

Alveolar and exhaled NO in relation to asthma characteristics – effects of correction for axial diffusion

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Abstract

Background: Inflammation in the small airways might contribute to incomplete asthma disease control despite intensive treatment in some subgroups of patients. Exhaled NO (FeNO) is a marker of inflammation in asthma and the estimated NO contribution from small airways (Calv_{NO}) is believed to reflect distal inflammation. Recent studies recommend adjustments of Calv_{NO} for trumpet model and axial diffusion (TMAD-adj). This study aimed to investigate the clinical correlates of Calv_{NO}, both TMAD-adjusted and unadjusted.

Methods: Asthma symptoms, asthma control, lung function, bronchial responsiveness, blood eosinophils, atopy and treatment level were assessed in 410 subjects, aged 10–35 years. Exhaled NO was measured at different flow-rates and Calv_{NO} calculated, with TMAD-adjustment according to Condorelli.

Results: Trumpet model and axial diffusion-adjusted Calv_{NO} was not related to daytime wheeze ($P = 0.27$), FEF₅₀ ($P = 0.23$) or bronchial responsiveness ($P = 0.52$). On the other hand, unadjusted Calv_{NO} was increased in subjects with daytime wheeze ($P < 0.001$), decreased FEF₅₀ ($P = 0.02$) and with moderate-to-severe compared to normal bronchial responsiveness ($P < 0.001$). All these characteristics correlated with increased FeNO (all $P < 0.05$). Unadjusted Calv_{NO} was positively related to bronchial NO flux (J'aw_{NO}) ($r = 0.22$, $P < 0.001$) while TMAD-adjCalv_{NO} was negatively related to J'aw_{NO} ($r = -0.38$, $P < 0.001$).

Conclusions: Adjusted Calv_{NO} was not associated with any asthma characteristics studied in this large asthma cohort. However, both FeNO and unadjusted Calv_{NO} related to asthma symptoms, lung function and bronchial responsiveness. We suggest a potential overadjustment by current TMAD-corrections, validated in healthy or unobstructed asthmatics. Further studies assessing axial diffusion in asthmatics with different degrees of airway obstruction and the validity of proposed TMAD-corrections are warranted.

Abbreviations

ACT, asthma control test; B-eos, blood eosinophils; Calv_{NO}, estimated alveolar nitric oxide concentration; FEF₅₀, forced expiratory flow at 50% of vital capacity; FeNO₅₀, standardized fractional exhaled nitric oxide at 50 ml/s; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; ICS, inhaled corticosteroid; J'aw_{NO}, bronchial flux; LLN, lower limits of normal; LTRA, leukotriene receptor antagonist; NO, nitric oxide; PD₂₀, provocative dose causing a fall of 20% in FEV₁; TMAD, trumpet shape of the airways and axial diffusion.

Chronic inflammation of the airways is the hallmark of asthma. However, diagnostic workup and tailoring of treatment strategy are traditionally based on symptoms and lung function tests (1). Despite intensified and sometimes costly treatment, a significant subgroup of patients with asthma do not achieve disease control (2). The development and standardization of methods to measure the fraction of exhaled nitric oxide (FeNO) has provided a reproducible and noninvasive means to obtain information about inflammatory activity, especially Th2-cytokine-driven, in the airways (3, 4).

Inflammation in peripheral airways (internal diameter of <2 mm) has been highlighted as an important aspect of

asthma (5–7). It has been speculated that one factor underlying failure to achieve asthma control could be peripheral inflammation that is not accessible to conventional therapies (5). With the availability of new extrafine drug formulations that can potentially reach farther into the airways, a clinically useful marker of inflammation in peripheral airways in asthma is needed to guide treatment decisions. The alveolar NO concentration (Calv_{NO}) has been proposed as such a marker (8).

Alveolar NO has been validated as a marker of alveolar inflammation in allergic alveolitis, and previous studies have shown raised alveolar NO in uncontrolled asthma (9), symptomatic asthma (10), asthma with nocturnal symptoms (11), and severe asthma (12). Further, alveolar NO levels decrease after oral corticosteroid treatment (12). However, recent mathematical modeling of NO transport in the lungs has indicated that axial molecular diffusion brings NO from the higher concentration in the airway lumen into the alveolar compartment during exhalation (13); thus, measurements would overestimate alveolar NO concentrations, especially in situations with raised bronchial NO flux. With models that compensate for axial diffusion (14, 15), more recent studies have presented variable results on alveolar NO (16–18). Moreover, the relation between alveolar NO and asthma characteristics has been little studied.

The primary aim of this study was to investigate the clinical signal provided by Calv_{NO} , by evaluating associations between Calv_{NO} and various asthma characteristics such as symptoms, asthma control, lung function, bronchial hyperresponsiveness, atopy, blood eosinophils, and treatment level, with estimates of Calv_{NO} calculated with and without correction for back diffusion. A secondary aim was to examine associations between standardized exhaled NO at 50 ml/s (FeNO_{50}), bronchial NO flux (J'_{awNO}), and the above-named asthma characteristics.

Material and methods

Subjects

A total of 410 subjects, aged 10–35 years, with physician-diagnosed asthma and daily treatment with inhaled corticosteroids (ICS) and/or oral leukotriene receptor antagonist (LTRA) during at least three of the preceding 12 months were included in this study. Subjects were recruited from both primary and secondary care in Uppsala, Sweden, between March 2010 and March 2012. Exclusion criteria were other chronic respiratory diseases, active tuberculosis, and recorded bloodborne disease.

Clinical asthma characteristics

Subjects responded to questions regarding asthma symptoms during the last 12 months (19). The degree of asthma control was assessed by asthma control test (ACT) (20). The subjects' use of ICS, LTRA, and any short courses of oral corticosteroids during the last three months was recorded in the interview. The prescribed daily dose of ICS was also collected from the subjects' medical record.

Exhaled NO and extended NO analysis

Exhaled NO was measured according to the American Thoracic Society/European Respiratory Society recommendations using a chemiluminescence analyzer (NIOX Flex; Aerocrine AB, Solna, Sweden) (21) and always before spirometry. A more detailed description of the patients' instructions and calibration is given in Patelis et al. (22). Measurements of exhaled NO were performed at four exhalation flow rates (50, 100, 200, and 300 ml/s) in random order, as previously described (23). Alveolar and central airway contributions to exhaled NO were calculated using the slope intercept model and the NO measurements at 100–300 ml/s (24). The Pearson correlation coefficient (r) between NO output and expiratory flow was calculated, and subjects were defined as model compliers if $r \geq 0.80$, as previously described by Mahut et al. (16). A total of 29 noncompliers were excluded because $\text{Calv}_{\text{NO}} < 0$ ($n = 4$), $J'_{\text{awNO}} < 0$ ($n = 4$), or $r < 0.8$ ($n = 21$). Estimated alveolar NO was adjusted for the trumpet shape of the airways and axial diffusion (TMAD) according to Condorelli (TMAD-adjusted $\text{Calv}_{\text{NO}} = \text{Calv}_{\text{NO}} - J'_{\text{awNO}}/860$) (15). The Condorelli method was chosen rather than the Kerckx method (14) because the Condorelli method gave fewer negative TMAD-adjusted Calv_{NO} (20 vs 47). Negative TMAD-adjusted Calv_{NO} values were assigned an arbitrary value of 0.01 to allow logarithmic transformation.

Lung function

Flow–volume curves were obtained according to American Thoracic Society recommendations (25) with a MasterScope spirometer (Erich Jaeger, Hoechberg, Würzburg, Germany). The lower limit of normal (LLN) for the ratio between forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) was calculated according to Hankinson (26). Subjects were subdivided according to normal or impaired lung function values ($\text{FEV}_1 < \text{or} \geq 80\%$, $\text{FVC} < \text{or} \geq 80\%$, $\text{FEV}_1/\text{FVC} < \text{or} \geq \text{LLN}$, forced expired flow at 50% of vital capacity (FEF_{50}) $< \text{or} \geq 50\%$). For subjects <18 years of age, Zapletal reference values for lung function were used (27), and for subjects >18 years of age, Hedenström reference values were used (28, 29).

Bronchial responsiveness

Methacholine provocation was performed with aerosol provocation system (Viasys Healthcare GmbH, Hoechberg, Germany) according to a simplified protocol described in detail elsewhere (22). Bronchial responsiveness was defined according to Schulze et al. (30) as normal when methacholine cumulative dose causing a fall in FEV_1 (PD_{20}) >1.0 mg, borderline-to-mild 0.3–1.0 mg, and moderate-to-severe <0.3 mg.

Blood eosinophil count

Blood eosinophils (B-eos) were counted at the Department of Clinical Chemistry at Uppsala University using a routine method (Cell-Dyn 4000, Abbott, IL, USA). Subjects were

divided into three groups according to the level of B-eos: normal ($<0.3 \times 10^9/l$), intermediate ($0.3\text{--}0.5 \times 10^9/l$) and high ($>0.5 \times 10^9/l$).

Atopy

IgE against a mix of aeroallergens (grass, tree and weed pollen, animal, mite, and mould allergens) (Phadiatop; Immunodiagnosics Thermofisher Scientific, Uppsala, Sweden) (31) was measured in all subjects except three. Subjects were defined as atopic if they had IgE antibodies against Phadiatop ≥ 0.35 kU/l.

Statistics

Statistical analyses were performed with STATA/IC 12.1 (StataCorp LP, College station, TX, USA) using log-transformed NO parameters. Unpaired *t*-test was used to compare NO parameters in dichotomized subject groups, linear regression models were applied for correlation between $J'aw_{NO}$ and $Calv_{NO}$, and multiple linear regression models

for differences in NO parameters for asthma characteristics adjusted for age, sex, height, and smoking. A *P*-value of <0.05 was considered significant.

Ethics

Uppsala Regional Ethical Review Board approved the study (approval number 2009/349), and all subjects and their legal guardians gave written informed consent.

Results

Description of the subjects

Most subjects were IgE-sensitized and their asthma well controlled. Demographic details and asthma characteristics are presented in Table 1. A total of 376 of 410 (91.7%) subjects were slope intercept model compliers. The noncompliers ($n = 29$) were younger and shorter and had a higher FEF_{50} than compliers ($P < 0.05$, Table 1). For all further analyses, results are based on model compliers only.

Table 1 Data presented as mean (SD) unless otherwise indicated

	All asthma subjects ($n = 410^*$)	Model compliers ($n = 376$)	Noncompliers ($n = 29$)	<i>P</i> -value
Age (years)	20.4 $\pm$ 7.1	20.7 $\pm$ 7.0	16.1 $\pm$ 5.9	<0.001
Female sex, n (%)	209 (51.0)	194 (51.6)	11 (37.9)	0.16
Height (cm)	166.8 $\pm$ 12.6	167.5 $\pm$ 12.2	159.8 $\pm$ 13.4	0.001
Weight (kg)	63.7 $\pm$ 16.5	64.4 $\pm$ 16.3	58.3 $\pm$ 16.6	0.05
Smoking, n (%)	18 (4.4)	18 (4.8)	0	0.20
ACT	20.6 ($\pm$ 3.4)	20.6 ($\pm$ 3.4)	19.9 ($\pm$ 3.7)	0.32
ACT ≥ 20 , n (%)	287 (70)	267 (71)	19 (65)	0.53
FEV ₁ (%pred)	92.0 $\pm$ 14.1	92.1 $\pm$ 13.9	94.3 $\pm$ 15.9	0.42
FVC (%pred)	99.6 $\pm$ 14.2	99.7 $\pm$ 14.1	102.2 $\pm$ 14.3	0.36
FEV ₁ /FVC (%)	81.4 $\pm$ 8.4	81.2 $\pm$ 8.4	84.0 $\pm$ 7.7	0.08
FEF ₅₀ (%pred)	82.0 $\pm$ 26.2	81.3 $\pm$ 25.5	94.7 $\pm$ 31.2	0.008
PD ₂₀ methacholine (mg)†	0.69 (0.52–0.93)	0.69 (0.51–0.94)	1.00 (0.28–0.60)	0.54
Atopic, n (%)	322 (79.1)	299 (79.5)	19 (65.5)	0.06
B-eos ($\times 10^9/l$)†	0.18 (0.17–0.20)	0.18 (0.17–0.20)	0.19 (0.13–0.28)	0.80
ICS-use				
no ICS, n (%)	81 (19.8)	71 (18.9)	10 (34.5)	0.22
ICS <500 , n (%)	244 (59.5)	227 (60.4)	15 (51.7)	
ICS 500–800, n (%)	53 (12.9)	49 (13.0)	3 (10.3)	
ICS >800 , n (%)	32 (7.8)	29 (7.7)	1 (3.4)	
LTRA, n (%)	74 (18.0)	65 (17.3)	6 (20.7)	0.64
FeNO ₅₀ (ppb)†	15.8 (14.6–17.0)	16.4 (15.1–17.7)	11.0 (8.1–14.8)	0.007
$J'aw_{NO}$ (pl/s)†		644 (584–710)		
$Calv_{NO}$ (ppb)†		2.7 (2.6–2.9)		
TMAD-adj. $Calv_{NO}$ (ppb)†		1.3 (1.1–1.5)		

ACT, asthma control test; ICS, inhaled corticosteroid; LTRA, leukotriene receptor antagonist; NO, nitric oxide; TMAD, trumpet model and axial diffusion.

Forced vital capacity (FVC) and FEV₁ presented as percentage predicted adjusted for age, sex, and height. Atopic = Phadiatop positive.

*Five individuals had a valid FeNO₅₀ measurement, but failed a set of measurements between 100 and 300 ml/s.

†Data presented as geometric means (95% confidence interval).

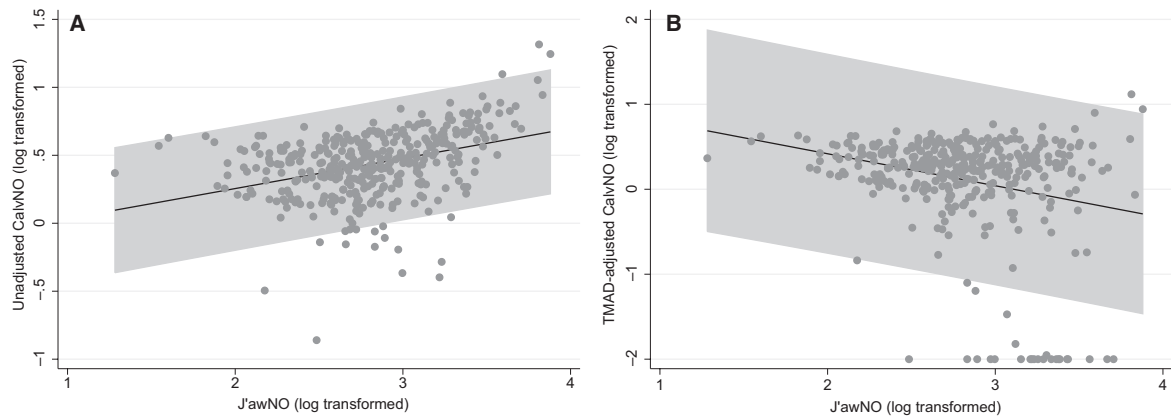


Figure 1 Regression plot of log-transformed $J'awNO$ and (A) log-transformed $CalvNO$ unadjusted. (B) log-transformed TMAD-adjusted $CalvNO$, with regression line (black) and 95% CI (gray area).

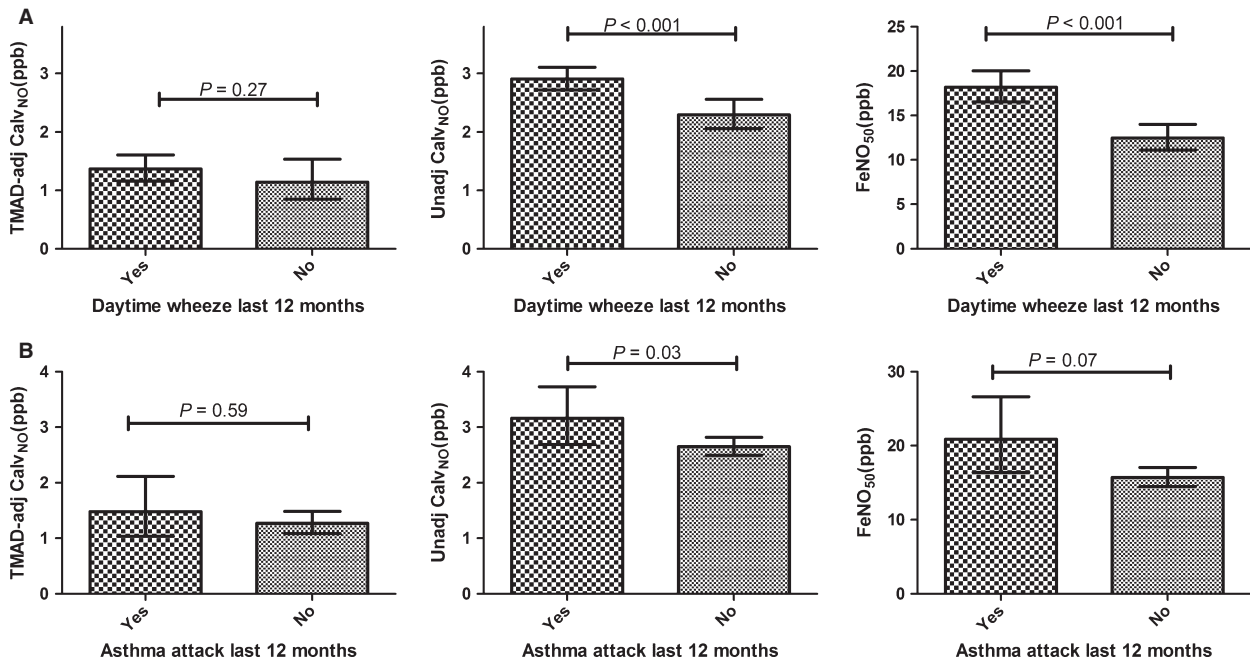


Figure 2 TMAD-adjusted $CalvNO$, unadjusted $CalvNO$, $FeNO_{50}$, and (A) daytime wheeze and (B) asthma attack during the last 12 months. Data presented as geometric means (95% confidence interval).

Correlation between unadjusted and TMAD-adjusted $CalvNO$ and $J'awNO$

Unadjusted $CalvNO$ was positively associated with $J'awNO$ ($r = 0.22$, $P < 0.001$). For TMAD-adjusted $CalvNO$, a negative correlation with $J'awNO$ was found ($r = -0.38$, $P < 0.001$), see Fig. 1. These results remained unchanged when subjects were divided into two groups: <18 years of age ($r = 0.25$, $P < 0.001$ and -0.41 , $P < 0.001$) or ≥ 18 years of age ($r = 0.20$, $P < 0.001$ and $r = 0.34$, $P < 0.001$).

NO parameters and asthma symptoms in the year preceding the study

TMAD-adjusted $CalvNO$ was similar for subjects who did and did not report any of the asthma symptoms studied. Unadjusted $CalvNO$ and $FeNO_{50}$ were higher in subjects with reported daytime wheeze in last 12 months ($n = 271$) compared with subjects without daytime wheeze ($n = 105$) ($P < 0.001$ for both) (Fig. 2), as was $J'awNO$ ($P < 0.001$). In subjects reporting an asthma attack in last 12 months

Table 3 Nitric oxide (NO) parameters in asthmatic individuals with normal and impaired FEV₁, FEV₁/FVC LLN, FEF₅₀. Data presented as geometric means (95% confidence interval)

	FEV ₁		FEV ₁ /FVC LLN		FEF ₅₀	
	≥80%	<80%	≥LLN	<LLN	≥50%	<50%
TMAD-adjusted	1.3 (1.1–1.5)	1.4 (1.0–2.0)	1.2 (1.0–1.4)	1.6 (1.3–2.0)	1.3 (1.1–1.5)	1.7 (1.1–2.7)
Calv _{NO} (ppb)						0.23
Unadjusted	2.7 (2.5–2.9)	2.9 (2.4–3.4)	2.7 (2.5–2.8)	3.0 (2.6–3.4)	2.7 (2.5–2.8)	0.02
Calv _{NO} (ppb)						
FeNO ₅₀ (ppb)	15.7 (14.5–17.1)	19.9 (16.1–24.6)	15.7 (14.5–17.1)	18.8 (15.5–22.8)	15.8 (14.6–17.1)	0.004
J'aw _{NO} (pl/s)	614 (552–682)	812 (633–1040)	624 (561–693)	716 (562–912)	622 (563–687)	0.03

FVC, forced vital capacity; LLN, lower limits of normal; TMAD, trumpet model and axial diffusion. Bold values show *P*-values <0.05.

Calv_{NO} and FeNO₅₀ were seen in subjects with raised level of B-eos compared with normal levels (Fig. 3B), and the same was found for J'aw_{NO} (*P* < 0.001). These results were consistent after adjustment for age, sex, height, and smoking.

NO parameters and atopy

TMAD-adjusted Calv_{NO} was similar in atopic and nonatopic asthmatics: 1.3 ppb (1.1–1.5) vs 1.5 ppb (1.2–2.0), *P* = 0.33. Unadjusted Calv_{NO} was increased in those with atopy: 2.8 ppb (2.7–3.0) vs 2.3 ppb (2.0–2.7), *P* = 0.01. Also, FeNO₅₀ was higher in atopic subjects, 17.9 ppb (16.4–19.5) vs 11.3 ppb (9.6–13.2), *P* < 0.001 as was J'aw_{NO}, *P* < 0.001. These results were consistent after adjustment for age, sex, height, and smoking.

NO parameters and medical treatment regimen

TMAD-adjusted Calv_{NO} was similar in the different treatment groups (Fig. 4). Unadjusted Calv_{NO} and FeNO₅₀ were decreased in asthma subjects on higher ICS dose (Fig. 4A), and the same was found for J'aw_{NO} (data not shown). FeNO₅₀ was decreased in subjects on LTRA treatment (Fig. 4B), and so was J'aw_{NO} (*P* = 0.001). Reported treatment with a short course of oral steroids during the last 3 months did not correlate with any NO parameter (data not shown). These results were consistent after adjustment for age, sex, height, and smoking.

Discussion

The main findings in our study were that when estimates of alveolar NO were adjusted for axial diffusion as recommended, no associations with asthma symptoms, asthma control, lung function, bronchial responsiveness, atopy, blood eosinophils, or treatment level could be found. On the other hand, increased FeNO₅₀ and unadjusted alveolar NO were significantly associated with reporting daytime wheeze last 12 months, FEF₅₀, bronchial responsiveness, atopy, and blood eosinophilia. Interestingly, the adjusted alveolar NO levels showed a negative correlation with bronchial NO flux, which raises the question of overadjustment.

This is the largest study to date investigating the clinical signal of alveolar NO in asthma. Our subjects were recruited both from primary and secondary care in Uppsala county and thus should be representative of individuals with asthma aged 10–35 years in Sweden, covering different asthma severities and underlying inflammatory subtypes. Most of our subjects were compliant with the slope intercept model, based on the quality measure suggested by Mahut et al. (16), and thus, only a fraction were excluded. Smokers were not excluded, but were only a small proportion (5%) of our subjects, which might be explained by the relatively low prevalence of smoking in the general population in Sweden (32). Another strength of the study is that we included both children and young adults, and we could demonstrate a similar pattern of results in both populations as no interaction with age group was found regarding the main outcomes. A limitation of the

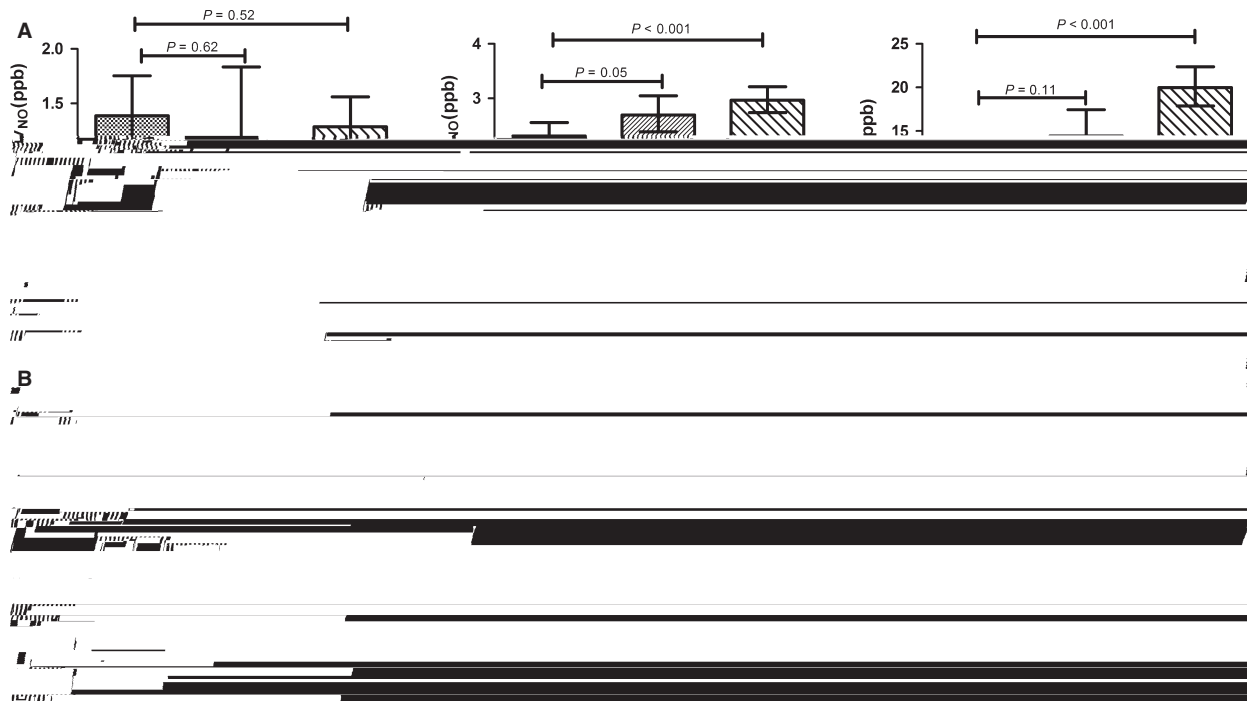


Figure 3 TMAD-adjusted CalvNO , unadjusted CalvNO , FeNO_{50} , and (A) level of bronchial responsiveness and (B) level of B-eos. Data presented as geometric means (95% confidence interval).

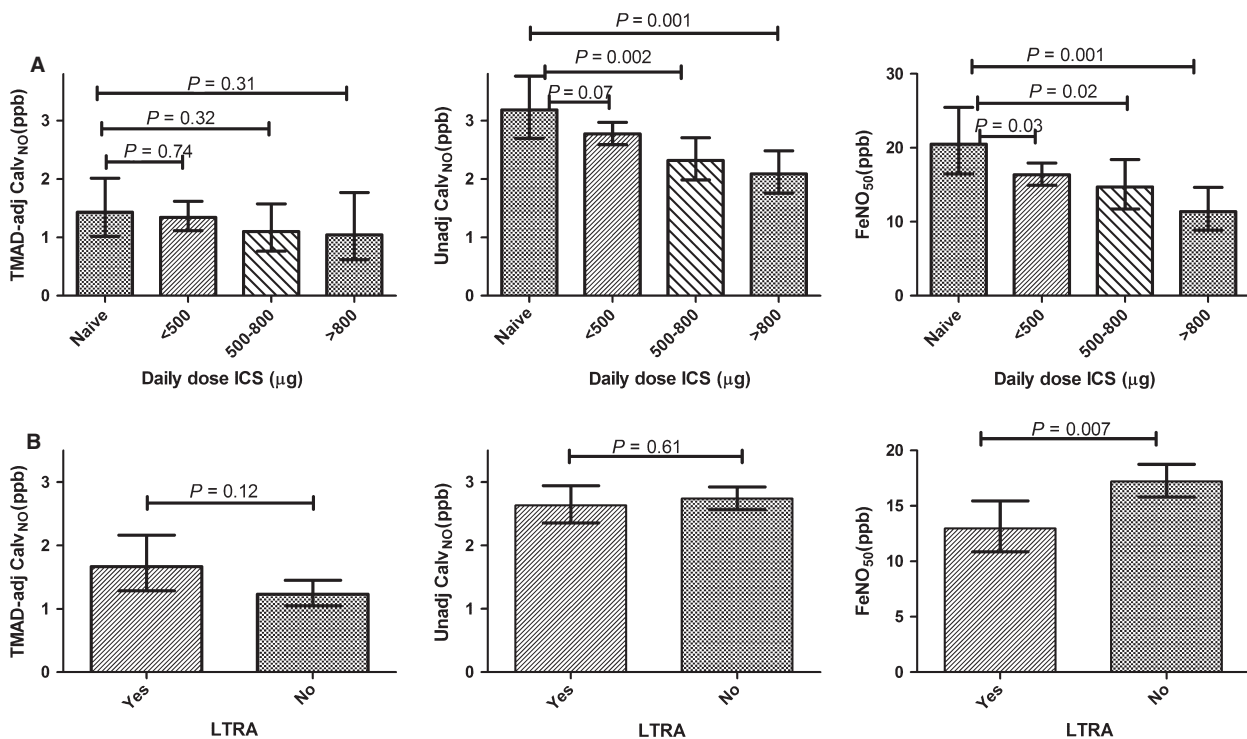


Figure 4 TMAD-adjusted CalvNO , unadjusted CalvNO , FeNO_{50} , and (A) inhaled corticosteroid (ICS) and (B) leukotriene receptor antagonist (LTRA) treatment. Data presented as geometric means (95% confidence interval).

study is the lack of physiological measurements of small airways function, for example, nitrogen washout tests or forced oscillation technique (33). As respiratory symptoms were assessed within the time frame of 12 months previous to the study, recall bias cannot be ruled out.

Our main finding was the lack of association between any of the above-mentioned asthma characteristics and TMAD-adjusted Calv_{NO} . Thus, our results corroborate those presented by Mahut et al. (16) where 200 individuals with age range, lung function, and medication level comparable with our cohort were evaluated for correlation between TMAD-adjusted Calv_{NO} and asthma control/severity. However, some smaller studies have presented association between TMAD-adjusted Calv_{NO} and medical treatment (17), and also asthma control (34).

Biopsy-verified distal airways inflammation is correlated with uncontrolled, severe asthma (35), and unadjusted Calv_{NO} has been reported to be increased in patients with uncontrolled, severe asthma (9, 12), making Calv_{NO} an appealing noninvasive marker of small airways inflammation. However, recent studies have highlighted the difficulties of using this marker: for example, alveolar NO can paradoxically increase after antiinflammatory treatment in a subgroup of asthmatics (36). This probably reflects that in more constricted peripheral airways, back diffusion would increase once treatment has relieved the peripheral obstruction. Moreover, the need to correct for axial diffusion should be smaller in patients with a larger degree of peripheral obstruction. It might therefore be argued that the axial diffusion adjustment is not a 'one size fits all' adjustment. The present study reports a negative correlation between TMAD-adjusted Calv_{NO} and J'_{awNO} , in contrast to the only two studies known to us that reported correlations between TMAD-adjusted Calv_{NO} and J'_{awNO} (16, 37). This has not been studied or reported before, but might signal an overadjustment of Calv_{NO} in our material. The original study by Condorelli et al. (15) was performed in eight healthy subjects, and the correction parameters were based on data from these subjects. However, both the degree and location of airways constriction vary from one individual to another in asthma, and the degree of peripheral airways constriction would interfere with the axial diffusion correction. Therefore, it would be relevant to investigate whether these corrections are valid in asthmatic individuals with different degrees of airway obstruction and whether corrections should take into account the degree and location of obstruction.

In our study, FeNO_{50} , J'_{awNO} , and unadjusted Calv_{NO} were correlated with daytime wheeze. As the recall period for asthma symptoms was 12 months prior to the measurements of exhaled NO, it could be argued that this symptom report mainly reflects a wheezing phenotype, and consequently its relation to FeNO_{50} , results in line with our report on the association between self-reported wheeze during the last 12 months and increased FeNO_{50} in a large population-based study (38). Exhaled NO was not correlated with asthma control defined by ACT score, which is in line with previous findings (16). Bronchial hyperresponsiveness was related to higher FeNO_{50} in our material, which is in

line with previous studies (39). However, the relation with raised unadjusted Calv_{NO} has not to our knowledge been previously reported. Impaired FEF_{50} related to increased FeNO_{50} , J'_{awNO} and unadjusted Calv_{NO} , in line with previous findings (40). FeNO_{50} was also related to FEV_1 . Atopy was related not only to increased FeNO_{50} and J'_{awNO} but also to unadjusted Calv_{NO} , which contradicts a previous study from our group (41). This might be explained by the population-based setting of the previous study and the relatively small size of the effect: a large population of patients with asthma as in the present study is probably required to find significant differences. The relation between higher unadjusted Calv_{NO} and history of asthma attack in the preceding year is a novel and interesting finding, suggesting that unadjusted Calv_{NO} might identify asthmatics at risk of asthma attack. However, we are cautious due to the design of our study as this information was retrospective and the difference in Calv_{NO} was small (<0.5 ppb) between subjects with and without an asthma attack previous year, casting doubt on the potential clinical applicability of this information.

In conclusion, no associations between TMAD-adjusted Calv_{NO} and asthma characteristics were found in this large asthma cohort study. Standard measures of exhaled NO, FeNO at 50 ml/s, as well as unadjusted Calv_{NO} correlated with reported daytime wheeze during last 12 months, lung function, bronchial responsiveness, atopy, blood eosinophilia, and treatment level, and the findings on the associations between unadjusted Calv_{NO} and bronchial hyperresponsiveness and atopy are novel. As we raise the potential problem that the suggested adjustments may lead to overadjustment, we conclude that further studies assessing axial diffusion in subjects with asthma and the validity of proposed adjustment algorithms are warranted. With the increased awareness of asthma as a disease also in the small airways and availability of therapies better able to reach the small airways, more studies are needed to establish the role of adjusted – or unadjusted – alveolar NO in identifying this phenotype and following up on the effects of treatment.

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Author contributions

C. Heijkenskjöld-Rentzhog, L. Nordvall, C. Jansson, Magnus P. Borres, K. Alving, and A. Malinovski contributed to the study design. C. Heijkenskjöld-Rentzhog, L. Nordvall, C. Jansson, K. Alving, and A. Malinovski were involved in the data acquisition. C. Heijkenskjöld-Rentzhog and A. Malinovski contributed to statistics. C. Heijkenskjöld-Rentzhog wrote the first draft of the manuscript. C. Heijkenskjöld-Rentzhog, L. Nordvall, C. Jansson, K. Alving, and A. Malinovski critically revised and approved the final version.

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Conflicts of interest

Kjell Alving is an employee of Aerocrine AB (producer of exhaled NO devices) and minority shareholder, but participated in the study with his role as adjunct professor at Uppsala University. Magnus Borres is an employee of Thermo Fisher Scientific, Immunodiagnosics, and participated in the study as adjunct professor at Uppsala University. None of the funding agencies or industry partners had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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