Therefore, we did not apply any correction in our data analysis.

This study has limitations. Cigarette smoking is known to interfere with exhaled nitric oxide concentration (36), and smoking had been previously shown to decrease C_{ANO} in asthma (37), which was not confirmed by our findings. Importantly, C_{ANO} was elevated in asthma, irrespective of the smoking status. Our cohort reflects a realistic asthmatic population in terms of smoking habits, as approximately one third of asthmatics were also reported to be current or former smokers in larger cohorts (38, 39). Cigarette smoking in asthma results in greater morbidity, uncontrolled and more severe disease, and accelerated decline in lung function (40). In addition, a recent publication described that one third of patients with severe

asthma were active or former smokers, who presented with fixed airflow limitation and clustered into clinical subgroups with either Th2-high or Th2-low signatures, underlining the potential therapeutic relevance of measuring inflammatory markers including exhaled NO in these populations (41). Furthermore, this is a single-centre observational study, and our results should be validated by future investigations. The cross-sectional nature of the NO measurements does not allow to draw conclusions regarding the repeatability of C_{ANO} in asthma, or how it reflects disease course or therapeutic interventions.

Conclusions

The present study describes a feasible protocol for extended NO analysis to calculate alveolar nitric oxide concentration, a marker of distal lung inflammation. Our method can successfully and reliably be applied to patients with asthma of differing severity including those with severe disease. Alveolar NO concentration shows a weak correlation to physiological measures of small airways dysfunction in asthma. The application of our protocol could facilitate understanding the role of C_{ANO} in phenotyping asthma.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Tables

Table 1. Patient characteristics

	Control, N=25	**Asthma, N=40**	**p-value**
Male/Female, N (%)	7/18 (28%/72%)	11/29 (27.5%/72.5%)	1.00
Age, years	39±14	44±17	0.17
Smoking status			
Non-smoker, N (%)	17 (68%)	28 (70%)	0.10
Ex-smoker, N (%)	3 (12%)	10 (25%)	
Current smoker, N (%)	5 (20%)	2 (5%)	
Pack-years	24±7	18±10	0.14
Blood eosinophil, %	1.4 /0.7-2.7/	4.2 /2.2-5.8/	<0.001
CRP, mg/L	2.0 /1.0-4.8/	2.6 /1.7-5.1/	0.47
FEV_1, %ref.	103±10	79±18	<0.001
FVC, %ref.	107±13	95±14	0.002

FEV_1/FVC	0.82±0.06	0.70±0.13	<0.001
$FEF_{25-75\%}$, %ref.	93±18	54±28	<0.001
RV/TLC, %	0.30±0.21	0.33±0.14	0.53
*K*CO, %ref.	97±19	98±17	0.89
*D*L,CO, %ref.	114±20	112±16	0.65
ICS, N (%)	NA	33 (82.5%)	NA
ICS dose, budesonide equivalent µg	NA	800 /340-800/	NA
LABA, N (%)	NA	31 (77.5%)	NA
LAMA, N (%)	NA	3 (7.5%)	NA
LTRA, N (%)	NA	14 (35%)	NA
Oral theophylline, N (%)	NA	3 (7.5%)	NA
Anti-IgE treatment, N (%)	NA	17 (42.5%)	NA

Data are presented as mean ± standard deviation or median /interquartile range/ and compared with unpaired t-test, Mann-Whitney or chi-square tests (categorical variables). CRP: C-reactive protein, *D*L,CO: diffusion capacity of the lung for carbon monoxide, ICS: inhaled corticosteroid, IgE: immunoglobulin E, $FEF_{25-75\%}$: forced expiratory flow at 25-75% of vital capacity, FEV_1: forced expiratory volume in 1 second, FVC: forced vital capacity, *K*CO: transfer coefficient of the lung for carbon monoxide, LABA: long-acting β2-agonist, LAMA: long-acting muscarinic antagonist, LTRA: leukotriene receptor antagonist, N: number, NA: not applicable, ref.: reference, RV: residual volume, TLC: total lung capacity.

Table 2. Percentage of technically acceptable exhaled NO manoeuvres with valid recordings at different exhalation flow rates

Exhalation flow, mL/s	**10**	**30**	**50**	**100**	**150**	**200**	**250**	**300**
Control subjects	20%	79%	100%	100%	100%	100%	98%	98%
Patients with asthma	8%	58%	100%	98%	98%	95%	90%	80%

Figure captions

Figure 1. Exhaled NO concentrations at multiple flow rates

Exhaled NO at different constant exhalation flows in controls (a) and patients with asthma (b). Lines show median values. Mann-Whitney test: $^{\#}p<0.001$ compared to corresponding NO concentration in controls.

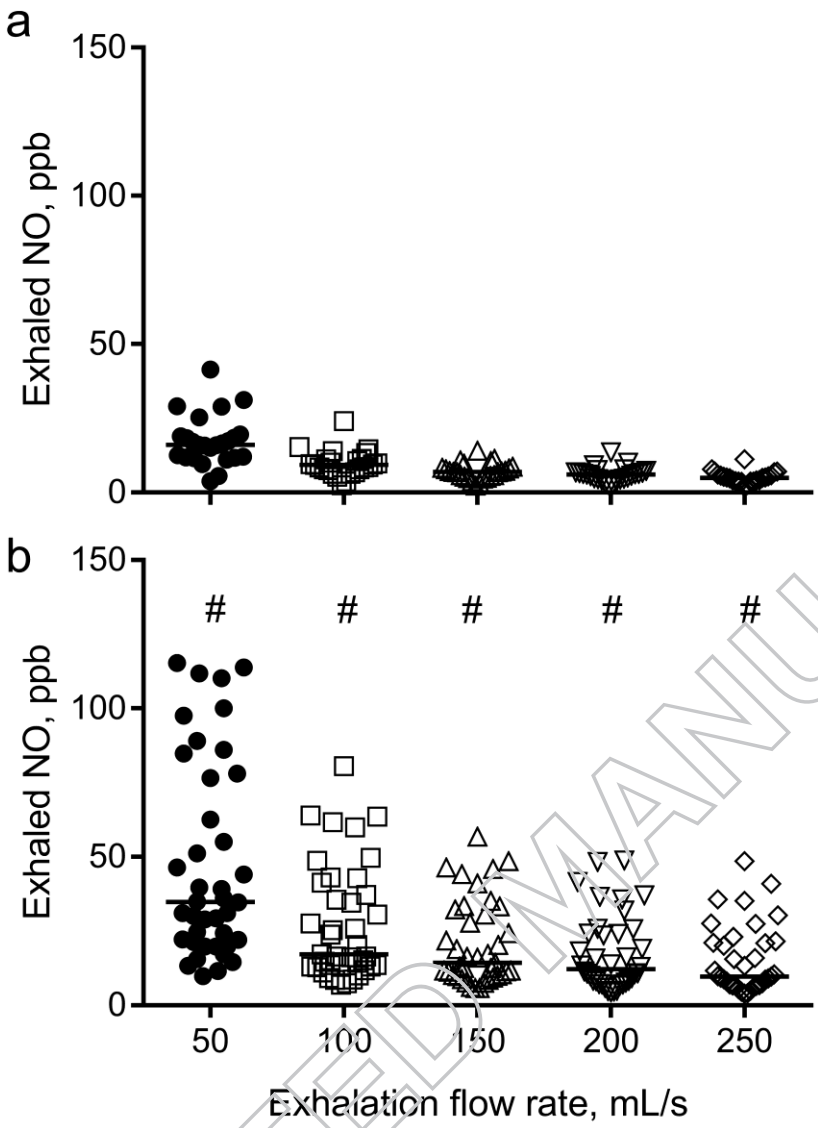

Figure 2. Intra-subject repeatability of J_{awNO} and C_{ANO}

The weekly intra-subject repeatability of total flux of NO in the conducting airway compartment (J_{awNO}; a) and alveolar nitric oxide concentration (C_{ANO}; b) in control subjects as shown by the Bland-Altman plot with mean difference and limits of agreement (mean difference ± 1.96 standard deviation).

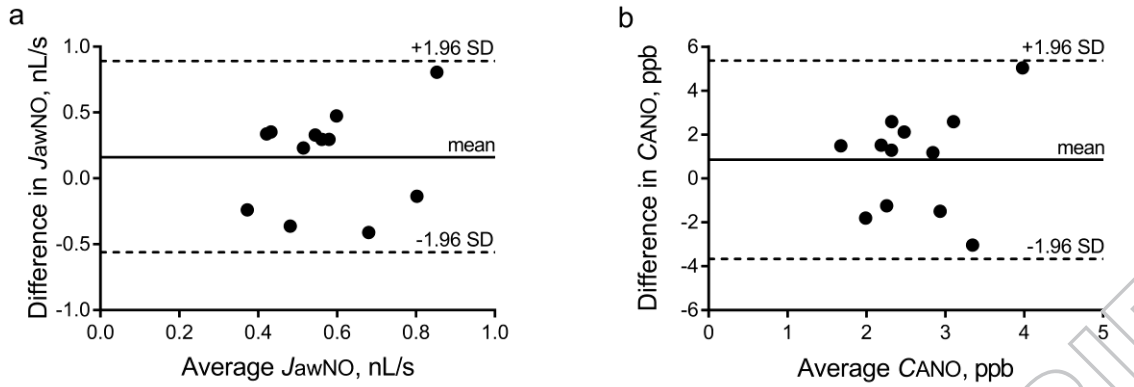

Figure 3. *J*awNO in patients with asthma

*J*awNO was increased in asthma (Mann-Whitney test; a), and strongly correlated with *F*ENO$_{50}$ (b). Spearman correlation: ***p<0.001

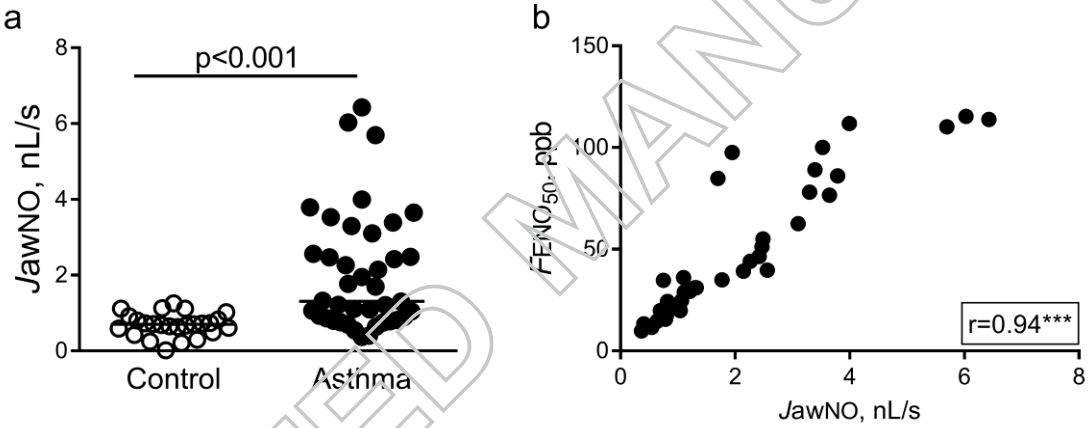

Figure 4. *C*ANO in patients with asthma

*C*ANO was increased in asthma (Mann-Whitney test; a), and correlated with FEF$_{25-75\%}$ % reference (b), RV/TLC (c) and *D*L,CO (d). Spearman correlation: *p<0.05, **p<0.01, ***p<0.001

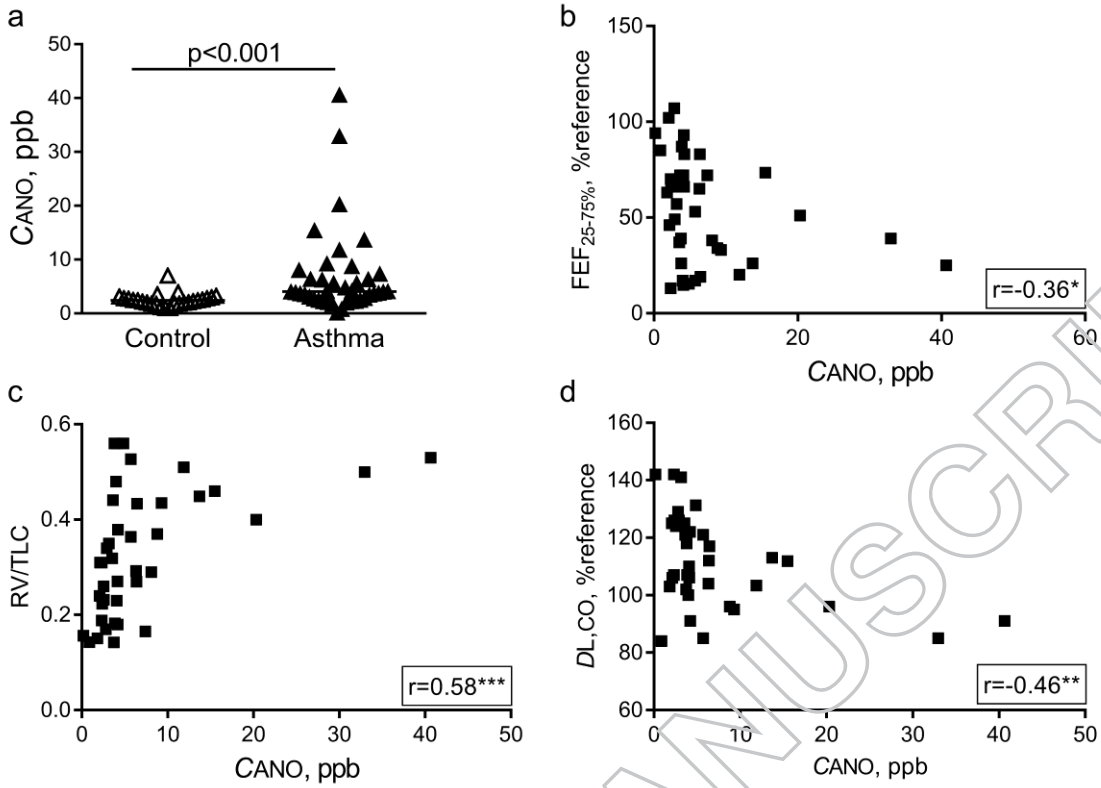

Figure 5. NO parameters in smokers and non-smokers

*J*awNO (a) and *C*ANO (b) were increased in ex- and current smoking patients with asthma compared to corresponding control subjects (Kruskal-Walllis with Dunn's post-hoc test).