

Acinar effect of inhaled steroids evidenced by exhaled nitric oxide

Alain Van Muylem, PhD, Yannick Kerckx, PhD, and Alain Michils, PhD *Brussels, Belgium*

Background: The effects of inhaled corticosteroids (ICSs) on distal lung inflammation, as assessed by alveolar nitric oxide concentration ($C_{A}NO$), are a matter of debate. Recently, a theoretic study suggested that acinar airway obstruction that is relieved by ICS treatment and associated with a decrease in fraction of exhaled nitric oxide (FeNO) concentration might, paradoxically, increase $C_{A}NO$. This increase could be a hallmark effect of ICSs at the acinar level.

Objective: In the light of this new hypothesis, we studied changes in $C_{A}NO$ and FeNO after administration of ICSs. **Methods:** $C_{A}NO$ and FeNO were measured before and after ICS treatment of 38 steroid-naïve patients with uncontrolled asthma who showed clinical improvement after ICS therapy.

Results: The average FeNO decreased from 78.3 to 28.9 ppb ($P < .001$); $C_{A}NO$ decreased from 7.7 to 4.3 ppb ($P = .009$). In 14 subjects (low-slope group), slope ($= \Delta C_{A}NO / \Delta FeNO$) values ($\Delta = \text{post-ICS} - \text{pre-ICS value}$) were less than the 95% normal CI (average $\Delta FeNO = -32.7$ ppb and average $\Delta C_{A}NO = +2.9$ ppb). In this group, baseline $C_{A}NO$ was abnormally low when FeNO was taken into account. In 11 subjects (the high-slope group), the slope was above the normal interval (average $\Delta FeNO = -42.5$ ppb and average $\Delta C_{A}NO = -14.7$ ppb). **Conclusion:** Opposite patterns (one that was predicted) can indicate peripheral actions of ICSs; this difference might account for conflicting data reported from studies using $C_{A}NO$ to determine the peripheral action of ICSs. We show that a low $C_{A}NO$ does not preclude distal inflammation. (J Allergy Clin Immunol 2010;■■■■:■■■-■■■.)

Key words: Exhaled nitric oxide, alveolar nitric oxide, asthma, inhaled corticosteroids, Asthma Control Questionnaire

Studies of postmortem tissue¹ and transbronchial biopsy samples indicated that asthma affects the small airways.² Noninvasive analysis of the function of these airways is complex, especially at peripheral zones, such as intra-acinar airways. One noninvasive

Abbreviations used

ACQ:	Asthma Control Questionnaire
$C_{A}NO$:	Alveolar nitric oxide concentration
FEF ₇₅ :	Forced expired flow at 75% of vital capacity
FeNO:	Fraction of exhaled nitric oxide
FVC:	Forced vital capacity
ICS:	Inhaled corticosteroid
J'awNO:	Apparent bronchial nitric oxide production
LABA:	Long-acting β_2 -agonist
NO:	Nitric oxide

approach for measuring distal inflammation³⁻⁸ involves quantification of alveolar nitric oxide (NO) by tracing exhaled NO.

The exact site of action of inhaled corticosteroids (ICSs) in patients with asthma is controversial. Although ICSs have anti-inflammatory effects on bronchial airways,^{9,10} their effects on distal inflammation, as assessed by alveolar nitric oxide concentration ($C_{A}NO$), are unclear. Lehtimäki et al¹⁰ did not find a difference in $C_{A}NO$ between asthmatic patients and control subjects at baseline or after ICS treatment. However, a cross-sectional study by Gelb et al⁴ revealed that overall $C_{A}NO$ in ICS-treated patients was greater than that of the control group, although there was wide scatter and overlap of the data from the 2 groups.

The effect of ICSs on lung periphery, from terminal bronchioles to alveoli, should be determined by taking interaction between $C_{A}NO$ and bronchial NO production into account. Recent analyses of NO transport indicated a link between fraction of exhaled nitric oxide (FeNO) concentration, which is measured to assess bronchial (including peripheral) NO production, and $C_{A}NO$.^{11,12} Because of the concentration gradient between pre-acinar bronchioles and alveoli, some NO molecules produced in the peripheral airways diffuse toward the alveoli (back diffusion) without being expired.^{13,14} This can result in a lower FeNO and a higher $C_{A}NO$ ^{13,14} compared with values predicted by perfectly mixed, 2-compartment models.

When intra-acinar peripheral bronchoconstriction develops, back diffusion is impaired by the reduced caliber of the airway,¹⁵ which hampers NO flux toward the alveolar zone. Compared with an unobstructed condition, FeNO increases (more NO molecules are available for expiration) and $C_{A}NO$ decreases.

Therefore reduction or elimination of bronchoconstriction by therapy would increase $C_{A}NO$ and decrease FeNO. Similar to an ICS-induced reduction in $C_{A}NO$, these changes might indicate activity of ICSs in the periphery and account for the observed absence of changes in $C_{A}NO$ after ICS treatment¹⁰ or the wide range of $C_{A}NO$ detected in ICS-treated and untreated patients with asthma.⁴

From the Chest Department, Cliniques Universitaires Erasme, Université Libre de Bruxelles.

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Reprint request: Alain Van Muylem, PhD, Chest Department, Cliniques Universitaires Erasme, Université Libre de Bruxelles, 808 Route de Lennik, B-1070 Brussels, Belgium. E-mail: avmuylem@ulb.ac.be.

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We evaluated changes in FeNO and C_A NO that occurred after treatment with an ICS (Turbuhaler, a powder inhaler; AstraZeneca, Södertälje, Sweden) that significantly improved control of asthma.

METHODS

Subjects

This prospective study included nonsmoking adult patients who were given new diagnoses of asthma based on standard criteria¹⁶ and treated at the Allergy and Asthma Clinic in the Chest Department of Erasme University Hospital (Brussels, Belgium). The patients had not received any treatment for asthma before the study began. Diagnosis of asthma was based either on the reversal of airflow obstruction after inhalation of a short-acting β_2 -agonist ($n = 16$) or on airway hyperresponsiveness ($n = 22$) depending on baseline spirometric values. All patients were atopic, as defined by at least 1 positive response on a skin prick test or RAST of common inhaled allergens. The local ethics committee approved the protocol, and each subject signed an informed consent form.

Study design

Patients were evaluated twice at a median interval of 3 weeks (2-6 weeks). Scores from an Asthma Control Questionnaire (ACQ), FeNO, C_A NO, and standard lung function (FEV₁, forced vital capacity [FVC], and forced expired flow at 75% of vital capacity [FEF₇₅]) were recorded at each examination. At the end of visit 1, asthma therapy was initiated in line with international guidelines and recommendations¹⁶ and regardless of ACQ scores or FeNO and C_A NO, which were recorded separately. Data from a total of 38 patients with significant improvements in asthma control after the prescribed treatment were analyzed (21 male subjects; age, 38 ± 14 years; age range, 18-67 years). Among these, 20 patients were treated with only ICSs (Pulmicort Turbuhaler, a dry-powder budesonide; 875 μ g beclomethasone equivalent per day on average), and 18 patients received a combination of ICSs (722 μ g beclomethasone equivalent per day on average) and a long-acting β_2 -agonist (LABA; Symbicort Turbuhaler, a dry-powder budesonide/formoterol; 1-2 inhalations twice daily).

NO

A chemiluminescence analyzer (LR 2000; Logan Research LTD, Rochester, United Kingdom) was used to measure NO levels in real time. The instrument was calibrated daily (111 ppb of NO in nitrogen). C_A NO and the apparent bronchial nitric oxide production (J'_{aw} NO) were calculated by using the multiframe method described by Pietropaoli et al.¹⁷ Tracings of exhaled NO were obtained for flow rates of 50, 175, and 300 mL/s. An extended discussion concerning the validity of the method is available elsewhere.¹¹ C_A NO was also calculated by using the method described by Tsoukias and George.¹⁸ In this study exhaled NO at 50 mL/s was referred to as FeNO, which is in line with American Thoracic Society/European Respiratory Society standards.¹⁹

Changes in FeNO and C_A NO

The ratio of ΔC_A NO to Δ FeNO (ΔC_A NO/ Δ FeNO; Δ is the change in concentration from before to after ICS therapy) was calculated to assess changes in C_A NO after ICS treatment compared with changes in FeNO for each subject. When plotting C_A NO as a function of FeNO, ΔC_A NO/ Δ FeNO corresponds to the slope of the line segment joining pre-ICS and post-ICS treatment values. The 95% CI of ΔC_A NO/ Δ FeNO for patients with stable and obstruction-free asthma¹¹ was derived by using the bootstrap method. Fig.E1 of the Online Repository showed the adjusted log-normal distribution of the slope (see the Methods section in this article's Online Repository at www.jacionline.org for details).

ACQ

Asthma control was assessed by using a short version of the ACQ, as created by Juniper et al,²⁰ that was translated into French. A score of less than 0.75 was defined as well-controlled asthma, whereas a score of greater than 1.5 was defined as poorly controlled asthma.²¹ A change of 0.5 in the ACQ score was considered clinically relevant.²¹

TABLE I. Baseline and post-ICS therapy results

	Baseline	Post-ICS	P value
ACQ score*	2.3 (1.0-5.0)	0.5 (0.0-1.8)	<.001
FEV ₁ (% predicted)†	86 $\pm$ 18	95 $\pm$ 13	<.001
FVC (% predicted)†	100 $\pm$ 25	108 $\pm$ 22	.007
FEV ₁ /FVC (%)†	73 $\pm$ 12	78 $\pm$ 9	<.001
FEF ₇₅ (% predicted)†	62 $\pm$ 25	81 $\pm$ 24	<.001
FeNO (ppb)‡	72.3 (50.0-104.7)	28.8 (16.6-49.9)	<.001
J'_{aw} NO (pL/s)†	3,346 $\pm$ 1,469	1,386 $\pm$ 795	<.001
C_A NO _a (ppb)‡	7.4 (2.9-18.6)	4.2 (1.6-11.6)	.009
C_A NO _b (ppb)‡	6.3 (1.9-20.3)	3.6 (1.2-11.1)	.006

C_A NO_a, Alveolar nitric oxide concentration obtained by using the Pietropaoli et al¹⁷ method; C_A NO_b, alveolar nitric oxide concentration obtained by using the Tsoukias et al¹⁸ method; FVC, Forced vital capacity; J'_{aw} NO, apparent bronchial production obtained by using the Pietropaoli et al¹⁷ method.

*Median (range)

†Mean $\pm$ SD.

‡Geometric mean (geometric interval).

Lung function

Spirometry was performed with a Zan 300 spirometer (Zan, Oberthulba, Germany). The prebronchodilator FEV₁ was used as an index of airway caliber. FEV₁, FVC, and FEF₇₅ values were expressed as a percentage of predicted value.²²

Statistics

FEV₁, FVC and FEF₇₅ were expressed as a percentage of the predicted value, and FeNO and C_A NO were log-transformed.²³ These variables were compared with paired *t* tests, unpaired *t* tests, and 1-way classification ANOVA (with *post hoc* comparisons in case of a significant global difference). ACQ scores were compared with Wilcoxon, Mann-Whitney *U*, and Kruskal-Wallis tests. The limits of significance were set at .05.

RESULTS

Table I shows average baseline and post-ICS values for ACQ scores, FeNO, J'_{aw} NO, C_A NO determined by using the methods of Pietropaoli et al¹⁷ (C_A NO_a) and Tsoukias et al¹⁸ (C_A NO_b), FEV₁, FVC, FEV₁/FVC, and FEF₇₅. All changes were significant. FeNO and J'_{aw} NO were correlated at baseline ($R^2 = 0.94$) and after ICS treatment ($R^2 = 0.96$). The C_A NO values calculated by using the methods of Pietropaoli et al¹⁷ (C_A NO_a) versus those calculated by using the methods of Tsoukias et al¹⁸ (C_A NO_b) did not differ significantly at baseline ($P = .54$) or after ICS treatment ($P = .61$). Therefore, except in Table I, only the C_A NO calculated by using the method of Pietropaoli et al¹⁷ is presented. Table II shows prebronchodilation and postbronchodilation pulmonary function values and post-ICS values for the 16 patients who were given diagnoses of asthma based on bronchodilation.

Improvements in asthma control

After ICS treatment, asthma control improved for all patients (their ACQ scores decreased by >0.5). Moreover, all but 5 patients had an ACQ score of less than 0.75, which indicates well-controlled asthma.

Changes in FeNO and C_A NO

Fig 1 shows FeNO (Fig 1, A) and C_A NO (Fig 1, B) before and after ICS therapy for each patient. Fig 1, C, shows ΔC_A NO/ Δ FeNO for each patient. A negative value means that C_A NO

TABLE II. Prebronchodilation, postbronchodilation, and post-ICS therapy results (n = 16)

	Prebronchodilation	Postbronchodilation	Post-ICS	P value*	P value†
FEV ₁ (% predicted)	65 ± 20	74 ± 22	90 ± 13	<.001	<.001
FVC (% predicted)	84 ± 22	92 ± 22	97 ± 20	<.001	<.001
FEV ₁ /FVC (%)	66 ± 14	65 ± 12	76 ± 10	.463	<.001
FEF ₇₅ (% predicted)	40.6 ± 17.7	49.7 ± 21.8	65 ± 24.1	.027	<.001

All variables are presented as means ± SDs. FVC, Forced vital capacity.

*Postbronchodilation compared with prebronchodilation.

†Post-ICS compared with prebronchodilation.

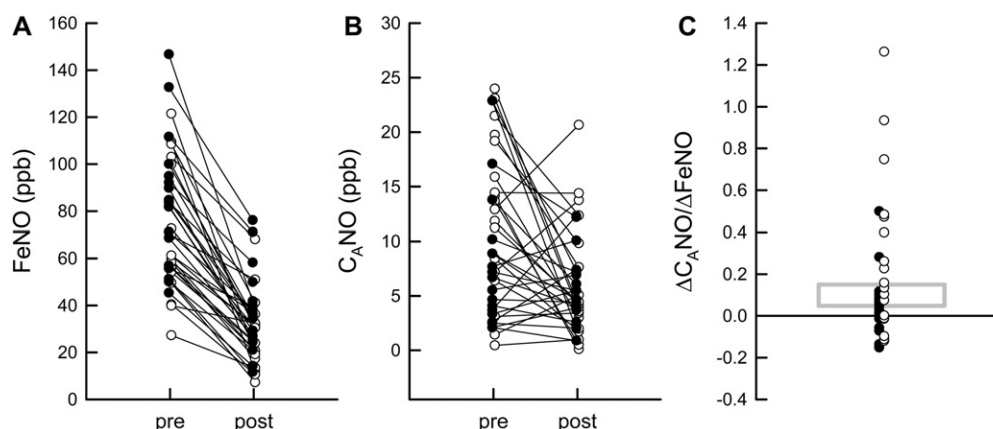


FIG 1. A, Pre-ICS and B, post-ICS therapy FeNO and C, $C_A NO$ values for each patient. C, $\Delta C_A NO/\Delta FeNO$ for each patient (Δ refers to the pre-ICS/post-ICS therapy difference). The gray rectangle indicates the 95% CI of $\Delta C_A NO/\Delta FeNO$ in patients with stable asthma. Solid and open symbols indicate treatments with ICSs alone or ICSs plus LABAs, respectively.

and FeNO changed in opposite directions, such as a decrease in FeNO and an increase in $C_A NO$. The 95% CI in a stable population of asthmatic patients (from 0.05 [5%] to 0.15 [15%], with a mean of 0.08 [8%]) is also shown. In 13 patients $\Delta C_A NO/\Delta FeNO$ values were inside the normal interval (normal slope group, median $\Delta C_A NO/\Delta FeNO = 0.08$). Two other groups can be defined with respect to the 95% CI: a high-slope group (n = 11, median $\Delta C_A NO/\Delta FeNO = 0.47$) and a low-slope group (n = 14, median $\Delta C_A NO/\Delta FeNO = -0.08$).

The baseline values were similar among the normal, low-slope, and high-slope groups for FEV₁ (81.9% ± 17.9%, 89.3% ± 11.9%, and 85.4% ± 23%, respectively; $P = .558$), forced vital capacity (FVC; 109.1% ± 25.0%, 96.9% ± 26.3%, and 95.9% ± 28.3%, respectively; $P = .342$), FEV₁/FVC (74.9% ± 8.5%, 70.9% ± 13.4%, and 72.9% ± 13.4%, respectively; $P = .683$), FEF₇₅ (44.8% ± 25.3%, 54.9% ± 27.1%, and 55% ± 22.5%, respectively; $P = .580$), and ACQ scores (1.7 [1-3.3], 2.5 [1.2-3.4], and 2.9 [1.4-5], respectively; $P = .065$). The corresponding post-ICS values were also similar among the groups for FEV₁ (94.3% ± 13.8%, 89.3% ± 11.9%, and 97.5% ± 10.5%, respectively; $P = .797$), FVC (100.8% ± 22.2%, 106.3% ± 22.9%, and 102.2% ± 26.2%, respectively; $P = .468$), FEV₁/FVC (78.9% ± 7.7%, 78.2% ± 7.4%, and 77.7% ± 6.9%, respectively; $P = .953$), FEF₇₅ (64.1% ± 26%, 74.1% ± 28%, and 70% ± 14.4%, respectively; $P = .624$), and ACQ score (0.6 [0.0-1.3], 0.6 [0.1-1.8], and 0.4 [0-1.2], respectively; $P = .744$). All differences from before to after ICS therapy were significant ($P < .001$), except for FVC in the low-slope group ($P = .038$) and FEV₁/FVC in the high-slope group ($P = .042$).

Table III presents the average baseline and post-ICS values for FeNO, J'awNO, and $C_A NO$. The last row shows the post-ICS therapy $C_A NO$ corrected for back diffusion contamination by bronchial NO. All changes from before to after ICS therapy were significant; most notable was the increase in $C_A NO$ in the low-slope group ($P < .001$). Baseline FeNO did not differ significantly between groups ($P = .073$), whereas baseline $C_A NO$ did differ significantly between groups ($P = .042$ and $P < .001$ for normal vs low- and high-slope groups, respectively; $P < 0.001$ for low-slope vs high-slope groups). Post-ICS values did not differ significantly between groups for FeNO ($P = .155$) or $C_A NO$ ($P = .093$).

In Fig 2 $C_A NO$ was plotted as a function of FeNO; it demonstrates the average trajectories from pre-ICS to post-ICS therapy values in the normal slope, high-slope, and low-slope groups. The shaded area corresponds to the 95% CI for $C_A NO$ as a function of FeNO derived from 24 patients with stable asthma and normal lung function.¹¹ This area indicates the expected range of $C_A NO$ values for a given FeNO when alveolar production is normal.

Treatment with ICSs versus ICSs plus LABAs

Baseline pulmonary function of patients receiving ICSs (n = 20) compared with ICSs plus LABAs (n = 18) differed in FEV₁ (92% ± 10% and 79% ± 22% of predicted value, respectively; $P = .024$) and FEF₇₅ (74% ± 24% and 49% ± 21% of predicted value, respectively; $P = .002$). After treatment, however, pulmonary functions were similar; FEV₁ values for the ICS and ICS plus LABA groups were 96% ± 14% and 95% ± 12% of

TABLE III. Baseline and post-ICS FeNO and C_ANO value in the normal, low-slope, and high-slope groups

	Normal group (n = 13)		Low-slope group (n = 14)		High-slope group (n = 11)	
	Baseline	Post-ICS	Baseline	Post-ICS	Baseline	Post-ICS
FeNO* (ppb)	79.6 (72.7-87.1)	24.7† (21.0-29.1)	78.5 (71.6-86.2)	36.0† (31.6-41.2)	58.2 (51.8-65.3)	25.9† (22.1-30.3)
J'awNO§ (pL/s)	3,732 ± 1,162	1,203 ± 777	3,928 ± 1,491	1,668 ± 731	2,534 ± 1,295	1,234 ± 924
C _A NO* (ppb)	7.1 (6.0-8.5)	2.9‡ (2.3-3.7)	3.7 (2.9-4.7)	6.6† (5.4-8.1)	18.4 (17-19.9)	3.7† (2.5-5.4)
C _A NO _{corr} * (ppb)	—	0.8 (0.5-1.3)	—	3.3 (2.3-4.7)	—	2.0 (1.2-3.2)

C_ANO_{corr}: Alveolar nitric oxide concentration corrected for back diffusion; J'awNO, apparent bronchial production obtained by using the Pietropaoli et al¹⁷ method.

*Geometric mean (geometric interval).

†P < .001, pre-ICS/post-ICS therapy difference.

‡P = .002, pre-ICS/post-ICS therapy difference.

§Mean ± SD.

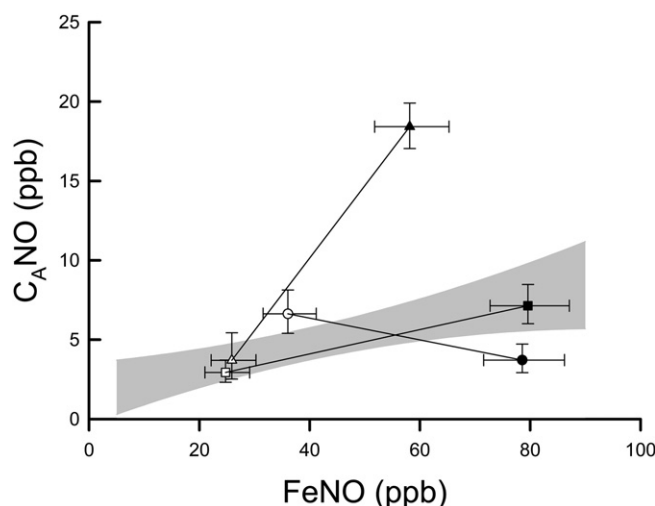


FIG 2. Pre-ICS/post-ICS average values ($\pm$ SEMs) of C_ANO versus FeNO trajectories for the normal (squares), low-slope (circles), and high-slope (triangles) groups. Solid and open symbols are the pre-ICS and post-ICS values, respectively. The shaded area corresponds to the C_ANO 95% CI as a function of FeNO derived from 24 patients with stable and unobstructed asthma.¹¹

predicted value, respectively ($P = .801$), and FEF₇₅ values were $86\% \pm 28\%$ and $77\% \pm 20\%$ of predicted value, respectively ($P = .307$).

Baseline FeNO values were comparable between groups receiving ICSs or ICSs plus LABAs; 80.0 ppb (58.4-109.8 ppb) and 64.6 ppb (43.3-96.4 ppb), respectively ($P = .074$). After ICS therapy, FeNO values were slightly less decreased in the group receiving ICSs only (34.0 ppb [20.9-55.2 ppb] vs 23.9 ppb [19.5-42.4 ppb], $P = .048$).

C_ANO values did not differ between the groups given ICSs or ICSs plus LABAs, either at baseline (6.0 ppb [2.8-12.9 ppb] vs 9.3 ppb [4.3-26.4 ppb], respectively; $P = .145$) or after treatment (4.9 ppb [2.6-9.4 ppb] vs 3.6 ppb [1.0-13.1 ppb], respectively; $P = .350$).

In the low- and high-slope groups 43% and 82% of the patients, respectively, received LABAs. This difference was not statistically different ($P = .099$, Fisher exact test).

DISCUSSION

This study provides evidence of the peripheral action of ICSs; we show that ICSs reduce distal inflammation at either the alveolar or preacinar and acinar airway levels. We detected a low

C_ANO in asthmatic patients that did not definitively preclude distal lung inflammation.

In our study FeNO and C_ANO decreased after ICS treatment, indicating the bronchial and alveolar effects of ICSs. However, on closer examination, when FeNO decreased, changes in C_ANO were widely scattered.

Given their interdependence, which was recently demonstrated in healthy subjects¹² and patients with stable and nonobstructive asthma,¹¹ C_ANO and FeNO must each be considered in interpreting changes in C_ANO. Molecular diffusion generates a back-diffusion flux (toward the alveoli) through acinar airways, and therefore alveolar and bronchial NO concentrations are related.^{13,14} This flux is proportional to the bronchoalveolar NO gradient and airway cross-sections; it removes NO molecules from expiration flux, thereby decreasing FeNO, and provides a positive source of NO for the alveolar compartment, thereby increasing C_ANO. If bronchial NO production decreases (decreasing FeNO), the reduction of the bronchoalveolar NO gradient decreases the back-diffusion flux, and as a result, C_ANO also decreases. Because of the interaction between FeNO and C_ANO, correction formulae were proposed to extract C_ANO specifically related to *in situ* production of NO.^{11,12} This correction was not applicable to our study, at least for baseline values, because the patients with asthma presented with obvious abnormal lung function before they were treated with ICSs. Even if an increase in the bronchoalveolar gradient before treatment may compensate for an impaired back-diffusion gradient, peripheral obstruction contradicts the assumptions that were made to derive the correction formulae. $\Delta C_{A}NO/\Delta FeNO$ (the ratio of pre-ICS vs post-ICS differences in C_ANO to pre-ICS vs post-ICS differences in FeNO) can be used to evaluate individual responses to ICSs in C_ANO with respect to changes in FeNO. In a group of patients with stable asthma who did not have changes in alveolar production of NO, this slope averaged 0.08¹¹; C_ANO increased (decreased) by 8% of the FeNO increase (decrease). In this study 13 of 38 patients had changes in FeNO versus C_ANO that were compatible with normal alveolar NO production and a decrease in C_ANO that resulted from reduced bronchial inflammation after ICS treatment. However, even in this case, the modulation of the alveolar concentration by bronchial NO change indicated that the latter originated peripherally at the onset of the acinus.¹⁴

In addition to this population, which had a normal slope, 2 groups of patients had different patterns of change in FeNO versus C_ANO. The group with a high $\Delta C_{A}NO/\Delta FeNO$ (high-slope group, $n = 11$) exhibited a high baseline level of C_ANO, which probably resulted from high production of alveolar NO that was

greatly reduced after ICS therapy, more than expected from the normal back-diffusion effect. This group displayed an obvious response to ICS therapy at the alveolar level, with normal recovery of $C_A\text{NO}$.

The group with a low $\Delta C_A\text{NO}/\Delta \text{FeNO}$ (low-slope group, $n = 14$) had abnormally low baseline $C_A\text{NO}$ and, in 12 of 14 patients, negative $\Delta C_A\text{NO}/\Delta \text{FeNO}$ values because of a paradoxical increase in $C_A\text{NO}$ after ICS treatment. A recently published theoretic study¹⁵ that addressed the issue of the effect of bronchoconstriction on FeNO might explain this finding; the study showed that FeNO increased significantly when bronchoconstriction occurred in intra-acinar airways around generation 18 of the Weibel model.²⁴ In this zone a reduction in airway cross-section impaired the above-described back-diffusion flux, thereby decreasing the effect of the bronchial NO production on the alveolar concentration. Therefore more molecules of NO were expired, increasing FeNO and reducing the bronchial source of the alveolar compartment; this decreased $C_A\text{NO}$. Consequently, with decreasing levels of obstruction, FeNO decreased and $C_A\text{NO}$ increased because of back-diffusion flux recovery, which resulted in a negative $\Delta C_A\text{NO}/\Delta \text{FeNO}$. The behavior of the low-slope group indicates obstruction of acinar airways, which is at least partly decreased by treatment with ICSs.

These normal, high-slope, and low-slope groups might represent distinct phenotypes that are characterized by different baseline $C_A\text{NO}$ values. One of these phenotypes might present distinct characteristics in a longitudinal context (eg, more prone to exacerbations and greater decreases in lung function over time). These phenotypes must be studied further, but our findings indicate that measurements of $C_A\text{NO}$ and FeNO provide useful phenotypic information that can be used in the clinic. Several studies have shown that FeNO alone might not clearly determine asthma severity in adults.²⁵

It is not clear why baseline levels of alveolar production differed between the groups, despite similar baseline levels of pulmonary function, exhaled NO, and asthma control. Although some studies have reported low $C_A\text{NO}$ values in patients with uncontrolled asthma,^{10,26} the dispersion of baseline $C_A\text{NO}$ values observed in this study was in agreement with the results of Gelb et al.⁴ These authors showed that in asthmatic patients who are or are not being treated with ICSs, $C_A\text{NO}$ values varied widely, and there was significant overlap between values from patients with asthma and healthy subjects; these findings indicate a wide distribution of alveolar production. Lehtimäki et al.²⁷ observed an association between increased alveolar NO levels and nocturnal symptoms in asthmatic patients. In this study only 3 of 38 patients were free from nocturnal symptoms (they had a nil value for this specific ACQ item) before treatment with ICSs. No difference in the magnitude of the nocturnal symptoms before therapy with ICSs was observed between the low- and high-slope groups ($P = .33$, Mann-Whitney U test).

In this study the combination of ICSs and LABAs had significant additional effects on standard pulmonary function: baseline FEV_1 and FEF_{75} values were lower among patients who received LABAs, but after treatment, these values were similar. In contrast, treatment with LABAs did not affect NO indices, except for FeNO, which remained slightly higher ($P = .048$) in patients treated with only ICSs. Furthermore, only 43% of patients in the low-slope group, who achieved relief from acinar obstruction, received LABAs. Relief from acinar obstruction might therefore result from the actions of ICSs.

Our data indicate that the peripheral lung, from the terminal bronchioles to the alveoli, is affected in patients with poorly controlled mild-to-moderate asthma. An acinar effect was detected in 66% of the patients, involving the acinar airways or alveolar zone. This confirms the results of Verbanck et al.,²⁸ who used the multibreath nitrogen washout technique to demonstrate abnormal acinar ventilation distribution in most patients with asthma. Further studies might compare expiratory flow-volume curves by using air and heliox to confirm peripheral involvement.

In the low- and high-slope groups, changes observed in patterns of $C_A\text{NO}$ were not compatible with a purely central effect of ICSs (no average changes in $C_A\text{NO}$ associated with decreases in FeNO). This contradicts the concept that ICSs, such as the dry-powder inhaler device (Turbuhaler), are centrally deposited. Although ICSs are spatially distributed to the large airways,²⁹ trace amounts of ICS particles might reach the lung's periphery. Alternatively, a systemic effect of ICSs cannot be excluded, as demonstrated for inhaled budesonide.³⁰

Based on the recently proposed relationship between FeNO and $C_A\text{NO}$, our results show that ICSs have peripheral activities (ie, at the alveolar or acinar airways) in patients with mild-to-moderate asthma who have significantly improved control after treatment. In addition, detection of a low level of $C_A\text{NO}$ in asthmatic patients who have high levels of exhaled NO should be interpreted cautiously because it could indicate peripheral inflammation.

Clinical implications: In patients with moderate or mild asthma, ICSs have positive effects on the preacinar and acinar airways and at the alveolar level. A low alveolar concentration of NO does not preclude peripheral inflammation.

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METHODS

Changes in FeNO and C_ANO

The ratio $\Delta C_A\text{NO}/\Delta \text{FeNO}$ (Δ refers to the change from pre-ICS to post-ICS values) was computed to assess the individual response of C_ANO to ICS treatment with respect to FeNO change. When plotting C_ANO as a function of FeNO, $\Delta C_A\text{NO}/\Delta \text{FeNO}$ corresponds to the slope (S) of the line segment joining pre-ICS and post-ICS values.

The problem arises of whether $\Delta C_A\text{NO}/\Delta \text{FeNO}$ is compatible with the normal interaction between bronchial NO production and the alveolar concentration (ie, with normal alveolar NO production and without obstruction). It is therefore difficult to longitudinally assess a normal range of $\Delta C_A\text{NO}/\Delta \text{FeNO}$ because ΔFeNO is often concomitant with asthma control and changes in lung function.

The relationship between FeNO and C_ANO was estimated transversally by the slope of a regression line calculated from 24 individual measurements of FeNO and C_ANO in patients with stable and obstruction-free asthma.^{E1} The 95% CI of the slope, as computed from this sample, might be the best possible estimator of a normal interval for $\Delta C_A\text{NO}/\Delta \text{FeNO}$; it was derived by using the bootstrap method.^{E2}

Bootstrap method

The bootstrap method allows empiric construction of the sampling distribution of the slope without an *a priori* parametric hypothesis. Briefly,

this method is a resampling procedure; 24 pairs of values are randomly drawn from the original sample (some pairs are drawn several times, and others are not drawn), constituting a new sample from which the slope is calculated. By repeating this procedure 1000 times, the sampling distribution of the slope is derived.

RESULTS

Fig E1 shows the observed frequency distribution. This distribution fitted a log-normal distribution with a mean of -2.53 (slope geometric mean, 0.08) and an SD of 0.029 ($P = .66$, χ^2 test). Based on this adjusted distribution, the 95% CI of the slope goes from 0.049 to 0.150.

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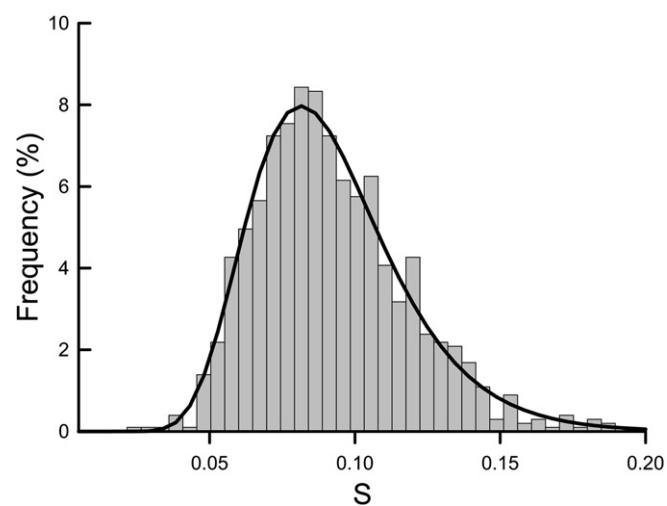


FIG E1. Gray bars, Distribution histogram of the slope (S). Solid line, Adjusted log-normal distribution.