

Inflammation and airway function in the lung periphery of patients with stable asthma

Sylvia Verbanck, PhD, Daniel Schuermans, RN, and Walter Vincken, MD, PhD *Brussels, Belgium*

Background: An important role for exhaled nitric oxide (NO) measurement could be in the distinction between proximal and peripheral lung contributions to inflammation, with a particular interest for the alveolar lung zone and its implication on airway function.

Objective: We aimed to isolate the acinar lung zone contribution to both inflammation and airway function to seek a relationship between them.

Methods: In 30 patients with asthma with an asthma control test score exceeding 20, indices of conductive and acinar ventilation heterogeneity (Scond, Sacin) were obtained from a multiple breath washout. NO production in the conductive airways ($J'aw_{NO}$), alveolar NO concentration (CA_{NO}), and the standard exhaled NO at 50 mL/s ($FENO_{50}$) were obtained from exhaled NO.

Results: Scond was consistently abnormal in all patients with stable asthma, but without any correlation to inflammation abnormality in that compartment ($J'aw_{NO}$). Sacin was particularly abnormal in the asthma subgroup receiving >500 μ g budesonide equivalent, and a correlation was found between Sacin and CA_{NO} ($r = 0.61$; $P = .015$); in this subgroup, a weak association was found between Scond and $J'aw_{NO}$ or $FENO_{50}$ ($r = 0.50$; $P = .059$ for both).

Conclusion: The persistent functional abnormality of small conductive airways in patients with stable asthma is largely independent of inflammation as measured by exhaled NO. In the alveolar compartment, a functional correlate of alveolar NO was found in a subgroup of patients with stable asthma on moderate-to-high maintenance doses of inhaled steroids. These patients in particular could benefit from novel therapies specifically aimed at improving airway functionality at the level of the acinar entrance and beyond. (*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

Key words: Small airways, exhaled nitric oxide, stable asthma, inflammation

Abbreviations used

ACT:	Asthma control test
CA_{NO} :	Alveolar nitric oxide concentration
CA_{NO}^{corr1} :	Corrected alveolar nitric oxide concentration as proposed by Condorelli et al ²⁷
CA_{NO}^{corr2} :	Corrected alveolar nitric oxide concentration as proposed by Kerckx et al ²⁸
$FENO_{50}$:	Fractional concentration of exhaled nitric oxide measured at expiratory flow rate of 50 mL/s
$J'aw_{NO}$:	Nitric oxide flux in the conductive airways
NO:	Nitric oxide
Sacin:	Acinar ventilation heterogeneity
Scond:	Conductive ventilation heterogeneity
Sn:	Normalized N_2 phase III slope in each expiration of a multiple breath N_2 washout
TO:	Lung turnover

There has been considerable interest for exhaled nitric oxide (NO) as an easy and non-invasive measure of airway inflammation, leading to detailed studies of confounding factors,^{1,2} a standardization document,³ and several reports of normal values.⁴⁻⁷ However, some of the initial enthusiasm has been tempered by recent studies indicating that exhaled NO does not provide an additional benefit to standard asthma control measures when guiding therapy (see, for example, Shaw et al⁸). An editorial by Stick and Franklin⁹ shared this skepticism but did recognize the potential interest of measuring exhaled NO in severe asthma. On the other hand, Taylor¹⁰ argued in favor of the standard exhaled NO obtained at 50 mL/s exhalation rate ($FENO_{50}$) on the basis of, for example, Michils et al,¹¹ who proposed to consider $FENO_{50}$ changes, rather than $FENO_{50}$ cut-off values, in the follow-up of individual patients. One problem with $FENO_{50}$ is that it is a global measure of NO production, making it potentially sensitive but less specific to characterize patients with asthma adequately. Several authors have therefore promoted the determination of alveolar and bronchial components of exhaled NO.¹²⁻¹⁶ These can be easily obtained with very little extra effort on the part of the patient¹⁷ by having the patient perform exhalations at various flow rates instead of just 1. The validity of compartmentalization of NO parameters has been confirmed experimentally, for instance by inducing conditions that are known to affect only NO concentration in the alveolar compartment.¹⁸

The inconsistent relationships between the various exhaled NO parameters and spirometric indices have prompted a search for alternative functional correlates of inflammation. Some authors have related exhaled NO parameters to indices of ventilation heterogeneity, such as the N_2 phase III slope of single breath N_2 washout. Battaglia et al¹⁹ found a correlation between exhaled

From the Respiratory Division, University Hospital UZ Brussel.

Supported by the Fund for Scientific Research—Flanders and the NO Microgravity Application Project of the European Space Agency.

Disclosure of potential conflict of interest: S. Verbanck has received research support from the Fund for Scientific Research and the European Space Agency. The rest of the authors have declared that they have no conflict of interest.

Received for publication June 25, 2009; revised October 28, 2009; accepted for publication October 30, 2009.

Reprint requests: Sylvia Verbanck, PhD, Respiratory Division, University Hospital UZ Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium. E-mail: sylvia.verbanck@uzbrussel.be.

0091-6749/\$36.00

© 2010 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2009.10.053

NO at 100 mL/s and N₂ phase III slope in patients with mild asthma. van Veen et al.¹² reported an association between alveolar airway inflammation and N₂ phase III slope, particularly in patients with asthma on high doses of inhaled steroids. It is prudent to point out that, very much like FENO₅₀ is a mixture of bronchial and alveolar NO, usually dominated by the former, the standard phase III slope of the vital capacity single breath washout is a mixture of conductive and acinar ventilation heterogeneity, usually dominated by the latter. What is currently lacking is a simultaneous measurement of inflammation and ventilation heterogeneity specific to each lung compartment to test, for instance, whether inflammation in the alveolar lung zone of a given patient is also reflected in airway dysfunction of this particular lung zone.

In the current study, we set out to identify conductive and acinar airway contributions to inflammation, and to search for their functional correlates in the corresponding lung compartments of patients with asthma that is well controlled according to Global Initiative for Asthma guidelines with varying doses of inhaled corticosteroids.²⁰ Inflammation was quantified by exhaled NO at different flow rates to obtain a measure of NO production in the conductive airways (J'aw_{NO}) and of alveolar NO concentration (CA_{NO}). Airway function at the corresponding lung depths was assessed by means of validated indices of conductive and acinar ventilation heterogeneity (Scond and Sacin) derived from the N₂ phase III slope of a multiple breath N₂ washout.²¹⁻²⁴ On the basis of the increasing Sacin values previously obtained in asthma patients with increasing disease severity, it was hypothesized that patients on high doses of standard inhaled corticosteroids would be distinguished by a more pronounced functional abnormality of the acinar lung zone, and that this could have an inflammatory basis. Hence, the primary objective was to search for a link between CA_{NO} and Sacin in these patients with asthma, but potential links between Scond and J'aw_{NO} or the more commonly used FENO₅₀ were also evaluated.

METHODS

The protocol was approved by the local ethics committee (#B14320071296), and informed consent was obtained from all participating subjects. A set of measurements was performed in patients with asthma and in a healthy control group. Measurements in the asthma group were done postdilution with 400 µg salbutamol, thereby minimizing variable smooth muscle contraction, to assess any residual structure-function abnormality in these patients with stable asthma. Of all measured parameters, normal values are available in the literature, and our control group merely served to verify that, in the absence of disease, no correlation was found between exhaled NO and ventilation heterogeneity parameters. To this aim, we deliberately chose a group of young never-smokers with no history of respiratory disease and with a negative histamine provocation test. From the Sacin and Scond data in a normal group (n = 63; age range, 19-51 years) from a previous study,²⁵ we had verified that there was no age dependence of the ventilation heterogeneity indices, but because of inconsistent reports of age dependence of exhaled NO parameters,⁴⁻⁷ the potential confounding factor of age on correlation analysis was avoided by the choice of a narrow age range for the control group (19-21 years).

All participating patients with asthma had been diagnosed and treated in our clinic according to standard criteria.²⁰ Inclusion criteria for the current study were no respiratory infection or asthma exacerbation in the 6 months before the study and an asthma control test (ACT) score exceeding 20.²⁶ During the study visit, 1 set of measurements on each test subject included, in this sequence, exhaled NO, spirometry, and multiple breath N₂ washout measurements. Spirometry was performed by using standard equipment (VmaxEncore; Viasys, Bithoven, The Netherlands).

Exhaled NO

The exhaled NO measurement started with a vital capacity inhalation of NO-free air (filter type A1B2E2K1HgCONO-P3; Draeger, Luebeck, Germany) followed by exhalation via 1 of 6 restrictors with the subject maintaining a mouth pressure of 15 cm H₂O, so as to obtain exhaled NO for 6 expiratory flows between 50 and 350 mL/s (2 tests per flow). Exhaled NO concentration was continuously recorded (CLD; EcoPhysicsAG, Duerten, Switzerland) such that an NO plateau value could be readily identified. From the plot of exhaled NO plateau value versus the inverse of the corresponding flow, alveolar NO concentration (CA_{NO}) and NO flux in the airway compartment (J'aw_{NO}) were first estimated as follows: CA_{NO} and J'aw_{NO} correspond to, respectively, intercept and slope of the exhaled NO versus flow⁻¹ plot, also considering the recommendation that only data points corresponding to flow >100 mL/s (flow⁻¹ < 0.01 s/mL), or at least in the linear part of the curve, should be used.¹⁷ One pitfall of determining the alveolar NO concentration is that it can be artificially increased in a clinical situation in which bronchial NO production is increased and part of this bronchial NO back-diffuses into the alveolar space. It has been suggested that this artefact can be corrected for, and we applied here 2 published correction methods:

$$CA_{NO}^{corr1} = CA_{NO} - J'aw_{NO}/740$$

as proposed by Condorelli et al.,²⁷ and

$$CA_{NO}^{corr2} = (CA_{NO} - 0.08 \times FENO_{50})/0.92$$

as proposed by Kerckx et al.²⁸

Ventilation heterogeneity

Instrumentation and theory underlying multiple breath N₂ washout tests of ventilation heterogeneity have been extensively reported elsewhere (see, for example, Verbanck et al.²¹). Three multiple N₂ breath washout tests were performed by each test subject or patient. From the N₂ tracings, N₂ phase III slopes were computed in subsequent expirations and normalized by mean expired N₂ concentration to obtain a normalized slope (Sn) for each breath; functional residual capacity is computed to obtain a measure of lung turnover (TO). From 3 multiple breath tests in each subject, an average Sn versus TO curve is obtained (an illustrative curve is shown in the Results section), from which indices Scond and Sacin are derived to represent the conductive and acinar components of ventilation heterogeneity, respectively. Scond is the slope of the Sn versus TO regression in the TO range 1.5 to 6, whereas Sacin is the slope of the first expiration minus Scond times the TO of the first breath. Because Scond and Sacin derive from N₂ phase III slopes, their value increases when ventilation heterogeneity increases.

Statistical analysis

Using Statistica5.1 (StatSoft, Tulsa, Okla), nonparametric tests (Mann-Whitney; Spearman rank correlation) were performed.

RESULTS

All patients had clinically diagnosed asthma and documented reversibility (n = 30; 14 women, 16 men; 44.5 ± 15.2 [SD] years; 172 ± 10 [SD] cm). All patients were atopic and had been treated by inhaled corticosteroids in combination with long-acting β₂ mimetics for at least 5 years. None of the patients were treated with oral corticosteroids. All patients with asthma were clinically stable, and their reported ACT score ranged from 20 to 25.²⁶ Two asthma subgroups were distinguished according to their daily use of inhaled steroids (with a cut-off at 500 µg budesonide equivalent). The first subgroup consisted of 15 patients receiving 160 to 400 µg budesonide equivalent dose per day (average, 301 µg); the second subgroup of 15 patients had a maintenance treatment of 640 to 1600 µg budesonide equivalent dose per day (average,

TABLE I. Spirometry, ventilation heterogeneity, and exhaled NO parameters

	Control	Asthma		P value
		<500 µg BUD equivalent	>500 µg BUD equivalent	
Mean ACT score	—	22.8	22.2	
Spirometry				
FEV ₁ (%pred)	104 ± 13	101 ± 16	88 ± 17*	.037
FEV ₁ /FVC(%)	86 ± 7	72 ± 11	66 ± 9	.050
FEF ₂₅₋₇₅ %pred)	98 ± 22	66 ± 21	47 ± 21*	.016
Ventilation distribution				
Sacin (L ⁻¹)	0.060 ± 0.019	0.085 ± 0.042	0.127 ± 0.053	.033
Scond (L ⁻¹)	0.030 ± 0.009	0.047 ± 0.021	0.057 ± 0.017	.074
FRC (mL)	3001 ± 919	3017 ± 797	3295 ± 876	>.1
Exhaled NO				
FENO ₅₀ (ppb)	16.9 ± 8.4	42.4 ± 41.4	27.3 ± 21.5	>.1
CA _{NO} (ppb)	2.34 ± 1.46	4.49 ± 4.46	3.53 ± 2.61	>.1
CA _{NO} ^{corr1} (ppb)	1.07 ± 1.71	1.19 ± 1.90	1.47 ± 1.45	>.1
CA _{NO} ^{corr2} (ppb)	1.36 ± 1.63	1.92 ± 2.33	1.93 ± 1.63	>.1
J'aw _{NO} (pl/s ⁻¹)	727 ± 424	1898 ± 1860	1188 ± 963	>.1

BUD, Budesonide; FEF₂₅₋₇₅, forced expiratory flow between expiration of 25% and 75% FVC; FRC, functional residual capacity; FVC, forced vital capacity; *pred*, predicted. Data are means ± SDs.

*Significant differences between the asthma subgroups (*P* values are from Mann-Whitney test).

1176 µg), 8 of whom were on 1000 µg fluticasone. The control group (*n* = 20; 14 women, 6 men; 19.9 ± 0.6 [SD] years; 172 ± 9 [SD] cm) showed normal values for indices of ventilation heterogeneity (Table I)—that is, below the upper limit of normal for Sacin (0.11–0.13 L⁻¹) and below the upper limit of normal for Scond (0.037–0.042 L⁻¹).^{22,23} The exhaled NO parameters for the control group also fell in the published normal ranges.^{4,5,17}

In the ventilation heterogeneity and exhaled NO curves, as presented across Figs 1 and 2, changes in the rate of rise of the curves reflect inflammatory or functional changes occurring in the conductive airway compartment of the lung. Changes in the intercept of either curve signal inflammatory or functional changes potentially taking place in the acinar airway compartment. Overall, the rate of rise of Sn versus TO (Fig 1) was increased between the control and asthma groups, resulting in an increased conductive ventilation heterogeneity (Scond). The offset of the entire Sn versus TO plot was also increased in the asthma group, reflecting an additional increase in acinar ventilation heterogeneity (Sacin). As an illustration of Scond and Sacin computation, the Scond value that would correspond to the average curves in this figure is 0.027 L⁻¹ (control) and 0.047 L⁻¹ (asthma); corresponding Sacin are 0.069 L⁻¹ (control) and 0.110 L⁻¹ (asthma). In the study, individual Scond and Sacin values are determined from the Sn versus TO plot for each test subject individually. Considering the entire asthma group, significant increases in Sacin (*P* = .002) and Scond (*P* < .001) were observed with respect to the control group; average values for the control group and the 2 asthma subgroups can be found in Table I.

Overall, the rate of rise of FENO versus flow⁻¹ plot (Fig 2) was increased between the control and asthma groups, reflecting an increased NO production in the airways (J'aw_{NO}). The offset of the entire FENO versus flow⁻¹ plot was also increased, pointing to an additional increase in alveolar NO concentration (CA_{NO}). As an illustration of J'aw_{NO} and CA_{NO} computation, the J'aw_{NO} values that would correspond to the average curves in this figure are 747 pl/s⁻¹ (controls) and 1532 pl/s⁻¹ (asthma); corresponding CA_{NO} values are 2.3 ppb (controls) and 4.1 ppb (asthma). In the study, individual J'aw_{NO} and CA_{NO} values are determined from the

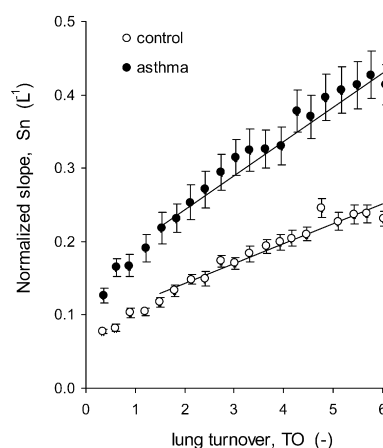


FIG 1. Sn versus TO plots from which indices of conductive and acinar ventilation heterogeneity (Scond, Sacin) can be computed (see text for details); averaged Sn values (±SEs) from the 20 control subjects and 30 patients with asthma.

FENO versus flow⁻¹ plot for each test subject individually. Considering the entire asthma group, significant increases in J'aw_{NO} (*P* = .009) and CA_{NO} (*P* = .03) were observed with respect to the control group; average values for the control group and the 2 asthma subgroups can be found in Table I. When correcting alveolar NO concentration to account for the back diffusion of increased NO production in the airway compartment, alveolar concentration values were smaller and very similar to each other (CA_{NO}^{corr1}, CA_{NO}^{corr2} in Table I).

In the control group, and in the asthma group as a whole, there were no significant correlations between inflammation and spirometry or ventilation heterogeneity. In particular, FENO₅₀ did not correlate with either FEV₁ or FEF₂₅₋₇₅, nor with either Scond or Sacin (*P* > .1 for all). In the entire asthma group, the correlation between Sacin and CA_{NO} was near the borderline of significance (*P* = .06). However, in the subgroup of patients with asthma on >500 µg budesonide equivalent, significant correlations existed

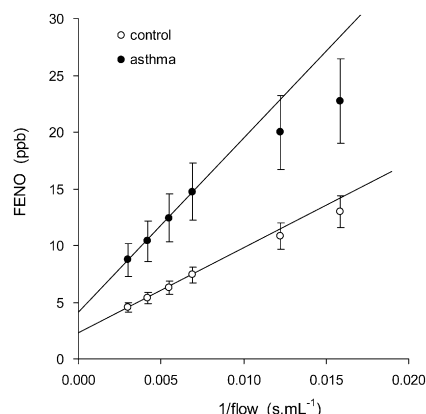


FIG 2. Exhaled NO concentrations (means + SEs) versus the reciprocal of flow in asthma (closed circle) and control (open circle) groups. From the slope and intercept of exhaled NO versus the reciprocal of flow, CA_{NO} and $J'aw_{NO}$ were estimated (see text for details).

between Sacin and CA_{NO} ($r = 0.61$; $P = .015$), between Sacin and CA_{NO}^{corr1} ($r = 0.57$; $P = .025$), and between Sacin and CA_{NO}^{corr2} ($r = 0.57$; $P = .027$). For the sake of comparison with previous studies, where CA_{NO} is most commonly used, individual data points are shown for CA_{NO} in Fig 3, A. The correlations between Scnd and $J'aw_{NO}$ (Fig 3, B) and between Scnd and $FENO_{50}$ failed to reach statistical significance ($r = 0.50$; $P = .059$ for both).

DISCUSSION

In this study we have first identified the conductive airways contribution to ventilation heterogeneity which was accompanied by, but largely unrelated to, an abnormally high airway NO production as measured from exhaled NO in patients with otherwise stable asthma. Possibly the conductive ventilation heterogeneity observed here was at least in part the functional consequence of a nonsteroid-responsive structural change associated with airway remodeling. If such remodeling is present early in the disease process and nonprogressive,²⁹ this could also explain that the Scnd values obtained here are very similar to those previously reported in nonsteroid-dependent mild asthma patients.²¹ Even in an asthma population in which both inflammation and ventilation heterogeneity were expected to be abnormal, Downie et al²³ observed that inflammation and conductive ventilation heterogeneity were independent determinants of bronchial hyperresponsiveness. In Downie et al,²³ exhaled NO was measured by the off-line method collecting NO at 1 expiratory flow rate (200 mL/s), such that no distinction could be made between bronchial and alveolar NO. van Veen et al¹² found that the bronchial component of exhaled NO in mild and more severe asthma patients did not correlate with the vital capacity single breath N_2 phase III slope, which is a global measure of ventilation heterogeneity. The separation of both inflammatory and functional noninvasive markers into alveolar and conductive compartments, and the potential correlations between them, constitutes the novelty of the current work. In this way, we were able to demonstrate, by noninvasive measurement, that the acinar airway dysfunction in more severe asthma patients could indeed have an inflammatory basis.

The correlation between acinar ventilation heterogeneity (Sacin) and alveolar NO (CA_{NO} , CA_{NO}^{corr1} , or CA_{NO}^{corr2}) was

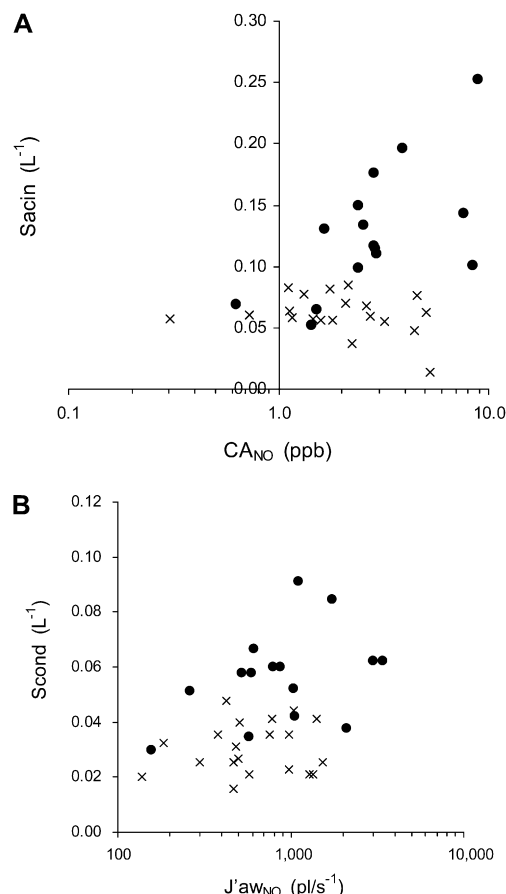


FIG 3. A, Scatter plot of acinar ventilation heterogeneity Sacin and CA_{NO} in subgroup of patients with asthma treated with $>500 \mu\text{g}$ budesonide equivalent daily dose (closed symbols) and controls (crosses). B, Scatter plot of conductive ventilation heterogeneity Scnd and $J'aw_{NO}$ in subgroup of patients with asthma treated with $>500 \mu\text{g}$ budesonide equivalent daily dose (closed symbols) and controls (crosses).

particularly marked in a subgroup of patients treated with moderate-to-high doses of inhaled steroids, who were also characterized by lower FEV_1 and greater Sacin (Table I). Despite some methodologic differences, van Veen et al¹² also reported correlations between alveolar NO and the standard phase III slope in the patients with severe asthma (postbronchodilator FEV_1 89% predicted; beclometasone dose, 1155 μg) and not in the patients with mild-to-moderate asthma (postbronchodilator FEV_1 102% predicted; beclometasone dose, 445 μg). Possibly, no further functional improvement can be obtained, even with high doses of standard inhaled steroids if these do not reach the affected lung zones in the patient with more severe asthma. One could even speculate that NO levels remain elevated in certain airway locations because these cannot be reached by anti-inflammatory medication as a result of local ventilation heterogeneity in these very locations. In any case, the correlation between function and inflammation of the acinar lung zone (Fig 3, A) indicates that there is an asthma subpopulation for whom alternative treatments (smaller particle inhaled steroids or oral steroids) aimed at further decreasing inflammation at the level of the respiratory bronchioles and beyond could also improve functionality there. Identifying such subgroups via Sacin and/or CA_{NO} may be valuable when assessing the peripheral effect of novel therapies in patients with asthma.^{30,31}

A significant degree of intersubject scatter has been reported for standard exhaled NO measurement $FENO_{50}$ in never-smokers,⁵ with regression equations accounting for no more than 10% of the $FENO_{50}$ variability. The largely unexplained intersubject variability in exhaled NO parameters but acceptable (intrasubject) reproducibility³² has led to the recommendation that the clinical use of exhaled NO parameters resides in considering relative (intrasubject) changes rather absolute cut-off values.¹¹ In this respect, the use of fixed cut-offs for exhaled NO parameters such as CA_{NO} could prove to be problematic to select patients eligible for more peripheral treatment, whereas CA_{NO} could still be suitable to track a therapeutic intervention in an individual patient. Considering that Sacin has a much lower intersubject variability and reported upper limits of normal ranging from 0.11 to 0.13 L⁻¹ across studies in different centers,^{22,23} Sacin may be the preferred parameter for identification of patients eligible for more peripheral airway treatment. Alternatively, one could take a pragmatic approach on basis of the current findings and simply select patients on maintenance treatment of high doses of steroids in an attempt to decrease further their CA_{NO} . Irrespective of the selection criteria, monitoring of intrasubject changes after novel peripheral treatment can then be done with either Sacin or CA_{NO} , and preferably with both.

Thus far, results from studies exploring the value of novel therapies on inflammation and function in the acinar lung zone have been inconclusive. van Veen et al¹² did not observe a difference between alveolar NO in patients with severe asthma whether or not their maintenance treatment included oral steroids. Similarly, Berry et al¹⁴ did not find a difference in alveolar NO between patients with refractory asthma on either inhaled or oral steroids, yet, alveolar NO was shown to be decreased following a step-up treatment with oral steroids, and not with doubling the dose of inhaled steroids. Gelb et al¹³ obtained significant decreases in CA_{NO} , but not in $J'aw_{NO}$, when patients with stable asthma on inhaled steroids were given prednisone for 10 days. Conversely, Fritscher et al³³ did not observe any changes in $J'aw_{NO}$ or CA_{NO} when patients with asthma were given montelukast in combination with fluticasone after a period of fluticasone alone. Finally, Cohen et al³⁴ studied patients with asthma with an elevated CA_{NO} . Seven of those patients receiving ciclesonide showed a CA_{NO} decrease, whereas the 8 patients in the placebo arm did not. This points to a key issue: there must be abnormality of inflammation and/or function in the alveolar compartment if a novel therapy is to elicit an improvement in such a peripheral lung zone at all. This is line with our previous study in which only those patients with asthma with abnormal Sacin at baseline showed an improvement in acinar airway functionality when switched from a standard budesonide to ultrafine beclomethasone dipropionate treatment.²⁴ In this respect, the outcome and success of novel therapies targeting the peripheral lung are critically dependent on the patient selection in terms of baseline inflammation and function of the acinar lung compartment.

Although the current study was focused on functionality and inflammation in the acinar lung zone, it is important to point out that any correlation between noninvasive measurement of ventilation heterogeneity and inflammation was confined to its corresponding lung compartment—for instance, Sacin did not correlate with $J'aw_{NO}$. No correlation was found between $J'aw_{NO}$ and any spirometric parameter, also in agreement with previous reports.^{13,15,16} Consistent with previous observations by others,^{12,13} $J'aw_{NO}$ was not different between asthma patients on maintenance

treatment of moderate-to-high versus low doses of inhaled steroids (Table I). One explanation for this relatively consistent $J'aw_{NO}$ behavior across studies and the poor association of $J'aw_{NO}$ with ventilation heterogeneity observed here can be sought in very recent lung modeling work by Suresh et al.³⁵ These authors have shown that local areas of high NO production can lead to either $J'aw_{NO}$ increases or $J'aw_{NO}$ decreases, even in the case of a homogeneously ventilated lung, and that $J'aw_{NO}$ response is further complicated by the ventilation heterogeneity (of the type reflected in Scond) across these areas. This effect may blur potential relationships between noninvasive measures of airway function and inflammation in the conductive airway compartment, particularly when the degree of inflammation is relatively mild and heterogeneously distributed across the bronchial airway tree. Possibly, in patients with uncontrolled asthma with more elevated $J'aw_{NO}$, a more distinct correlation with Scond could occur.

All measurements performed here were noninvasive and based on lung modeling,^{27,28,36} whereby airway inflammation and function are attributed to conductive and acinar lung zone compartments. One could suspect a mathematical link between the respective lung zone components of exhaled NO and ventilation heterogeneity, owing to the lung modeling on which they are based. By observing the absence of correlation between CA_{NO} and Sacin and between Scond and $J'aw_{NO}$ in a young healthy control group, we verified that no such mathematical link existed. One could also wonder whether CA_{NO} and Sacin correspond to exactly the same part of the acinar lung zone and whether Scond and $J'aw_{NO}$ correspond to exactly the same part of the conductive lung zone. On basis of lung modeling and the respective diffusion fronts^{2,36} for NO and O₂, it can be argued that the dividing line for the inflammatory as well as the functional parameters must be located in the vicinity of the acinar entrance. The fact that in control and asthma groups, no correlations existed between $J'aw_{NO}$ and Sacin, nor between CA_{NO} and Scond, lends support to the fact that inflammatory and functional parameters are confined to their respective lung zones.

In summary, the current data complement earlier studies in pointing to a relatively fixed degree of conductive airway abnormality across patients with stable asthma of different asthma severities. We have now shown that the conductive airway abnormality is only poorly associated to inflammation as inferred by the bronchial component of exhaled NO. Despite being stable, the patients with more severe asthma in terms of FEV₁ showed an increased acinar component of airway dysfunction that was clearly linked to inflammation in that lung compartment. In this respect, valuable information could be lost if only $FENO_{50}$, and not its component CA_{NO} , were determined when developing novel therapies, particularly those that are preferentially targeted to the acinar lung zone.

Key messages

A noninvasive assessment of airway function and airway inflammation in stable asthma shows the following:

- Airway function in the conductive lung zone of patients with stable asthma is consistently abnormal and only poorly associated with bronchial NO.
- Airway function in the acinar lung zone is particularly abnormal in the patients with more severe asthma and significantly correlated with alveolar NO concentration.

REFERENCES

1. Malinovschi A, Janson C, Holm L, Nordvall L, Alving K. Basal and induced NO formation in the pharyngo-oral tract influences estimates of alveolar NO levels. *J Appl Physiol* 2009;106:513-9.
2. Verbanck S, Kerckx Y, Schuermans D, Vincken W, Paiva M, Van Muylem A. Effect of airways constriction on exhaled nitric oxide. *J Appl Physiol* 2008;104:925-30.
3. American Thoracic Society; European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;171:912-30.
4. Travers J, Marsh S, Aldington S, Williams M, Shirtcliffe P, Pritchard A, et al. Reference ranges for exhaled nitric oxide derived from a random community survey of adults. *Am J Respir Crit Care Med* 2007;176:238-42.
5. Olin AC, Bake B, Torén K. Fraction of exhaled nitric oxide at 50 mL/s: reference values for adult lifelong never-smokers. *Chest* 2007;131:1852-6.
6. Haight RR, Gordon RL, Brooks SM. The effects of age on exhaled breath nitric oxide levels. *Lung* 2006;184:113-9.
7. Högman M, Lafi H, Meriläinen P, Bröms K, Malinovschi A, Janson C. Extended NO analysis in a healthy subgroup of a random sample from a Swedish population. *Clin Physiol Funct Imaging* 2009;29:18-23.
8. Shaw DE, Berry MA, Thomas M, Green RH, Brightling CE, Wardlaw AJ, et al. The use of exhaled nitric oxide to guide asthma management: a randomized controlled trial. *Am J Respir Crit Care Med* 2007;176:231-7.
9. Stick S, Franklin P. NO more dogma: nitric oxide marker in asthma. *Am J Respir Crit Care Med* 2009;179:87-8.
10. Taylor DR. Exhaled nitric oxide: still alive, not laid to rest. *Am J Respir Crit Care Med* 2009;179:88-9.
11. Michils A, Baldassarre S, Van Muylem A. Exhaled nitric oxide and asthma control: a longitudinal study in unselected patients. *Eur Respir J* 2008;31:539-46.
12. van Veen IH, Sterk PJ, Schot R, Gauw SA, Rabe KF, Bel EH. Alveolar nitric oxide versus measures of peripheral airway dysfunction in severe asthma. *Eur Respir J* 2006;27:951-6.
13. Gelb AF, Taylor CF, Nussbaum E, Gutierrez C, Schein A, Shinar CM, et al. Alveolar and airway sites of nitric oxide inflammation in treated asthma. *Am J Respir Crit Care Med* 2004;170:737-41.
14. Berry M, Hargadon B, Morgan A, Shelley M, Richter J, Shaw D, et al. Alveolar nitric oxide in adults with asthma: evidence of distal lung inflammation in refractory asthma. *Eur Respir J* 2005;25:986-91.
15. Lehtimäki L, Kankaanranta H, Saarelainen S, Turjanmaa V, Moilanen E. Inhaled fluticasone decreases bronchial but not alveolar nitric oxide output in asthma. *Eur Respir J* 2001;18:635-9.
16. Brindicci C, Ito K, Barnes PJ, Kharitonov SA. Differential flow analysis of exhaled nitric oxide in patients with asthma of differing severity. *Chest* 2007;131:1353-62.
17. George SC, Hogman M, Permutt S, Silkoff PE. Modeling pulmonary nitric oxide exchange. *J Appl Physiol* 2004;96:831-9.
18. Verbanck S, Kerckx Y, Schuermans D, de Bisschop C, Guénard H, Naeije R, et al. The effect of posture-induced changes in peripheral nitric oxide uptake on exhaled nitric oxide. *J Appl Physiol* 2009;106:1494-8.
19. Battaglia S, den Hertog H, Timmers MC, Lazeroms SP, Vignola AM, Rabe KF, et al. Small airways function and molecular markers in exhaled air in mild asthma. *Thorax* 2005;60:639-44.
20. Global Initiative for Asthma. Global strategy for asthma management and prevention: NHLBI/WHO workshop report. Washington (DC): US Government Printing Office; 2002. NHLBI pub no. 02-3659.
21. Verbanck S, Schuermans D, Paiva M, Vincken W. Nonreversible conductive airway ventilation heterogeneity in mild asthma. *J Appl Physiol* 2003;94:1380-6.
22. Verbanck S, Schuermans D, Noppen M, Van Muylem A, Paiva M, Vincken W. Evidence of acinar airway involvement in asthma. *Am J Respir Crit Care Med* 1999;159:1545-50.
23. Downie SR, Salome CM, Verbanck S, Thompson B, Berend N, King GG. Ventilation heterogeneity is a major determinant of airway hyperresponsiveness in asthma, independent of airway inflammation. *Thorax* 2007;62:684-9.
24. Verbanck S, Schuermans D, Paiva M, Vincken W. The functional benefit of anti-inflammatory aerosols in the lung periphery. *J Allergy Clin Immunol* 2006;118:340-6.
25. Verbanck S, Schuermans D, Meysman M, Paiva M, Vincken W. Noninvasive assessment of airway alterations in smokers: the small airways revisited. *Am J Respir Crit Care Med* 2004;170:414-9.
26. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, et al. American Thoracic Society/European Respiratory Society Task Force on Asthma Control and Exacerbations. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 2009;180:59-99.
27. Condorelli P, Shin HW, Aledia AS, Silkoff PE, George SC. A simple technique to characterize proximal and peripheral nitric oxide exchange using constant flow exhalations and an axial diffusion model. *J Appl Physiol* 2007;102:417-25.
28. Kerckx Y, Michils A, Van Muylem A. Airway contribution to alveolar nitric oxide in healthy subjects and stable asthma patients. *J Appl Physiol* 2008;104:918-24.
29. Bush A. How early do airway inflammation and remodeling occur? *Allergol Int* 2008;57:11-9.
30. Menzies D, Nair A, Meldrum KT, Fleming D, Barnes M, Lipworth BJ. Simvastatin does not exhibit therapeutic anti-inflammatory effects in asthma. *J Allergy Clin Immunol* 2007;119:328-35.
31. Brindicci C, Ito K, Barnes PJ, Kharitonov SA. Effect of an inducible nitric oxide synthase inhibitor on differential flow-exhaled nitric oxide in asthmatic patients and healthy volunteers. *Chest* 2007;132:581-8.
32. Kharitonov SA, Gonio F, Kelly C, Meah S, Barnes PJ. Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. *Eur Respir J* 2003;21:433-8.
33. Fritscher LG, Rodrigues MT, Zamel N, Chapman KR. The effect of montelukast on exhaled nitric oxide of alveolar and bronchial origin in inhaled corticosteroid-treated asthma. *Respir Med* 2009;103:296-300.
34. Cohen J, Douma WR, ten Hacken NHT, Vonk JM, Oudkerk M, Postma DS. Ciclesonide improves measures of small airway involvement in asthma. *Eur Respir J* 2008;31:1213-1220.
35. Suresh V, Shelley DA, Shin HW, George SC. Effect of heterogeneous ventilation and nitric oxide production on exhaled nitric oxide profiles. *J Appl Physiol* 2008;104:1743-52.
36. Verbanck S, Paiva M. Model simulations of gas mixing and ventilation distribution in the human lung. *J Appl Physiol* 1990;69:2269-79.