Exhaled nitric oxide: A biomarker integrating both lung function and airway inflammation changes

Amaryllis Haccuria, MD,[a,b] Alain Michils, MD, PhD,[a] Sébastien Michiels, MD,[a] and Alain Van Muylem, PhD[a] *Brussels and Namur, Belgium*

Background: The increased fraction of exhaled nitric oxide (FENO) values observed in asthmatic patients are thought to reflect increased airway inflammation. However, FENO values can be affected by airway caliber reduction, representing a bias when using FENO values to assess asthma control.

Objective: We sought to determine the effect of changes in both airway caliber and inflammation on FENO values using the allergen challenge model.

Methods: FEV_1 and FENO values were measured during early airway responses (EARs) and late airway responses after challenge with house dust mite allergens in 15 patients with mild allergic asthma. Helium and sulfur hexafluoride (SF_6) phase III expired concentration slopes (S_{He} and S_{SF6}, respectively) from single-breath washout tests were measured to identify sites of airway constriction.

Results: In EARs, FEV_1 and FENO value decreases reached 36.8% and 22%, respectively ($P < .001$). ΔS_{He} was greater than ΔS_{SF6} (+189.4% vs +82.2%, $P = .001$). In late airway responses FEV_1 and FENO value decreases reached 31.7% and 28.7%, respectively ($P < .001$), with the same ΔS_{He} and ΔS_{SF6} pattern (+155.8% vs +76%, $P = .001$). Eight hours after the EAR, FEV_1 was still decreased ($P < .001$), whereas FENO values had returned to baseline. At 24 hours, FEV_1 had returned to baseline, with FENO values increased by 38.7% ($P = .04$).

Conclusion: In patients with mild allergic asthma, airway caliber changes modulate changes in FENO values resulting from airway inflammation. Therefore FENO should no longer be considered solely an inflammation biomarker but rather a biomarker that integrates both airway inflammation and lung function changes. Furthermore, early and late phases resulting from allergen exposure were shown to involve similar lung regions. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Allergen challenge, bronchoprovocation, fractional exhaled nitric oxide, asthma, house dust mite*

Abbreviations used
CANO: Alveolar nitric oxide concentration
EAR: Early airway response
FENO: Fraction of exhaled nitric oxide
He: Helium
IS: Inflammatory status
LAR: Late airway response
NO: Nitric oxide
SF_6: Sulfur hexafluoride
S_{He}: Helium phase III expired concentration slope
S_{SF6}: Sulfur hexafluoride phase III expired concentration slope

Asthma is a chronic inflammatory airway disorder characterized by variable airflow obstruction, airway hyperresponsiveness, and respiratory symptoms.[1] Given these phenotypic elements, accurate assessment of asthma control might be improved by using a multidimensional approach including airway inflammation markers. Randomized trials have shown that asthma management that considered surrogate inflammatory markers, such as sputum eosinophil counts and airway hyperresponsiveness, improved asthma control.[2,3] The degree of airway inflammation in asthmatic patients might also be reflected by fraction of exhaled nitric oxide (FENO) values.[4,5] Nitric oxide (NO) detected in expired air is synthesized from L-arginine by constitutive and inducible NO synthase enzymes, which are expressed in resident and inflammatory cells and activated by inflammatory cytokines.[6] However, in addition to changes in airway inflammatory status (IS), some confounding factors (eg, anti-inflammatory medications, diet, and smoking) have been identified and reported in current guidelines.[7] Furthermore, airway caliber reduction might also influence FENO values. Indeed, several studies have revealed FENO values to be affected by reduction in FEV_1 caused by airway challenges.[8-11] Moreover, the site of airway constriction appears to be relevant, with a deeper FENO value reduction reported to be associated with more peripheral airway constriction.[8]

Overall, those data suggest that the FENO value as an airway inflammation marker was significantly affected by a noninflammatory event commonly observed in asthmatic patients (ie, FEV_1 decrease). This might represent a significant bias in using FENO values to assess asthma control over time.

Therefore our study sought to clarify the interaction between these 2 factors using the allergen challenge model, with both airway constriction and airway inflammation purposefully induced.

METHODS

Subjects

Patients with intermittent or mild persistent asthma associated with house dust mite allergy were recruited from the outpatient asthma clinic (CUB Erasme University Hospital, Brussels, Belgium). Asthma was diagnosed

From [a]the Chest Department, Erasme University Hospital, Université Libre de Bruxelles, Brussels, and [b]the Chest Department, Centre Hospitalier Régional de Namur, Namur.

Chiesi provided a grant for the Immunobiology of Asthma Unit.

Disclosure of potential conflict of interest: A. Haccuria has received research support from and has a board membership with Chiesi. A. Michils has received research support from Chiesi, Novartis, and AstraZeneca; is on advisory boards for Chiesi, Novartis, and AstraZeneca; has received payment for lectures from AstraZeneca, Stallergenes, and Chiesi; and has received travel support from Novartis and Chiesi. A. Van Muylem has received research support and payment for lectures from Chiesi. S. Michiels declares that he has no relevant conflicts of interest.

Received for publication June 27, 2013; revised November 28, 2013; accepted for publication December 10, 2013.

Corresponding author: Alain Van Muylem, PhD, Chest Department, CUB Erasme, Université Libre de Bruxelles, 808, route de Lennik, 1070 Brussels, Belgium. E-mail: avmuylem@ulb.ac.be.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2013.12.1070

according to standard criteria,[1] and sensitivity to house dust mite was evaluated by using skin prick tests or RASTs with commercially available extracts. Patients had no recent history of smoking or upper airway infection. At least 2 weeks before the challenge procedure, asthma treatment had to be discontinued, except for on-demand inhaled short-acting β_2-agonists. The study protocol was approved by the local ethics committee, and all patients provided written informed consent.

Study design

This prospective open study was carried out in the lung function laboratory of the Chest Department of Erasme University Hospital. FENO values, spirometric parameters, and ventilation distribution using single-breath washout tests were assessed. The measurements were taken before the allergen challenge and then after the test every 15 minutes for 1 hour and then every hour for at least 8 hours, with 1 final measurement taken 24 hours after the procedure.

Study procedures

Allergen challenge. Allergen challenge was performed by using the 2-minute tidal breathing technique at 15-minute intervals, according to established protocols.[12] After an initial saline challenge to ensure FEV_1 stability, increasing concentrations (0.2, 0.8, and 3.2 IR/mL) of the allergen extract (*Dermatophagoides pteronyssinus* 100%; Stallergenes, Antony, France) were administered with a nebulizer (Respironics SideStream Disposable Kit; Respironics, Herrsching, Germany) with an output of 8 L/min. The procedure was stopped when FEV_1 decreased by greater than 20% of the postsaline value. Once a threshold of a 20% FEV_1 decrease was reached, spirometry was monitored every 15 minutes for 1 hour, with the early asthmatic response determined as the maximum percentage decrease in FEV_1. Five patients received 32 mg of methylprednisolone and 400 μg of salbutamol at 380 minutes after allergen challenge for safety reasons, whereas 8 patients received those drugs at 480 minutes (8 hours) after allergen challenge, according to the initial protocol.

NO. FENO values were measured before any forced expiratory maneuvers by using a daily calibrated LR 2000 chemiluminescence analyzer (Logan Research, Rochester, United Kingdom), with online measurement of a single exhalation at a flow rate of 50 mL/s.[7] Exhaled NO levels were read when the plateau of 70% to 80% of the CO_2 curve was reached. FENO values were expressed in parts per billion. The alveolar nitric oxide concentration (CANO) was calculated by using the multiflow method described by Pietropaoli et al.[13] Exhaled NO tracings were obtained for flow rates of 130, 180, and 250 mL/s. The method's validity for evaluating CANO values has been previously established.[14]

Lung function. Lung function was measured with a Zan 500 spirometer (Zan, Oberthulba, Germany), according to standard guidelines.[15]

Ventilation distribution tests with inert gases. Ventilation distribution was assessed by using single-breath washout tests. Subjects were connected to a double bag-in-box system through a non-rebreathing valve with a 20-mL instrumental dead space. Patients inhaled a gas mixture containing 2 inert gases, 5% helium (He) and 5% sulfur hexafluoride (SF_6) in oxygen (O_2), from functional residual capacity to 1 L more than functional residual capacity and then exhaled gases at a constant flow of approximately 0.40 L/s to residual volume.

He and SF_6 concentrations were recorded as a function of expired volume immediately after each set of FENO and spirometric measurements (calibration cylinders: 6% CO_2, 15% O_2, 79% N_2 and 5% He, 5% SF_6, and 90% O_2; Messer Belgium, Zwijdrecht, Belgium).

The He phase III expired concentration slope (S_{He}) and SF_6 phase III expired concentration slope (S_{SF6}) were calculated by using a computerized linear regression (concentration vs expired volume) between 35% and 80% of the expired volume. He and SF_6 slopes were expressed in percentage per liter. S_{He} and S_{SF6} were multiplied by −15.6 for comparison to be made with published nitrogen slope data.[16] The test was performed in triplicate with a variation coefficient not exceeding 10%.

Analog signals of N_2, He, and SF_6 concentrations; flow; and volume were sampled at a 50-Hz frequency by using a 12-bit analog-numeric convertor, and digitized signals were saved on a computer for processing.

Model of eosinophilic inflammation. We propose a simple model of FENO value change over time in a subject as the linear combination of the change in airway caliber, as assessed by the change in FEV_1 and the change in eosinophilic IS reflected by epithelial NO production over the same period:

$$\Delta FENO/\Delta t = \alpha.\Delta FEV1/\Delta t + \Delta IS/\Delta t \quad (1)$$

The linear relationship between $\Delta FENO$ and ΔFEV_1 values was observed during bronchoprovocation challenges (unpublished data from Michils et al[16]). On the other hand, simulation of NO production and transport with models incorporating convection and diffusion transport showed that FENO values and epithelial NO production (reflecting IS) are linearly related.[14]

Assuming no change in IS occurring during the decrease in FENO values in the early airway response (EAR; $\Delta IS/\Delta t = 0$), individual values of α can be derived. For each time point, $\Delta FENO/\Delta t$ and $\Delta FEV_1/\Delta t$ are computed as the difference between the values at the considered time point and at the preceding time point divided by the time difference between the 2 time points. The amount of $\Delta IS/\Delta t$, assessing the change in eosinophilic IS, can thus be derived by equation 1.

Statistical analysis

Paired *t* tests were used to assess changes between baseline and values at each time point (EAR, recovery, late airway response [LAR], 8 hours, and 24 hours). Holm-Bonferonni corrections[17] were applied to *P* values to account for multiple testing. FENO values were log-transformed.

Paired *t* tests were used to compare S_{He} and S_{SF6} changes at each time point from baseline and expressed as percentages. Statistical significance was set at .05 (2-tailed). The Statistica 6.1 program (StatSoft France, Maisons-Alfort, France) was used for the analyses.

We used R software[18] to perform a mixed-model analysis of the relationship between $\Delta IS/\Delta t$ and time. We considered time a fixed effect and the by-subject model coefficients as random effects; that is, the model took account of the variability of the adjustment among subjects. Potential autocorrelation and change in variance with time were included in the model. The sample size allows including the considered model parameters with a power equal to 0.95 if the conditional squared multiple correlation coefficient[19] is at least 0.4.[20]

RESULTS

Patients' characteristics

Fifteen patients (mean age, 26 years; 11 men and 4 women) were included, with 13 exhibiting both EARs and LARs (dual responders). Baseline mean FEV_1 was 94.9% of predicted value.

Lung function and FENO values

For FENO values, spirometric values (FEV_1), S_{He}, and S_{SF6}, 5 time points after the allergen challenge were taken into account: EAR, recovery phase, LAR, and 8 and 24 hours after challenge. Fig 1 shows the mean changes in percentage from baseline of FEV_1 (Fig 1, black line and circles) and FENO (Fig 1, blue line and circles) values at each time point. The red line shows the evolution of the IS, as deduced from equation 1 (see the "Model of eosinophilic inflammation" subsection). The dashed lines (FEV_1 and FENO) between 8 and 24 hours (gray zone) and the question mark (inflammation) indicate that although the boundary values are known for FEV_1 and FENO, the exact profiles are unknown in this interval.

Table I displays the changes in percentages from baseline for FEV_1 and in absolute values for the other parameters at different time points, and the FENO value is presented as the geometric mean (geometric interval). With *m* and SD signifying the mean and the standard deviation of log-transformed FENO values, the