

In moderate-to-severe asthma patients monitoring exhaled nitric oxide during exacerbation is not a good predictor of spirometric response to oral corticosteroid

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Background: The importance of monitoring exhaled nitric oxide (NO) in asthma remains controversial.

Objective: To measure exhaled NO, postnebulized albuterol/ ipratropium spirometry, and Asthma Control Test (ACT) during asthma exacerbation requiring 8- to 10-day tapering oral corticosteroid in nonsmoking patients with moderate-to-severe asthma on moderate-dose inhaled corticosteroid and long-acting β_2 -agonist but not maintenance oral corticosteroid.

Methods: After measuring the fraction of exhaled NO (F_{NO} [ppb]) at 50, 100, 150, and 200 mL/s, the total F_{NO} at 50 mL/s (ppb), large central airway NO flux (J'_{awNO} [nL/s]), and peripheral small airway/alveolar NO concentration (C_{ANO} [ppb]) were calculated and corrected for NO axial back-diffusion. Outpatient exacerbation required the patient with asthma to be afebrile with normal chest x-ray and white blood cell count.

Results: Group 1 included 17 patients (6 men) with asthma, age 52 ± 12 years, studied at baseline, during 18 exacerbations with *abnormal* FENO at 50 mL/s, J'awNO, and/or C_{ANO}, and post 8- to 10-day tapering 40 mg prednisone (recovery). Baseline: IgE, 332 ± 243 Kμ; total blood eosinophils, 304 ± 266 cells/μL; body mass index, 28 ± 6 ; ACT, 16 to 19; and FEV₁, 2.5 ± 0.7 L ($86\% \pm 20\%$ predicted); exacerbation: FEV₁, 1.7 ± 0.4 L ($60\% \pm 17\%$) ($P < .001$); recovery: FEV₁, 2.5 ± 0.7 L ($85\% \pm 13\%$) ($P < .001$). Group 2 included 11 (7 men) similarly treated patients with asthma, age 49 ± 14 years, studied at baseline, during 15 exacerbations with *normal* FENO at 50 mL/s, J'awNO, and C_{ANO}. Baseline: IgE, 307 ± 133 Kμ; total blood eosinophils, 296 ± 149 cells/μL; body mass index, 28 ± 6 ; ACT, 16 to 19; and FEV₁, 2.7 ± 0.9 L ($71\% \pm 12\%$ predicted); exacerbation: FEV₁, 1.7 ± 0.6 L ($54\% \pm 19\%$) ($P < .006$); recovery: FEV₁, 2.7 ± 0.9 L ($70\% \pm 14\%$) ($P = .002$). On comparing group 1 versus group 2, there was no significant

difference for baseline IgE, eosinophils, body mass index, and ACT and similar significant ($\leq .006$) decrease from baseline in FEV₁ (L) during exacerbation and similar increase ($\leq .006$) at recovery.

Conclusions: Increased versus normal exhaled NO during outpatient exacerbation in patients with moderate-to-severe asthma on inhaled corticosteroid and long-acting β_2 -agonist but not maintenance oral corticosteroid does not preclude a robust clinical and spirometric response to tapering oral prednisone. (J Allergy Clin Immunol 2012;■■■:■■■-■■■.)

Key words: Asthma, exhaled nitric oxide, asthma mechanisms

Measurement of fraction of exhaled nitric oxide (FENO [ppb]) at 50 mL/s is a relatively simple, reproducible, and noninvasive biomarker test for monitoring the arginine-nitric oxide synthase pathway that signals airway inflammation.¹ Increased FENO at 50 mL/s in asthma has been thought to reflect predominantly eosinophilic-mediated inflammation in central airways, although the supporting data are only modest.¹⁻⁵ Increased FENO has also been reported to identify an increased response to inhaled corticosteroid (ICS) in ICS-naïve adult patients with asthma but not to reduce exacerbations.³ The currently accepted method of measuring FENO at a single constant expiratory flow rate, usually 50 mL/s, is incapable of separating whether the source of increased NO production is located in the large central airways versus peripheral small airways/alveolar or both.⁶ Therefore, several investigators have developed newer techniques to discriminate NO gas exchange between large central airways and peripheral smaller airways/alveolar compartments.⁷⁻¹²

By using a 2-compartment model developed by Tsoukias and George,¹¹ central airway NO flux and peripheral airway/alveolar concentration can be estimated by measuring F_{ENO} at multiple expiratory flows and plotting NO output versus expiratory flow. The slope of the linear regression line between NO output and flow reflects peripheral small airway/alveolar NO concentration (C_{ANO} [ppb]), whereas the Y intercept reflects large central airway NO flux (J' _{awNO} [nL/s]).¹¹ Gelb et al^{13,14} and others^{8,12,15-21} using this model¹¹ have previously reported NO gas exchange in central as well as in peripheral airway/alveolar sites in patients with mild^{8,12,15,18-21} and moderate-to-severe^{8,13,14,16,17,20} clinically stable asthma. However, 2 groups independently^{12,22} have also demonstrated, both experimentally and theoretically, axial back-diffusion (against the direction of exhalation) of NO from proximal to peripheral airway/alveoli. Furthermore, axial back-diffusion may contaminate peripheral airway/alveolar levels, leading to an underestimation of central airway NO flux and an

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Abbreviations used

ACT:	Asthma Control Test
C _{ANO} :	Peripheral small airway/alveolar nitric oxide concentration
CT:	Computed tomography
FENO:	Fraction of exhaled nitric oxide
FVC:	Forced vital capacity
ICS:	Inhaled corticosteroid
J' _{awNO} :	Large central airway nitric oxide flux
LABA:	Long-acting β_2 -agonist
MDI:	Metered-dose inhaler
NO:	Nitric oxide

overestimation of peripheral airway/alveolar NO levels. None of the above asthma studies^{8-11,13-21} corrected for NO axial back-diffusion.^{12,22}

Gelb et al²³ recently reported outpatient studies in acute asthma using the 2-compartment NO model¹¹ with correction for axial back-diffusion of NO.²² In 13 of the 15 evaluated adults with asthma, there was increased NO flux in predominantly central airway sites in patients with clinically stable, moderate-to-severe asthma at baseline with abnormal spirometry despite moderate-dose ICS and long-acting β_2 -agonist (LABA).²³ NO production in central airway sites was further increased in the same 13 of the 15 patients during exacerbation prior to the initiation of oral corticosteroid with robust clinical and spirometric response following an 8-day tapering course. The absence of increased C_{ANO} was in contrast to our previous observations of increased C_{ANO} in patients with clinically stable asthma^{13,14} even after correction for axial NO back-diffusion.²² This suggests that peripheral airway/alveolar NO concentration (C_{ANO}) in adults with acute asthma may be normal. Moreover, in 2 sentinel cases, we noted similar robust clinical and spirometric improvement during exacerbation in which both central and peripheral airway/alveolar NO concentration was normal at baseline as well as during exacerbation when compared with that in the 13 patients with asthma with predominantly increased central airway NO flux.²³

The current prospective outpatient study used the 2-compartment model after correcting for NO axial back-diffusion.^{11,22} We planned to study clinically stable, nonsmoking, asthma patients with moderate-to-severe expiratory airflow limitation despite treatment with moderate-dose ICS and LABA but no oral corticosteroid maintenance. Asthma patients were to be evaluated at baseline and subsequently during exacerbation and following recovery. Our goal was to evaluate the clinical and spirometric response in a larger group of patients with asthma who maintained normal NO gas exchange during exacerbation and compare their recovery response to that of patients with asthma with increased NO gas exchange during exacerbation. We hypothesized that while the difference in NO gas exchange during exacerbation may potentially reflect the source (eg, eosinophilic vs neutrophilic inflammation¹), it would not preclude a subsequent robust clinical and spirometric response to treatment with add-on oral corticosteroid during exacerbation in patients with moderate-to-severe asthma. This hypothesis has not been investigated previously.

METHODS**Patient selection**

We recruited adult patients followed in our referral clinic with moderate-to-severe persistent asthma who were on maintenance combination

moderate-dose ICS/LABA for at least 2 years. This included either fluticasone propionate 250 μ g/salmeterol 50 μ g twice daily (Advair 250/50; GlaxoSmithKline, Research Triangle Park, NC) or budesonide 160 μ g/formoterol 4.5 μ g 2 inhalations twice daily (Symbicort 160/4.5; Astra-Zeneca, Wilmington, Del). Smoking history was less than 5 pack-years, and currently all were nonsmokers for at least 5 years. All patients with asthma were clinically stable for at least 8 weeks and were monitored in our tertiary referral outpatient clinic for 2 or more years. Their asthma symptoms and spirometry were optimal when compared with clinical and spirometric results obtained over the previous 2 or more years. When needed for acute relief, medical therapy included short-acting inhaled β_2 -agonist (albuterol sulfate metered-dose inhaler [MDI] or solution) and inhaled antimuscarinic agent ipratropium bromide (Atrovent MDI or solution; Boehringer-Ingelheim Pharmaceuticals, Inc, Ridgefield, Conn). All patients with asthma were off oral corticosteroids, antibiotics, and leukotriene-modifying agents for at least 8 weeks before entry into the study. Serum eosinophil and IgE levels were measured only at baseline. Asthma Control Test (ACT) at baseline was used to characterize clinical status.²⁴ All patients with asthma had demonstrated bronchodilator reversibility within the past 2 years defined as an increase of 200 mL or more in FEV₁ and 12% or more 15 minutes following the intake of 180 μ g albuterol via an MDI with a tube spacer. The definition of moderate-to-severe asthma was consistent with clinical and spirometric National Asthma Education and Prevention Program guidelines.²⁵ This study was approved by both Lakewood Regional Medical Center Institutional Review Board and Western Institutional Review Board, Olympia, Wash, and registered as NCT#00576069 and NCT#01225913. These data have not been previously published.

Normal controls

Normal values for FENO were obtained from 40 normal subjects (20 men), age 57 ± 9 years (similar age as of patients with asthma who were studied), who were asymptomatic, healthy lifelong nonsmokers with no history of lung disease and not on any medications.²⁶ No provocative bronchoconstrictor challenge tests were obtained. All asthmatic subjects and normal subjects studied had given informed consent for participation.

Measurement of exhaled nitric oxide gas exchange

All subjects abstained from food and coffee for 2 hours and alcohol for 12 hours prior to studies. Exhaled nitric oxide was measured in triplicate prior to spirometry at 4 separate constant expiratory flow rates (50, 100, 150, and 200 mL/s), and the mean of 3 values obtained within 10% of each other was reported by using a Sievers NOA 280 chemiluminescence analyzer with varying expiratory airflow resistors (GE Analytical Instruments, Inc, Boulder, Colo) as previously described.^{13,14,23} Furthermore, to avoid nasal NO contamination, a mouth pressure of more than 5 cmH₂O was used, as previously recommended.^{13,14,23} The technique of Tsoukias and George¹¹ was used to calculate J'_{awNO} (nL/s) (y intercept) and steady-state C_{ANO} (ppb) (slope) by using a linear regression line for each subject with a minimum of 3 expiratory flow rate data points and an r² of 0.9 or more. Correction was made for the potential underestimation of large airway NO flux due to axial back-diffusion of NO by using the method of Condorelli et al²² by multiplying J'_{awNO} by a factor of 1.7. Furthermore, to adjust for possible spurious overestimation of values for peripheral lung C_{ANO}, the initial uncorrected J'_{awNO} (nL/s) was divided by a correction factor of 0.53 and subtracted from the initial uncorrected C_{ANO}.²² This could yield negative or near-zero values for peripheral lung C_{ANO}.²² This correction factor was determined in normal subjects and patients with mild asthma by using flow rates ranging from 50 to 250 mL/s.²² Increased J'_{awNO} (nL/s) and/or C_{ANO} was defined as values more than 1.64 SD from age-matched controls.²⁵ Investigators responsible for measuring FENO gas exchange and lung function (C.F.T., R.M., and D.H.S.) were blinded to the therapeutic intervention. Spirometry and NO gas exchange were measured at the initiation of the study when the patients with asthma were clinically stable at baseline, and subsequently prior to the initiation of oral corticosteroid during exacerbation, and then during recovery immediately after treatment with an 8-day course of tapering oral corticosteroid.

Lung computed tomography studies

Our goal was to include only those patients with asthma who were without clinically occult significant lung computed tomography (CT) scored emphysema or bronchiectasis. Therefore, all patients with asthma had high-resolution, thin-section scans of the lung obtained by using a helical 64-slice multidetector-row CT (Model Sensation 64; Siemens, Malvern, Pa). Images were obtained at 5-mm collimation at intervals of 6 mm by using 120 kVp and varying the current (mA) depending on patient size. Reconstructed 1-mm slices were obtained every 9 mm by using a window width of 850 HU and a level of -600 HU with edge-enhancing algorithm. Images were scored by a radiologist (Mark J. Schein, MD) 0 to 100, none to worst emphysema, by using picture templates we previously validated by using inflated whole lung specimens.²⁷

Lung function studies

When clinically stable at baseline, and during recovery following treatment with oral corticosteroid, patients with asthma were instructed to continue all their medications except to withhold MDI albuterol sulfate and/or ipratropium bromide 6 hours prior to testing and LABA dry powder inhaler 24 hours prior to testing. Obviously, during asthma exacerbation, medications were not withheld. Our techniques for measuring spirometry, thoracic gas volumes, and airway resistance by using plethysmography and diffusing capacity have been previously published.^{13,14,23,25}

Protocol: Therapeutic intervention with oral corticosteroid

All potential patients with asthma for this study were followed in our outpatient referral clinic and were eligible for this prospective, single-blind, open-label patient study and were continued on maintenance combination ICS plus inhaled LABA and off oral corticosteroid for 6 weeks or more. Concurrent acute relief medications when needed included aerosolized (MDI) and/or nebulized albuterol sulfate, and ipratropium bromide. No allergy tests were done at baseline. Definition of asthma exacerbation requiring oral corticosteroids included increasing complaints of cough $\pm$ sputum, chest tightness, wheezing, and shortness of breath for 1 day or more. These symptoms were not relieved despite at least 3 separate treatments with MDI and/or nebulized 2.5 mg albuterol sulfate and 0.5 mg ipratropium bromide within 8 hours, and there was a concomitant decrease of 0.3 L or more in FEV₁ from baseline. In addition, the absence of both fever and acute infiltrate on a chest roentgenogram and normal white blood cell count were required. No laboratory study was initiated to definitively determine the etiology of any potential superimposed acute bronchitis. The decision to initiate oral corticosteroids was determined by the emergency ward or attending physician who was initially unaware of the study until they or the patient with asthma informed the principal investigator. All patients with asthma who prospectively had an acute exacerbation with normal NO gas exchange were consecutively included in this 2-year study, whereas those with abnormal NO gas exchange were recruited and consecutively included toward the latter part of the study only because of the increased incidence of this phenotype.²³ All patients with asthma were identically treated with an 8- to 10-day tapering course of oral prednisone starting with 40 mg. Concurrent use of antibiotics was optional. FENO was not measured to guide clinical therapy.

Statistical methods

Data for patients with asthma were initially analyzed to determine normality distribution by using the Shapiro Wilks test. Because of nonparametric data, ANOVA using ranks with repeated measurement was used, with clinical status including stable baseline, exacerbation, and recovery as the within factor, and Mann-Whitney test was used to compare nonparametric data. Paired *t* test was used to compare change in clinical status within the same group. Values for *P* < .05 were considered significant. We needed to sample a minimum of 14 asthma exacerbations with normal NO gas exchange to

achieve 80% power (20% beta type 2 error), with alpha 5% (type 1 error), to detect no significant spirometric difference during asthma baseline-to-exacerbation-to-recovery with test value 0.8 L and sample average 0.9 L and SD of 0.15 L when compared with patients with asthma with abnormal NO gas exchange during exacerbation. This would avoid both falsely rejecting the null hypothesis (type 1 error) and falsely accepting it (type 2 error).

RESULTS

Baseline studies

We prospectively screened 96 patients with moderate-to-severe asthma²⁵ during 110 exacerbations over 2 years and included in this study all 11 consecutively studied patients with asthma during 15 exacerbations who had normal NO gas exchange compared with age-matched controls (group 2).²⁶ The comparator group included 17 consecutively studied patients with moderate-to-severe asthma²⁵ with 18 exacerbations, all with abnormal NO gas exchange (group 1). This study included 28 patients (15 men) with moderate-to-severe asthma,²⁵ age 50 ± 13 years, who had been treated with inhaled combination moderate-dose ICS and LABA for 3.5 ± 0.5 years. Only 2 patients in each group were on budesonide 160 μ g/formoterol 4.5 μ g 2 inhalations bid, and the remainder on fluticasone propionate 250 μ g/salmeterol 50 μ g bid. All patients with asthma were off oral corticosteroid for 6 weeks or more. Only 14% of all the patients with asthma and 16% of all the exacerbations evaluated had normal NO gas exchange during exacerbation. Results of baseline spirometry following the intake of 180 μ g albuterol by an MDI/tube spacer and exhaled NO are presented in the accompanying Fig 1 and Table I.

There were 17 patients (6 men) with asthma, age 52 ± 13 years, in group 1 with increased NO ($>$ normal²⁶ mean $+1.64$ SD) in central airway and/or peripheral/alveolar sites during 18 spontaneous exacerbations. Baseline ACT²⁴ was scored between 16 and 19. Outpatient 8- to 10-day tapering prednisone for exacerbation was prescribed 2.5 ± 2.3 times a year for the past 2 years. Baseline vital capacity was 3.1 ± 1.0 L ($93\% \pm 21\%$ predicted), total lung capacity 5.7 ± 1.3 L ($110\% \pm 12\%$ predicted), residual volume 2.6 ± 0.8 L ($139\% \pm 26\%$ predicted), functional residual capacity 3.3 ± 0.7 L ($127\% \pm 37\%$ predicted), specific conductance 0.13 ± 0.06 lps/cmH₂O/L ($54\% \pm 23\%$ predicted), single breath diffusing capacity 23 ± 9 mL/min/mmHg ($102\% \pm 35\%$ predicted), and diffusing capacity/alveolar volume 5.0 ± 0.4 mL/min/mmHg/L ($128\% \pm 11\%$ predicted). High-resolution, thin-section lung CT emphysema scores²⁷ were 9 ± 5 , indicating no or trivial emphysema, and no bronchiectasis was noted in the patients with asthma. At baseline, serum IgE was 332 ± 243 k μ /L (normal <114 k μ /L) and total blood eosinophil count was 304 ± 266 cells/ μ L (normal <500 cells/ μ L). Body mass index was 28 ± 6 .

There were 11 (7 men) patients with asthma, age 49 ± 14 years, in group 2 with normal NO in central airway and/or peripheral/alveolar sites during 15 exacerbations. Baseline ACT²⁴ was scored between 16 and 19, and outpatient corticosteroid usage was similar to that in group 1. Baseline vital capacity was 3.6 ± 1.1 L ($87\% \pm 14\%$ predicted), total lung capacity 6.4 ± 1.6 L ($106\% \pm 16\%$ predicted), residual volume 2.7 ± 0.8 L ($139\% \pm 33\%$ predicted), functional residual capacity 3.5 ± 0.9 L ($115\% \pm 29\%$ predicted), specific conductance 0.11 ± 0.08 lps/cmH₂O/L ($49\% \pm 36\%$ predicted), single breath diffusing capacity 24 ± 11 mL/min/mmHg ($90\% \pm 23\%$ predicted),

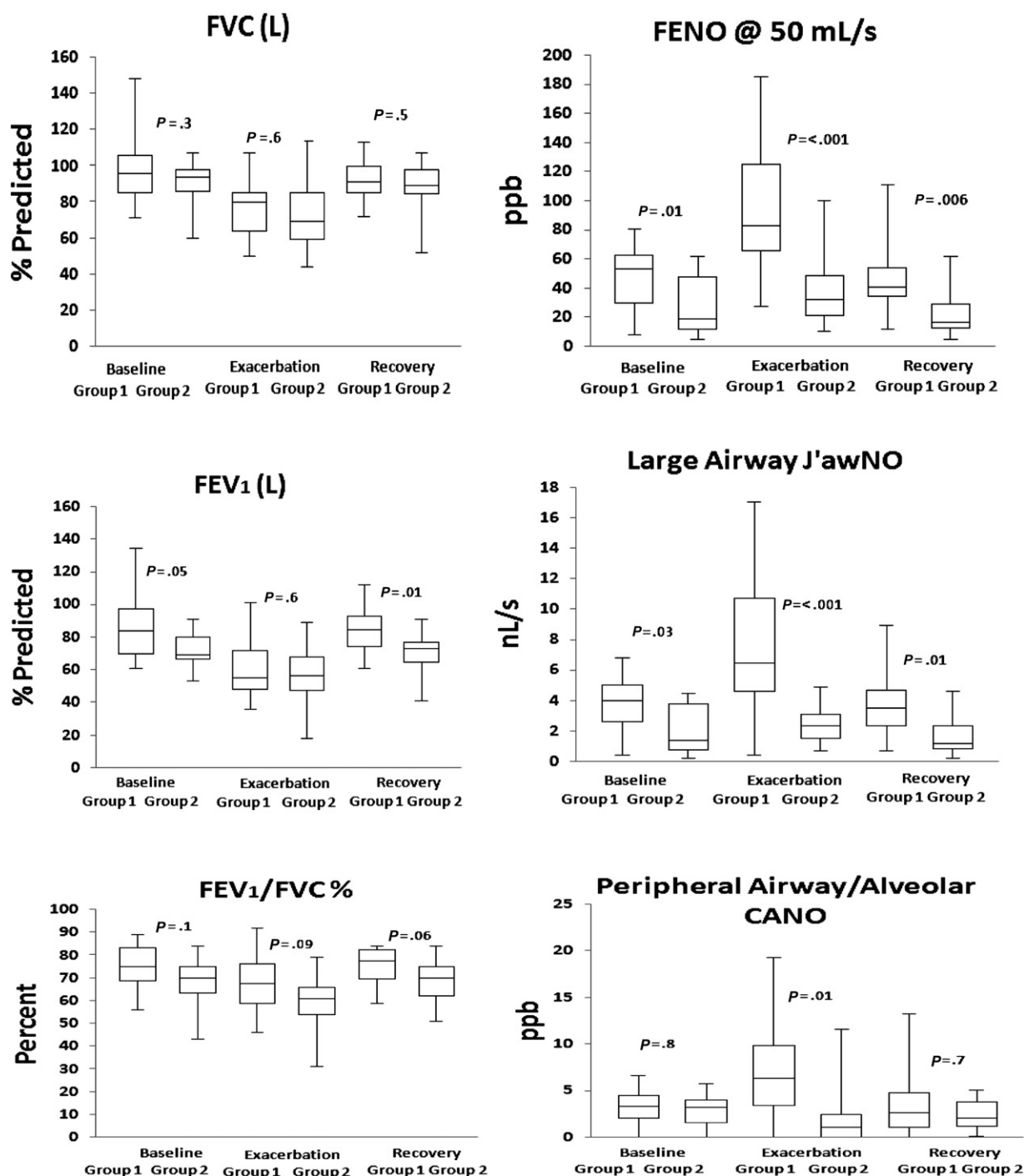


FIG 1. There were no significant differences for spirometry between group 1 and group 2 at baseline, exacerbation, and recovery except for FEV₁ (L) during recovery. However, spirometry was significantly decreased in both groups 1 and 2 during exacerbation compared with baseline and recovery. FENO at 50 mL/s and J'awNO in group 1 was significantly greater at baseline compared with group 2, and during exacerbation, FENO at 50 mL/s, J'awNO, and CANO increased significantly compared with baseline in group 1 and also compared with group 2. During recovery, FENO at 50 mL/s, J'awNO, and CANO decreased significantly compared with exacerbation in group 1 and FENO at 50 mL/s and J'awNO in group 1 still remained significantly increased compared with group 2.

and diffusing capacity/alveolar volume 5.0 ± 1.4 mL/min/mmHg/L ($119\% \pm 29\%$ predicted). These lung function studies were not significantly different from group 1. High-resolution, thin-section lung CT emphysema scores²⁷ were 8 ± 5 , indicating

none or trivial emphysema, and no bronchiectasis was noted in the 11 patients with asthma. At baseline, serum IgE level was 307 ± 133 k μ /L and total blood eosinophil count was 296 ± 149 cells/ μ L. Body mass index was similar to that of group 1.

TABLE I. Baseline spirometry and FENO at 50 mL/s, J'awNO, and C_{ANO} in group 1 and group 2 asthma patients

	FVC (L)	FVC% predicted	FEV ₁ (L)	FEV ₁ % predicted	FEV ₁ /FVC	FENO at 50 mL/s (ppb)	J'awNO (nL/s)	C _{ANO} (ppb)
Group 1								
Mean	3.4	98.0	2.5	86.0	74.6	47.2	3.7	3.1
SD	0.9	20.2	0.7	19.8	9.4	23.2	1.9	2.1
Median	3.4	95.5	2.3	83.5	75.0	52.8	4.0	3.3
Quartile 1	2.6	85.5	2.1	69.8	68.6	30.0	2.6	2.0
Quartile 3	4.0	105.5	2.9	97.3	83.0	62.8	5.0	4.4
Group 2								
Mean	4.0	90.1	2.7	72.9	68.4	28.8	2.1	2.9
SD	1.2	11.9	0.9	11.0	11.4	21.5	1.6	1.8
Median	3.9	94.0	2.3	69.0	70.0	19.0	1.4	3.2
Quartile 1	3.1	86.0	2.0	66.5	63.5	11.6	0.8	1.5
Quartile 3	4.2	97.5	3.4	80.0	75.0	47.3	3.8	3.9
Group 1 vs group 2 <i>P</i> value	.2	.3	.7	.05	.12	.01	.03	.8

Group 1 asthma patients were defined as having increased NO gas exchange during exacerbation, whereas group 2 asthma patients had normal values. At baseline, group 1 had significantly increased FENO at 50 mL/s and large airway J'awNO than did group 2.

TABLE II. Spirometry and FENO at 50 mL/s, J'awNO, and C_{ANO} in group 1 and group 2 asthma patients during spontaneous exacerbation

	FVC (L)	FVC% predicted	FEV ₁ (L)	FEV ₁ % predicted	FEV ₁ /FVC	FENO at 50 mL/s (ppb)	J'awNO (nL/s)	C _{ANO} (ppb)
Group 1								
Mean	2.6	76.1	1.7	59.9	67.6	94.5	7.5	6.9
SD	0.6	15.8	0.4	16.8	11.5	44.3	4.4	5.8
Median	2.5	80.0	1.6	55.0	67.5	82.4	6.5	6.3
Quartile 1	2.2	64.3	1.6	47.8	58.8	65.7	4.6	3.4
Quartile 3	3.1	85.1	1.9	71.5	76.0	125.0	10.7	9.8
Baseline vs exacerbation <i>P</i> value	<.001	<.001	<.001	<.001	.04	.001	.04	.01
Group 2								
Mean	3.0	73.0	1.8	54.6	59.7	37.9	2.5	2.3
SD	1.0	21.7	0.7	18.7	11.2	26.6	1.4	3.3
Median	3.1	69.0	1.9	56.0	61.0	32.0	2.3	1.1
Quartile 1	2.2	59.5	1.4	47.5	54.0	21.4	1.5	0.0
Quartile 3	3.6	85.5	2.2	67.5	66.0	48.1	3.1	2.4
Baseline vs exacerbation <i>P</i> value	<.001	.002	<.006	<.006	.08	.2	.5	.5
Group 1 vs group 2 <i>P</i> value	.3	.6	.6	.6	.09	<.001	<.001	.01

Group 1 asthma patients had significantly increased FENO at 50 mL/s, J'awNO, and C_{ANO} during exacerbation than at baseline and also compared with group 2, whereas group 2 had normal values similar to those at baseline.

Baseline NO studies

At baseline, group 1 patients with asthma when compared with both age-matched normals²⁶ and group 2 patients with asthma had significantly higher values for FENO at 50 mL/s (ppb) ($P = .01$), J'awNO (nL/s) ($P = .03$) but not C_{ANO} (ppb) ($P = .8$). In group 1, 9 of the 17 patients with asthma had an abnormal FENO, 3 C_{ANO}, and 4 J'awNO (see Table I and Fig 1).

Spirometry and NO production during asthma exacerbation prior to the initiation of oral corticosteroid

During asthma exacerbation compared with baseline, there was a significant similar decrease in spirometry following 180 µg albuterol using an MDI/tube spacer: forced vital capacity (FVC) (L) ($P < .001$) and FEV₁ (L) ($P < .006$) in both groups 1 and 2. However, there was a significant increase in FENO at 50 mL/s ($P = .001$) (15 of 17 abnormally increased), J'awNO ($P = .004$) (13 of 17 abnormally increased), and C_{ANO} ($P = .01$) (12 of 17 abnormally

increased) in group 1 versus group 2. Group 2 exacerbation values for exhaled NO were similar to normal values at baseline. Two patients with asthma during 2 separate exacerbations had unexplained completely opposite exhaled NO response (see Table II and Fig 1).

Spirometry and NO production in recovery following 8- to 10-day oral corticosteroid

After 10 ± 6 days in group 1 and 8 ± 3 days in group 2 following the initiation of a 8- to 10-day tapering course of 40 mg oral prednisone, there was a significant increase in both FVC (L) ($P \leq .006$) and FEV₁ (L) ($P \leq .002$) in both groups 1 and 2 compared with values obtained during acute exacerbation. In group 1, there was also a significant decrease in FENO at 50 mL/s ($P < .001$), J'awNO ($P = .002$), and C_{ANO} ($P = .04$) compared with values obtained during exacerbation. In group 1, 3 of the 17 patients with asthma still had abnormally increased J'awNO and 3 C_{ANO}. In group 2, values for exhaled NO were similar to those at baseline, exacerbation, and recovery. The robust clinical

TABLE III. Spirometry and FENO at 50 mL/s, J'_{awNO} , and C_{ANO} in group 1 and group 2 asthma patients during recovery

	FVC (L)	FVC% predicted	FEV ₁ (L)	FEV ₁ % predicted	FEV ₁ /FVC	FENO at 50 mL/s (ppb)	J'_{awNO} (nL/s)	C_{ANO} (ppb)
Group 1								
Mean	3.3	91.7	2.5	84.2	75.2	43.7	3.6	3.3
SD	0.9	10.7	0.8	13.0	8.1	24.6	2.3	3.3
Median	3.4	91.0	2.5	84.5	77.5	40.7	3.5	2.6
Quartile 1	2.4	85.0	2.0	74.0	69.5	34.1	2.3	1.1
Quartile 3	4.0	100.0	2.9	92.5	82.5	53.5	4.6	4.7
Exacerbation to recovery <i>P</i> value	<.001	<.001	<.001	<.001	.03	<.001	.002	.04
Group 2								
Mean	3.8	87.3	2.7	70.6	67.9	23.8	1.8	2.3
SD	1.2	14.0	0.9	12.8	10.3	17.6	1.5	1.5
Median	3.9	89.0	2.4	73.0	70.0	16.0	1.2	2.0
Quartile 1	2.7	84.5	1.9	64.5	62.0	12.5	0.8	1.2
Quartile 3	4.4	97.5	3.4	76.5	75.0	29.0	2.3	3.8
Exacerbation vs recovery <i>P</i> value	.006	.006	.002	.002	.008	.07	.2	.9
Group 1 vs group 2 <i>P</i> value	.2	.5	.8	.01	.06	.007	.01	.7

Group 1 asthma patients had significantly decreased FENO at 50 mL/s, J'_{awNO} , and C_{ANO} during recovery than during exacerbation. Group 1 still had significantly increased FENO at 50 mL/s and J'_{awNO} but not C_{ANO} than did Group 2. Group 2 had normal values similar to those at exacerbation and baseline.

response from exacerbation to recovery was similar between group 1 and group 2 (see Table III and Fig 1).

DISCUSSION

Spirometry and exhaled NO at multiple expiratory flow rates were measured to determine the contribution of central versus peripheral airway/alveolar NO production sites using the 2-compartment model¹¹ with correction for NO axial back-diffusion²² during treatment for acute exacerbation in patients with moderate-to-severe asthma.²⁵ Results demonstrated at baseline, despite relative clinical stability and ACT score of 16 to 19, that there was significantly abnormal spirometry in both groups consistent with National Asthma Education and Prevention Program criteria.²⁵ Abnormally increased J'_{awNO} and C_{ANO} was noted in only 6 of the 17 patients with asthma in group 1. During asthma exacerbation, with greater expiratory airflow limitation, J'_{awNO} as well as C_{ANO} increased only in group 1. Thirteen of the 17 patients with asthma in group 1 had an abnormal J'_{awNO} and/or 12 C_{ANO} . During recovery following oral corticosteroid intervention, clinical status and spirometry improved similarly to baseline values in both groups whereas J'_{awNO} and C_{ANO} decreased significantly in group 1 to baseline values. However, isolated J'_{awNO} in 3 and isolated C_{ANO} in 3 patients with asthma remained abnormal. Furthermore, 2 patients with asthma during 2 separate exacerbations had unexplained completely opposite exhaled NO response.

This study provides increasing evidence that acute asthmatic attacks with normal exhaled NO may involve a presumed noneosinophilic-mediated inflammatory pathway, possibly neutrophilic^{28,29} but of uncertain source. Most importantly, the clinical and physiologic response is just as robust to tapering oral corticosteroid therapy, similar to that in patients with asthma with increased exhaled NO, as we originally observed in 2 sentinel cases.²³

Central and peripheral airway/alveolar NO production

NO is produced by resident cells, for example, airway epithelial cells, airway and circulatory endothelial cells, and trafficking

inflammatory cells but presumably not neutrophils, in both large and small airways/alveoli.¹ The upregulation of endogenous NO by overexpressed inducible nitric oxide synthase, or less likely constitutive nitric oxide synthase,¹⁻⁶ plays a critical role in regulating airway function and acts as a gaseous signaling molecule. We suspect in both clinically stable and unstable group 1 patients with asthma that moderate-dose ICS (fluticasone propionate 250 µg/salmeterol 50 µg twice daily or budesonide 160 µg/formoterol 4.5 µg 2 inhalations twice daily) is probably unable to sufficiently suppress increased bronchoreactive inducible nitric oxide synthase and/or resident constitutive nitric oxide synthase in the larger airways to effectively reduce bronchial wall NO production and also in the peripheral airways/alveoli to maintain normal C_{ANO} .

The present study also reinforces our earlier observations^{13,14} and contradicts in part our recent findings²³ that following correction for NO axial back-diffusion, the site of inflammation during asthma exacerbation is predominantly localized in large airways. In the present study, 12 of the 17 patients with asthma in group 1 developed abnormally increased C_{ANO} during exacerbation, whereas 13 of the 17 had abnormally increased J'_{awNO} . Upon re-examination of our original data,^{13,14} and after correcting for axial NO back-diffusion,²² peripheral C_{ANO} still remained increased in 30% of the patients with asthma.¹³ The discrepancy with present results may in part be related to different asthmatic cohorts and less likely to therapeutic regimen. As we noted previously,²³ prior studies uncorrected for axial NO back-diffusion^{12,22} noted increased central airway NO flux and increased C_{ANO} in clinically stable patients with asthma with mild^{15,18-21} and moderate-to-severe obstruction^{13,14,16,17} despite ICS. Review of published data from van Veen et al¹⁶ suggests normal peripheral C_{ANO} production after we corrected for NO axial back-diffusion.²² Alternatively, we have previously emphasized²³ that published results, also without correction for axial NO back-diffusion^{12,22} by Högman et al,⁸ Brindicci et al,²⁰ and Shin et al,²¹ noted increased central NO flux but normal peripheral C_{ANO} in clinically stable patients with asthma with mild²¹ and moderate-to-severe^{8,20} expiratory airflow limitation despite ICS. Kerckx et al¹² noted normal peripheral airway/alveolar NO concentration in patients with

mild asthma after correction for NO axial back-diffusion. Brindicci et al²⁰ reported increased C_{ANO} during exacerbation in patients with moderate-to-severe asthma; however, reexamination of their published data²⁰ suggests normal C_{ANO} after correction for NO back-diffusion. Moreover, either an increase in peripheral airway/alveoli NO tissue production as an inflammatory response (increased inducible nitric oxide synthase and/or constitutive nitric oxide synthase) or unlikely reduced peripheral diffusing capacity for NO, or both, could account for increased C_{ANO} . Alternatively, Verbanck et al noted³⁰ that peripheral bronchoconstriction could potentially cause a paradoxical decrease in C_{ANO} by reducing axial back-diffusion of NO toward the lung periphery and increasing large airway J'_{awNO} .

The reduction of increased J'_{awNO} and C_{ANO} following oral corticosteroid during recovery in the present study is similar to our previous experience¹³ and that of Berry et al¹⁷ in patients with clinically stable asthmas prior to correction for NO axial back-diffusion and our study with correction.²³ Furthermore, in the present study, 2 patients with asthma during 2 separate exacerbations had completely opposite exhaled NO response with similar robust improvement following therapeutic intervention. The increase, if any, in peripheral lung C_{ANO} in clinically stable and especially unstable patients with asthma is only modestly downregulated by inhaled compared with systemic corticosteroids.^{13,14,17,23}

Clinical implications

By using asthma treatment guidelines including spirometry,^{25,31} several investigators^{2,5,31-36} have addressed the clinical role of monitoring induced sputa eosinophils,² and add-on monitoring of exhaled NO at a single expiratory airflow rate, usually at 50 mL/s, to help guide inhaled and/or systemic corticosteroid dosing in clinically stable and unstable children^{32,33,35} and adults^{2,34,36} with asthma. Results of 6 double-blind randomized control trials³²⁻³⁷ were equivocal. However, Gibson³⁸ has raised multiple issues related to study design that challenged the validity of the conclusions. Yet, after meta-analyses of the trials,³²⁻³⁷ Pet-sky et al conclude that routine serial measurements of FENO for clinical asthma management does not appear warranted.³⁹ The present study is an extension of our original investigations^{13,14,23,40} to define and partition central and peripheral airway/alveolar sites of inflammation and the response to therapy in patients with moderate-to-severe asthma²⁵ on moderate-dose ICS and LABA but no maintenance oral corticosteroid. In contrast, Dweik et al⁴¹ studied patients with severe asthma²⁵ on high-dose ICS (≥ 1 mg daily) and LABA and maintenance oral corticosteroid for 6 months or more per year. They⁴¹ noted that the subset of patients with asthma in Severe Asthma Research Program Cluster 5 with FENO of more than 35 ppb at 50 mL/s identified the most atopic with sputa eosinophilia of more than 2%, most bronchoreactive and reversible FEV₁ with bronchodilators, yet clinically most worrisome requiring frequent emergent care. However, in patients with moderate asthma with similar incidence of increased FENO of more than 35 ppb at 50 mL/s, a subset of difficult-to-manage patients with asthma was not identified.⁴¹ Furthermore, it should be emphasized that increased FENO at 50 mL/s, a surrogate of predominantly central airway increased NO flux, correlates only modestly with induced sputa eosinophilia of more than 3% ($r^2 = 0.26$, $P < .001$) and with sensitivity

of 71% and specificity of 72%⁴² and $r^2 = 0.29$, $P = .007$, with tissue eosinophilia in treated patients with asthma with severe refractory asthma.⁵

Measurement of exhaled NO complements spirometry, because it provides a potential opportunity to try to localize the site of inflammation. In asthma, increased FENO at 50 mL/s usually reflects a central airway NO source but does not clearly discriminate between central and peripheral airway/alveolar NO.⁶ Moreover, as previously emphasized, it only modestly discriminates between sputa and tissue eosinophilic and neutrophilic inflammation.^{1-5,42} And based on the present study, it does not identify a phenotype that is uniquely responsive to oral corticosteroid during asthma exacerbation. Furthermore, the conclusions of the meta-analyses by Petsky et al³⁹ suggest that routine serial measurements of FENO for clinical asthma management does not appear helpful. Alternatively, we have previously reported that baseline elevated exhaled NO independent of abnormal FEV₁ (L) in clinically stable, treated, patients with moderate asthma, similar to baseline values in group 1, markedly increased the relative risk for future asthma exacerbation.¹⁴ Dweik et al⁴¹ reported in patients with severe asthma that increased exhaled NO is an independent marker of a reactive and worrisome phenotype. However, McGrath et al⁴³ reported that approximately half the patients with mild-to-moderate asthma were persistently noneosinophilic on induced sputa and had normal FENO at 50 mL/s, and this phenotype also responded poorly to anti-inflammatory therapy. Reductions of less than 80% predicted in FEV₁ (L) and FEV₁/FVC ratio of less than 75% do not discriminate between large and small airway sites of airflow obstruction. Furthermore, if the FEV₁/FVC ratio is less than 75%, the FEF_{25-75%} will almost always be abnormal.⁴⁴ However, if the FVC (L) is less than 80% predicted, it implies premature airway closure due either to intrinsic small airway obstruction and/or loss of lung elastic recoil or both. But, it does not exclude small airway closure extending to larger airways.

Summary

We studied clinically stable adults with moderate-to-severe asthma²⁵ on moderate-dose ICS and LABA but not on maintenance oral corticosteroid. During outpatient asthma exacerbation, add-on oral tapering corticosteroid provided a robust clinical and spirometric response regardless of normal versus increased FENO at 50 mL/s, central J'_{awNO} , and/or peripheral airway/alveoli C_{ANO} . These results confirm and extend our sentinel observations in 2 cases²³ and suggest that routine measurement of exhaled NO gas exchange including FENO at 50 mL/s, peripheral/alveoli (C_{ANO}), and central or large airway NO (J'_{awNO}) during acute asthma exacerbation does not provide clinically useful therapeutic information regarding the potential response to oral corticosteroids.

Key message

- Routine measurement to determine normal versus increased exhaled NO gas exchange during outpatient exacerbation in patients with moderate-to-severe asthma does not provide clinically useful therapeutic information regarding potential robust clinical and spirometric response to oral corticosteroids.

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