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Original Research

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A Novel Approach to Partition Central and Peripheral Airway Nitric Oxide

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Background: **Determining the site of airways inflammation may lead to the targeting of therapy. Nitric oxide (NO) is a biomarker of airway inflammation and can be measured at multiple exhalation flow rates to allow partitioning into bronchial (large/central airway maximal nitric oxide flux [J'awNO]) and peripheral (peripheral/small airway/alveolar nitric oxide concentration [CANO]) airway contributions by linear regression. This requires a minimum of three exhalations. We developed a simple and practical method to partition NO.**

Methods: **In 29 healthy subjects (FEV_1, 97% ± 3% predicted), 13 patients with asthma (FEV_1, 90% ± 4% predicted), 14 patients with COPD (FEV_1, 59% ± 3% predicted), and 12 patients with cystic fibrosis (CF) (FEV_1, 60% ± 3% predicted), we measured the area under the curve of the NO concentration/exhalation time plot (AUC-NO) at exhalation flow rates of 50, 100, 200, and 300 mL/s. We determined the change of the total AUC-NO production (ΔAUC-NO) among the four different exhalation flow rates and compared these levels to CANO and J'awNO indices measured conventionally by linear regression.**

Results: **The change in AUC-NO between increasing exhalation flow rates of 50 to 200 mL/s ($\Delta AUC\text{-}NO_{50\text{-}200}$) was strongly correlated with J'awNO in all patient groups as follows: healthy subjects ($r = 0.94$, $P < .001$), patients with asthma ($r = 0.98$, $P < .001$), patients with COPD ($r = 0.93$, $P < .001$), and patients with CF ($r = 0.74$, $P < .05$). In all subjects, AUC-NO at an exhalation flow rate of 200 mL/s ($AUC\text{-}NO_{200}$) correlated with CANO ($r = 0.69$, $P < .01$).**

Conclusions: **The bronchial production of NO can be determined by measuring $\Delta AUC\text{-}NO_{50\text{-}200}$, whereas $AUC\text{-}NO_{200}$ measures its peripheral concentration. This approach is simple, quick, and does not require sophisticated equipment or mathematical models.** ***CHEST 2014; 145(1):113–119***

Abbreviations: AUC = area under the curve; AUC-NO = area under the curve of the nitric oxide concentration/exhalation time plot; CANO = peripheral/small airway/alveolar nitric oxide concentration; CF = cystic fibrosis; FENO = fraction of exhaled nitric oxide; J'awNO = large/central airway maximal nitric oxide flux; NO = nitric oxide; ppb = parts per billion

Nitric oxide (NO) is a biomarker of airways inflammation and an adjunct in the diagnosis and management of respiratory diseases such as asthma,[1-4] as approved by the Food and Drug Administration.[5] The single exhalation breath measurement of NO (fraction of exhaled NO [FENO]) is simple, reproducible, and noninvasive, and most notably, the methodology for its detection has been standardized.[6] Currently, there is immense interest in assessing the predominant site of airways inflammation (peripheral vs central) in various pulmonary diseases, and this may be important in targeting antiinflammatory therapy.[7-9]

This interest has encouraged the development of mathematical models to interpret the flow-dependent physiology of NO dynamics,[10,11] and the measurement of exhaled NO at different exhalation flow rates has

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been used to partition peripheral (peripheral/small airway/alveolar NO concentration [CANO]) from central (large/central airway maximal NO flux [J'awNO]) airways NO production. The latter technique is based on a two-compartment model,[10] which contrary to the single-compartment model, accounts for the production and contribution of central airways NO, explaining the exhalation flow dependency of this gas.[11] This approach requires a minimum of three exhalation flows, and both J'awNO and CANO are calculated by a linear regression analysis. This technique has been used to show elevated J'awNO in patients with mild to moderate asthma[10] and CANO in patients with refractory asthma,[12] older healthy subjects,[13] patients with COPD,[14] patients with cystic fibrosis (CF),[15] and patients with scleroderma.[16]

We revisited and simplified the method for NO partitioning from the two-compartment model to show that the complicated mathematical equations used in the linear regression method could be replaced with the measurement of the area under the curve (AUC) of the NO concentration/exhalation time plot (AUC-NO) obtained at one slow (50 mL/s) and one fast (200 mL/s) patient exhalation flow. In addition, we compared this new technique with a method described by Condorelli et al,[17] which takes into account the axial back diffusion of bronchial NO into the alveolar space.

Materials and Methods

Patients

Twenty-nine healthy volunteers (20 men; mean age, 38 ± 2 years; FEV_1, 97% ± 3% predicted) were enrolled in the study (Table 1). We also studied 13 steroid-naive patients with asthma (eight men; mean age, 48 ± 8 years; FEV_1, 90% ± 4% predicted) whose condition was diagnosed according to American Thoracic Society criteria[18]; 14 patients with COPD (10 men; mean age, 63 ± 2 years; FEV_1, 59% ± 3% predicted), all ex-smokers with a ≥ 20-pack-year smoking history and without a history of allergic disease or reversibility of airflow obstruction (> 15% or > 200 mL after 400 μg salbutamol through a metered-dose inhaler); and 12 patients with CF (eight men; mean age, 21 ± 4 years; FEV_1, 60% ± 3% predicted). The study was approved by the local research ethics committee (reference number 08/H0709/2).

Patients with a respiratory tract infection or respiratory disease exacerbation were excluded from the study. Active and passive smokers (exposure for > 0.5 h/d) were excluded.

Exhaled NO Measurement

Multiple Flows Linear Regression Method: Exhaled NO concentrations were measured by a chemiluminescence analyzer (NIOX; Aerocrine) at expiratory flow rates of 50, 100, 200, and 300 mL/s by applying resistances of 50, 100, 200, and 300 cm H_2O/mL/s to maintain the target exhalation flow rates. Patients inhaled NO-free air and exhaled through a fixed flow restriction to increase pressure in the mouth up to 10 cm H_2O, which is effective in closing the soft palate[19-21] and isolating the nasopharynx. Dead space air was excluded from the analysis. The analyzer was calibrated with a known NO concentration (200 parts/million). Every subject performed two exhalations at each exhalation flow.

The bronchial production of NO (J'awNO) and its alveolar concentration (CANO) were calculated by linear regression according to the equation of Tsoukias and George,[10] where the slope and the intercept of the regression line between NO output and exhalation flow indicates CANO and J'awNO, respectively. In addition, J'awNO and CANO were also calculated using the Condorelli adjustment for the axial diffusion of NO as follows[17]: $J'awNO = 1.7 \times (I)$ and $CANO = [(S) - (I)]/(740\ mL/s)$, where S is the slope and I is the y-intercept by simple linear regression.

Dual Flows Measurement by AUC Method: The database containing the measurements of CANO and J'awNO calculated by the linear regression method was downloaded from the NO analyzer and transferred to a computer. For each expiratory flow rate (50, 100, 200, and 300 mL/s), the point-by-point values of the NO concentrations were plotted graphically against the exhalation time (up to 10 s) (Fig 1). The AUC-NO was calculated by the average of two measurements at each expiratory flow rate with Prism version 5.03 (GraphPad Software, Inc) (Fig 1). The software calculated the AUC by the trapezoidal method. This process was straightforward and took < 1 min to compute.

We then studied the change in the AUC-NO between increasing exhalation flow rates of 50 to 100 mL/s ($\Delta AUC\text{-}NO_{50\text{-}100}$), 50 to 200 mL/s ($\Delta AUC\text{-}NO_{50\text{-}200}$), and 50 to 300 mL/s ($\Delta AUC\text{-}NO_{50\text{-}300}$). We compared the average AUC-NO values with the J'awNO and CANO values calculated by conventional linear regression and with values calculated by the Condorelli adjustment method with the same data as described previously.

Lung Function Tests

All patients underwent pulmonary function testing. Testing included spirometry and lung volumes by body plethysmography (Jaeger Master Laboratory Compact Transfer; Erich Jaeger Ltd).

Statistical Analysis

Comparisons between patient groups were made by one-way analysis of variance with Bonferroni correction. Kruskal-Wallis one-way analysis of variance was used to analyze nonparametric data. Correlations are presented as nonparametric Spearman rank correlation coefficients. Data were expressed as mean ± SEM and CIs of differences. Significance was defined as $P < .05$. Agreement between methods was confirmed with Bland-Altman plots.

Results

Single-Breath FENO and Linear Regression Method

FENO was significantly elevated in patients with asthma (73.23 ± 11.19 parts per billion [ppb], $P < .01$), whereas it was significantly reduced in patients with CF (11.79 ± 2.14 ppb, $P < .01$) compared with healthy subjects (34.68 ± 3.59 ppb). Patients with COPD (37.30 ± 10.97 ppb) had similar levels to those of the healthy subjects (Fig 2A).

J'awNO was significantly elevated in patients with asthma (189.00 ± 28.76 ng/s, $P < .01$) but significantly reduced in patients with CF (33.00 ± 5.93 ng/s, $P < .05$) compared with healthy subjects (57.69 ± 6.52 ng/s). Patients with COPD had comparable values to the healthy subjects (51.21 ± 9.89 ng/s, $P > .05$) (Fig 2B).