

Alveolar Concentration and Bronchial Flux of Nitric Oxide: Two Linear Modeling Methods Evaluated in Children and Adolescents With Allergic Rhinitis and Atopic Asthma

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Summary. Objective: Alveolar concentration ($C_A\text{NO}$) and bronchial flux ($J_{\text{aw}}\text{NO}$) of nitric oxide (NO) characterize the contributions of peripheral and proximal airways to exhaled NO. Both parameters can be estimated using a two-compartment model if the fraction of NO in orally exhaled air (FE_{NO}) is measured at multiple constant expiratory flow rates (V). The aim of this study was to evaluate how departures from linearity influence the estimates of $C_A\text{NO}$ and $J_{\text{aw}}\text{NO}$ obtained with the help of linear regression analysis of the relationships between FE_{NO} and $1/V$ (method P), and between the NO output ($V_{\text{NO}} = FE_{\text{NO}} \times V$) and V (method T). Furthermore, differences between patients with atopic asthma (AA) and allergic rhinitis (AR) and between methods P and T were assessed. Design: Measurements of FE_{NO} were performed with a chemiluminescence analyzer at five levels of V ranging from 50 to 250 ml/sec in school children and adolescents with mild to moderate-severe AA treated by inhaled corticosteroids ($N = 42$) and AR ($N = 20$). Results: Violation of the linearity condition at $V \leq 100$ ml/sec caused shifts between methods with regard to the partition of exhaled NO into alveolar ($C_A\text{NO}$: $P > T$) and bronchial ($J_{\text{aw}}\text{NO}$: $T > P$) components. Both methods gave similar results in the linear range of 150–250 ml/sec: The mean ratios P/T and limits of agreement calculated in AA and AR patients were 1.03 (0.49–1.56) and 1.07 (0.55–1.59) for $C_A\text{NO}$ and 1.03 (0.73–1.33) and 0.99 (0.90–1.10) for $J_{\text{aw}}\text{NO}$, respectively. No significant differences between AA and AR were found in $C_A\text{NO}$ and $J_{\text{aw}}\text{NO}$ calculated in the linear range by the T method {medians (inter-quartile ranges): 1.7 ppb (0.9–3.9) vs. 2.3 ppb (0.8–3.7), $P = 0.91$; 1,800 pl/sec (950–3,560) vs. 1,180 pl/sec (639–1,950), $P = 0.061$ }. However, the flow-dependency of the estimates was markedly higher in AA than in AR patients: $C_A\text{NO}$ was decreased 2.8-fold vs. 1.5-fold and $J_{\text{aw}}\text{NO}$ was increased 1.5-fold vs. 1.2-fold in the linear range as compared to the range of 50–250 ml/sec. In both groups, the median standard errors (SE) of the $J_{\text{aw}}\text{NO}$ estimates were similar for the methods P and T and small (<15%) regardless of the range for expiratory flows. The precision of $C_A\text{NO}$ estimates was less in all ranges. For both methods, the SE of the estimates obtained in the range of 150–250 ml/sec exceeded 50% in asthmatics and 30% in AR patients, respectively. The results show that FE_{NO} has to be measured at several expiratory flows ≥ 100 ml/sec for the accurate estimation of $C_A\text{NO}$ and $J_{\text{aw}}\text{NO}$ using linear methods P and T in children and adolescents with AA and AR. A stepwise procedure for detecting nonlinearity and evaluating the quality of FE_{NO} measurements is suggested. **Pediatr Pulmonol.** © 2012 Wiley Periodicals, Inc.

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INTRODUCTION

Since the first report that nitric oxide (NO) can be detected in the exhaled breath of animals and humans, a large body of knowledge has accumulated on its biochemical and anatomical origin as well as its multiple biological functions in the respiratory system.^{1,2}

Moreover, sensitive and reliable analyzers have been developed for measuring of the fraction of NO in orally exhaled air (FE_{NO}). Current guidelines recommend a single-breath technique with on-line detection of $FE_{NO,50}$ during exhalation at a flow rate of 50 ml/sec.³ The analysis of $FE_{NO,50}$ is the first rapid and completely noninvasive method which reflects the presence and severity of eosinophilic inflammation in the airways. Furthermore, this method correlates with eosinophil counts in induced sputum or bronchoalveolar lavage fluid collected from patients with atopic asthma (AA).^{4,5} The most valuable benefit of using $FE_{NO,50}$ in children is in distinguishing chronic respiratory symptoms due to AA from those of other etiology. The monitoring of $FE_{NO,50}$ is also helpful in predicting steroid responsiveness as well as successful steroid reduction or withdrawal.^{6,7}

Exhaled NO originates from small airways and alveoli and from central airways. During exhalation, the concentration of NO in the lumen of bronchi (C_{NO}) progressively increases above that in the alveolar air ($C_{A,NO}$) because NO diffuses from the bronchial wall into the lumen. The amount of NO added to the luminal air per unit of time (bronchial flux of NO, $J_{aw,NO}$) is proportional to the difference between the airway wall concentration ($C_{aw,NO}$) and luminal concentration of NO and the proportionality factor is the airway capacity for radial diffusion of NO ($D_{aw,NO}$). The $J_{aw,NO}$ is diffusion rate-limited. Consequently, the FE_{NO} decreases and the volume of NO exhaled per unit of time (NO output, V_{NO}) increases nonlinearly with increasing an expiratory flow rate (V). Determining of FE_{NO} at multiple expiratory flows and using the two-compartment model or more complex models of pulmonary NO exchange enables the estimation of flow-independent parameters $C_{A,NO}$, $C_{aw,NO}$, and $D_{aw,NO}$ that characterize the production of NO in the peripheral respiratory tract and the bronchi.^{8,9} At higher expiratory flows, linear approximations of nonlinear relationships between FE_{NO} and the reciprocal value of V or between V_{NO} and V enable the estimation of $C_{A,NO}$ and $J_{aw,NO}$ as the intercept and slope parameters from the linear regression analysis.^{8,10}

The results of some^{11–15} but not all studies¹⁶ with asthmatic children and adults suggest that increased $C_{A,NO}$ reflects more severe asthma, its poor control, and may help to predict asthma exacerbation. Recently, it has been shown that a combined evaluation

of $C_{A,NO}$ and $J_{aw,NO}$ allows separation of asthma patients according to the pattern of exhaled NO into four categories characterized by distinct clinical features including sensitivity to inhaled corticosteroids (ICS) and atopy.¹⁷

Flow-independent NO parameters have been estimated by linear and nonlinear methods in healthy children¹⁸ and children with allergy and asthma as well as with other diseases.¹⁹ Contrary to the measurement of $FE_{NO,50}$; however, these methods are less well standardized. There is a paucity of studies comparing various modeling methods and examining the influence of factors such as the range and number of expiratory flows on the results. The present study performed the on-line measurement of FE_{NO} at five expiratory flows ranging from 50 to 250 ml/sec in school children and adolescents with AA and allergic rhinitis (AR), all of whom presented for a scheduled visit to the outpatient allergy department. The aim was to characterize and compare two linear methods as to their compliance with the linearity assumption and as to the influence of the range and number of expiratory flows on the estimates of $C_{A,NO}$ and $J_{aw,NO}$ and their standard errors. Moreover, the differences between AA and AR patients in alveolar and bronchial NO parameters were assessed including their putative dependence on the range of expiratory flows and modeling method used in the estimation.

MATERIALS AND METHODS

Study Design

The study was approved by the Regional Ethics Committee of the Faculty of Medicine and informed consent was obtained from parents and from children older than 12 years of age. Children and adolescents were recruited consecutively who presented for a scheduled visit to the Allergy outpatient department at the University Hospital in Hradec Králové, Czech Republic. The clinical history of patients was known from four to eight visits to the department, all of which had occurred during the last 2 years preceding the study. All subjects had prior experience with the measurement of $FE_{NO,50}$. At the time of investigation, all patients and their parents were interviewed by the same experienced physician about the symptoms of asthma and allergy and the use of medication since the last visit. All patients underwent a measurement of exhaled NO at multiple expiratory flows followed by spirometry and skin-prick testing.

Study Population

Fifty-nine patients aged between 8 and 18 participated in the study. Children and adolescents with AA ($n = 42$) had mild or moderate-severe persistent disease

according to the Global Initiative for Asthma guidelines criteria.²⁰ AR was diagnosed in 79% of asthmatics. The second group under investigation included 20 patients with AR. The AR patients either have never been diagnosed with asthma according to their medical record ($n = 14$) or their asthma was in remission ($n = 6$). The patients and/or parents did not report asthma symptoms (recurrent coughing, wheezing or shortness of breath, no tolerance of physical activity) when asked by the physician during pre-study visits and on the day of investigation and AR children with asthma in the past were prescribed no medication for asthma in the last year. All patients with asthma had been taking ICS for ≥ 6 months prior to the study and no patient has experienced an asthma exacerbation requiring systemic corticosteroids in the last year. At the time of the investigation, a majority of the patients from both groups were treated with nasal corticosteroids and oral or local H₁-antihistamines (Table 1). No patient had a respiratory infection in the month prior to the study. All subjects were nonsmokers.

Procedures

Measurements of FE_{NO} were performed with the help of a chemiluminescence analyzer CLD88sp (Ecomedics AG, Duernten, Switzerland) using the single-breath on-line method in accordance with international recommendations.³ Zero-point calibration of the analyzer with NO-scrubbed air and calibration of the flow meter was performed on each study day. A two-point calibration was repeated weekly and a certified calibration gas with 1 ppm NO balanced with N₂ (Linde-Technoplyn, Prague, Czech Republic) was used for that purpose.

Subjects inhaled NO-free air to total lung capacity and exhaled against a pressure of 10–20 cm H₂O into

an exhalation circuit of the analyzer, which consisted of an ultrasonic flow meter, two-way valve, and sampling port. A visual feedback on a computer monitor helped the subjects to maintain exhalation at five expiratory flow rates of 50, 100, 150, 200, and 250 ml/sec, respectively. The exhaled air was sucked into the analyzer at a flow rate of 330 ml/min. Both the expiratory flow and FE_{NO} versus time curves were recorded. The exhalation time was set at 12 sec for children >12 years of age and 8 sec for younger children. Subjects were allowed to rest for at least 30 sec between exhalations. The mean FE_{NO} value during the first 3-second window (2-second window for children younger than 12 years of age) was recorded, with a stable guideline-derived FE_{NO} plateau value during the exhalation lasting 6 sec as a minimum (4 sec in children younger than 12 years of age). Two measurements at each flow approved by the software of the analyzer were required. If the FE_{NO} values differed by more than 15% (1.5 ppb for values less than 10 ppb) the patient performed a third exhalation. Measurement repeatability was assessed using the relative difference (in percent of the mean) between the results of two consecutive measurements or the two best results of FE_{NO} and V_{NO} at each single expiratory flow.

The atopy status was evaluated using the skin prick test with 12 common aeroallergens with negative and positive controls (Alyostal, Stallergènes, Paris, France). Spirometry was undertaken according to American Thoracic Society standards²¹ using a spirometer Master-screen (Jaeger, Hochberg, Germany). The best value of three maneuvers was expressed as a percentage of the predicted value.

Estimation of Alveolar Concentration and Bronchial Output of NO

The relationship between FE_{NO} and V derived from the two-compartment model is described by an exponential equation:⁹

$$\begin{aligned} \text{FE}_{\text{NO}} = & C_{\text{aw}}\text{NO} \\ & \times \left[1 - \exp\left(-\frac{D_{\text{aw}}\text{NO}}{V}\right) \right] + C_{\text{A}}\text{NO} \\ & \times \exp\left(-\frac{D_{\text{aw}}\text{NO}}{V}\right) \end{aligned} \quad (1)$$

At higher flow rates, V is much higher than D_{aw}NO and the relationship can be approximated as follows:

$$\text{FE}_{\text{NO}} = C_{\text{A}}\text{NO} + (C_{\text{aw}}\text{NO} - C_{\text{A}}\text{NO}) \times \frac{D_{\text{aw}}\text{NO}}{V} \quad (2)$$

As originally suggested by Pietropaolli et al.¹⁰ and Tsoukias et al.,⁹ the estimates of C_ANO and J_{aw}NO can

TABLE 1—Patient Characteristics

	Atopic asthma (n = 42)	Allergic rhinitis (n = 20)	P-value
Age (years)	13 ± 2.9	13 ± 3.4	0.76
Gender, M/F (n)	26/16	11/9	0.57
Height (cm)	158 ± 15	154 ± 16	0.46
Weight (kg)	53 ± 18	49 ± 14	0.52
Inhaled CS (%)	100	0	<0.0001
Nasal CS (%)	55	47	0.77
Oral H ₁ -antihistamines (%)	67	65	1.0
FEV ₁ (%)	101 ± 14	110 ± 16	0.070
MEF ₅₀ (%)	91 ± 22	99 ± 21	0.28
FE _{NO50} (ppb)	29 (19–62)	23 (13–29)	0.075

Data are shown as the arithmetic mean ± SD, FE_{NO} is given as the median (inter-quartile range).

be obtained under such conditions with the help of the linear Equations (3) and (4):

$$FE_{NO} = C_A NO + J_{aw} NO \times \left(\frac{1}{V} \right) \quad (3)$$

$$V_{NO} = J_{aw} NO + C_A NO \times V \quad (4)$$

where

$$J_{aw} NO = (C_{aw} NO - C_A NO) \times D_{aw} NO \text{ and } V_{NO} = FE_{NO} \times V.$$

The estimation using Equations (3) and (4) is designated here as linear methods P and T, respectively. What is a high enough expiratory flow to allow for the use of linear approximation depends of the subjects bronchial NO output: With high $J_{aw} NO$ linearity is

reached at higher flows, and with lower $J_{aw} NO$ the linearity is reached at lower flows. In most subjects, the flows ≥ 50 ml/sec are required. Figure 1 illustrates the aforementioned nonlinear relationships as well as the estimation of $C_A NO$ and $J_{aw} NO$ with the help of linear regression analysis of the data obtained at higher flow rates.

The plateau values of FE_{NO} (V_{NO}) from the two correctly performed measurements at each of the five expiratory flow rates (50, 100, 150, 200, and 250 ml/sec) were plotted against the actual, time-averaged values of the flow rates (the reciprocal values of the flow rates) obtained using an ultrasonic flow meter. Simple linear regression was calculated. Thereafter, the lower limit of the flow range was gradually increased from 50 to 100 to 150 ml/sec with the higher limit fixed at 250 ml/sec. The calculation was then repeated. For each of the three ranges, the point estimates of the slope and y intercept

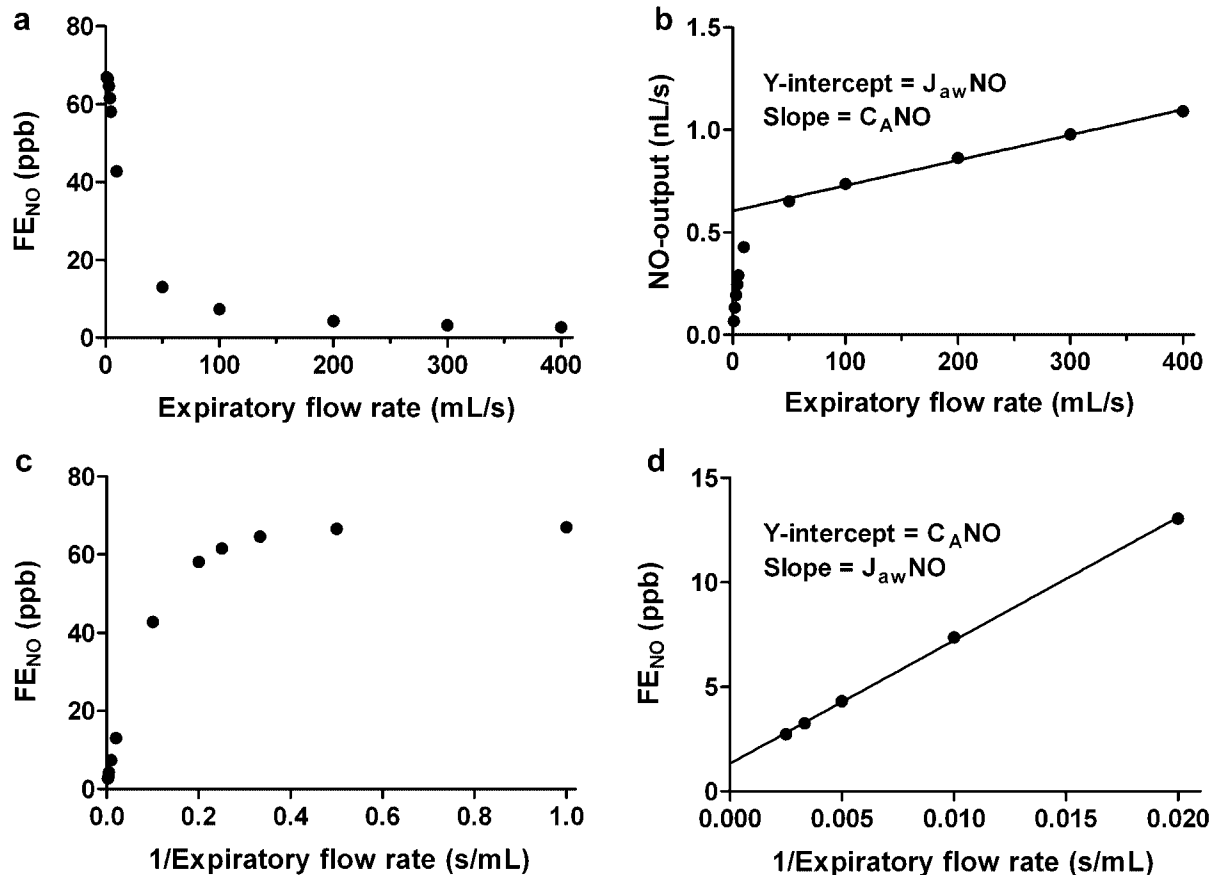


Fig. 1. The nonlinear relationships between the expiratory flow rate and (a) the fraction of nitric oxide (NO) in the exhaled air (FE_{NO}) and (b) the NO-output. c: The FE_{NO} plotted against the reciprocal value of expiratory flow rate. The data were simulated using the two-compartment model equations for a subject with the alveolar NO concentration ($C_A NO$) of 1.1 ppb, the bronchial wall NO concentration of 67 ppb, and the airway capacity for radial NO diffusion of 10 pl/sec/ppb. The estimation of the bronchial flux of NO ($J_{aw} NO$) and $C_A NO$ with the help of linear regression analysis of the data obtained at higher flow rates is shown in (b) and (c) (see the Materials and Methods Section).

and the standard errors of the estimates were calculated.

Statistical Analysis

All variables were assessed for normality of distribution. If the distribution was normal or near normal, the arithmetic mean and standard deviation (SD) were used as characteristics of a central value and spread of the data. The descriptive statistics of the variables with a skewed distribution (FE_{NO} , V_{NO} , C_{ANO} , J_{awNO}) was given as the median and inter-quartile range (25th–75th percentile). The influence of the expiratory on FE_{NO} and V_{NO} and of the range for expiratory flows on the estimates for C_{ANO} and J_{awNO} and their standard errors were analyzed, separately in the groups of asthmatics and AR patients by means of the Friedman's test and Dunn's multiple-comparison test. The between-group comparisons were performed with Kruskal–Wallis test followed with Dunn's multiple-comparison test. A value of $P < 0.05$ was considered significant. Based on the results obtained with the help of the method T in a similar population of children with well-controlled asthma and in atopic healthy children,¹¹ the study of 36 patients with AA and 17 with AR would have a power of 90% to detect a between-group difference in C_{ANO} of 1 ppb and a difference in J_{awNO} of 900 pl/sec. The intra-class correlation coefficients of two repeated measurements reported in the same study were 0.992 for J_{awNO} and 0.809 for C_{ANO} , respectively.¹¹ Thus, the detectable within-group differences were expected to be correspondingly less. Statistical analysis was performed with Statistica (version 8.0, StatSoft, Inc., Tulsa, OK).

RESULTS

The demographic characteristics of patients with AA and AR were comparable (Table 1). All asthmatics were taking low to medium doses of ICS either as a monotherapy ($n = 17$) or in a fixed combination with long-acting β_2 -agonists ($n = 25$). The mean $\pm$ SD daily dose of ICS was 251 ± 122 mcg budesonide or the equivalent. At the time of the investigation, asthma was well controlled in all but three patients who required an increased dose of ICS. The mean FEV_1 was marginally lower ($P = 0.070$) and the median FE_{NO} detected at the expiratory flow of 50 ml/sec tended to be higher ($P = 0.075$) in asthmatics as compared to patients with AR. The differences, however, did not reach statistical significance (Table 1).

Measurement repeatability was similar in both groups. The maximum relative differences in the flow rates between two successive exhalations and their maximum deviations from the target values were less than 15% for 50 ml/sec and less than 10% for higher flow

rates, respectively. The median (range) relative differences between two accepted measurements of FE_{NO} were 7% (1–15%) at 50 ml/sec, 5% (0.1–13%) at 100 ml/sec, 6% (0.3–14%) at 150 ml/sec, 5% (0.9–13%) at 200 ml/sec, and 5% (0.1–12%) at 250 ml/sec, respectively. The variable V_{NO} was characterized with similar measurement repeatability (data not shown). According to the results of the analysis of variance for repeated measurements performed after logarithmic transformation, both FE_{NO} and V_{NO} were highly dependent on the expiratory flow ($P < 0.0001$). No significant differences in either FE_{NO} or V_{NO} were found between asthmatics and AR patients at any expiratory flow (Fig. 2).

Influence of the Range of Expiratory Flows on the Estimates of NO Parameters

The gradual increase of the lowest expiratory flow from 50 through 100 to 150 ml/sec with the highest flow fixed at 250 ml/sec resulted in a gradual decrease of C_{ANO} and increase of J_{awNO} (Table 2). This effect was higher in asthmatics than in patients with AR. The median C_{ANO} obtained in asthmatics using the three highest flow rates (150–200–250 ml/sec) was 67% less (method P) and 60% less (method T), respectively, than that derived from all five flow rates (50–100–150–200–250 ml/sec). Only a 40% (method P) and 28% decrease (method T) was found in AR patients. The estimates of J_{awNO} increased by 50 and 17% in asthmatics and AR patients, respectively. The influence of the selected range for expiratory flows was similar for methods P and T (Table 2).

Across all ranges for the expiratory flow and likewise for the methods P and T, the uncertainty of estimation was higher for C_{ANO} than J_{awNO} . The gradual increase of the lower limit of the flow range led to a parallel increase in the standard errors of the estimate (SE) and this effect was more remarkable for C_{ANO} (an 2.9-fold increase) than for J_{awNO} (an 1.7-fold increase) (Table 3).

The percent differences between the observed values of FE_{NO} and V_{NO} and those predicted from the linear regression (the percent y-residuals) were evaluated as indicators of the deviation in relation to the linearity assumption. Visual assessment of the plots of the percent residuals and the Friedman's test performed separately for the groups of AA and AR patients revealed that the linearity improved if the lower limit of the range for flows was increased from 50 to 150 ml/sec (Fig. 3).

Comparison of Linear Modeling Methods

For the purpose of method comparison, the within-subject ratios (P/T) were calculated using the estimates of C_{ANO} and J_{awNO} obtained with methods P and T for each flow range (Fig. 3). Unlike the differences

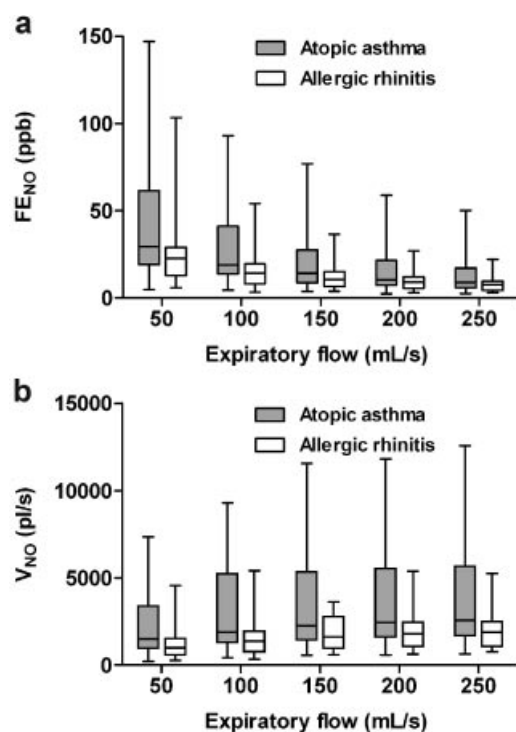


Fig. 2. The influence of the expiratory flow rate on (a) the fraction of nitric oxide (NO) in the exhaled air (FE_{NO}) and (b) the output of NO (V_{NO}) evaluated in children with atopic asthma and allergic rhinitis using a single-breath on-line method of NO detection. In both groups of patients, FE_{NO} and V_{NO} were highly flow-dependent ($P < 0.0001$, Friedman's test). The differences between asthmatics and allergic rhinitis patients in FE_{NO} and V_{NO} did not reach significance at any expiratory flow ($P > 0.05$, Dunn's multiple comparison test).

(P–T), the ratios showed normal or near-normal distribution with no relationship to the absolute values of the variables (data not shown). In the range of 50–250 ml/sec, the mean ratio P/T of $C_{A}NO$ values was significantly higher than one, indicating that method P produced

higher estimates of $C_{A}NO$ by 38% in asthmatics ($P < 0.001$) and by 23% in AR patients ($P < 0.005$), respectively. However, the ratio decreased as the lower limit of the range for flows increased in both asthmatics and AR patients. Methods P and T yielded comparable estimates of $C_{A}NO$ in the interval of 150–250 ml/sec (Fig. 4). In this interval, the mean ratio of $C_{A}NO$ values and the limits of the 95% confidence interval for the ratio (the limits of agreement) reached 1.03 (0.49–1.56) in asthmatics and 1.07 (0.55–1.59) in AR patients, respectively.

Unlike for $C_{A}NO$, the ratio P/T of the $J_{aw}NO$ values observed in asthmatics was significantly less than one in the range of 50–250 ml/sec. The estimated $J_{aw}NO$ using method P was 12% less than the result of method T ($P < 0.001$). The ratio P/T of $J_{aw}NO$ values increased with the increasing lower limit of the flow range ($P < 0.001$), and the methods produced similar results at higher flow ranges (Fig. 4). There was even more agreement between $J_{aw}NO$ estimates by methods P and T in patients with AR. In this group, the effect of the range for expiratory flows on the P/T ratio of $J_{aw}NO$ values was not significant ($P = 0.19$). In the highest interval for the expiratory flow, the mean ratio of $J_{aw}NO$ values and the limits of agreement were 1.03 (0.73–1.33) in asthmatics and 0.99 (0.90–1.10) in AR patients, respectively (Fig. 4).

Comparison of NO Parameters between Asthmatics and Allergic Rhinitis Patients

Regardless of the range of expiratory flows and the method of estimation used, there were no significant differences in the $C_{A}NO$ and $J_{aw}NO$ values between patients with asthma and AR (Table 3). However, due to the unequal effect of the range of expiratory flows in the groups under comparison, the percent differences in the median values changed considerably. The median

TABLE 2—The influence of the Range and Number of Expiratory Flows on the Estimates of the Alveolar Concentration ($C_{alv}NO$) and Bronchial Flux ($J_{aw}NO$) of NO Observed in Children With Atopic Asthma and Allergic Rhinitis Using the Linear Methods P and T

Method	Expiratory flows		Atopic asthma (n = 42)		Allergic rhinitis (n = 20)	
	Range (ml/sec)	n	$C_{alv}NO$ (ppb)	$J_{aw}NO$ (pL/sec)	$C_{alv}NO$ (ppb)	$J_{aw}NO$ (pL/sec)
P1	50–250	5	5.5 (2.9–11)	1,170 (655–2,260)	4.0 (2.7–5.5)	1,000 (600–1,270)
P2	100–250	4	3.2 (1.9–5.5) ^{P1}	1,610 (990–3,260) ^{P1}	3.0 (2.2–4.4)	1,022 (681–1,570)
P3	150–250	3	1.8 (0.8–4.3) ^{P1, P2}	1,800 (962–3,380) ^{P1, P2}	2.4 (0.8–3.9) ^{P1}	1,175 (825–1,962) ^{P1}
P-value*			<0.0001	<0.0001	<0.005	<0.01
T1	50–250	5	4.3 (2.2–8.3)	1,310 (758–2,590)	3.2 (2.6–4.3)	1,010 (551–1,390)
T2	100–250	4	2.7 (1.6–5.1) ^{T1}	1,580 (998–3,340) ^{T1}	3.0 (2.1–3.9)	1,070 (604–1,620)
T3	150–250	3	1.7 (0.9–3.9) ^{T1, T2}	1,800 (950–3,560) ^{T1, T2}	2.3 (0.8–3.7) ^{T1}	1,180 (639–1,950) ^{T1}
P-value*			<0.0001	<0.0001	<0.01	<0.01

The data are shown as the median (inter-quartile range); n: The number of expiratory flows.

^{P1, P2, T1, T2}: Significant differences ($P < 0.05$) indicated by the Dunn's post-test.

*P-value from the Friedman's test with the range for flows as a fixed effect.

TABLE 3—The Influence of the Range and Number of Expiratory Flows on the Standard Errors (SE) of the Estimates of Alveolar Concentration ($C_{\text{alv}}\text{NO}$) and Bronchial Flux ($J_{\text{aw}}\text{NO}$) of NO Observed in Children With Atopic Asthma and Allergic Rhinitis Using the Linear Methods P and T

Method ^a	Expiratory flows		Atopic asthma (n = 42)			Allergic rhinitis (n = 20)		
	Range (ml/sec)	n	r	$C_{\text{alv}}\text{NO}$ SE (%)	$J_{\text{aw}}\text{NO}$ SE (%)	r	$C_{\text{alv}}\text{NO}$ SE (%)	$J_{\text{aw}}\text{NO}$ SE (%)
P1	50–250	5	0.98 (0.95–0.99)	18 (13–26)	7.8 (4.7–12)	0.98 (0.97–0.99)	18 (11–24)	6.9 (5.0–8.1)
P2	100–250	4	0.98 (0.96–0.99)	27 (17–39) ^{P1}	7.2 (5.3–13)	0.98 (0.96–0.99)	17 (14–42)	8.2 (5.1–12) ^{P1}
P3	150–250	3	0.97 (0.94–0.99)	52 (30–112) ^{P1, P2}	13 (7.4–19) ^{P1, P2}	0.97 (0.92–0.99)	33 (26–55) ^{P1, P2}	12 (7.5–26) ^{P1}
P-value*			<0.01	<0.0001	<0.0001	0.06	<0.0001	<0.001
T1	50–250	5	0.91 (0.86–0.94)	19 (14–28)	9.7 (7.4–13)	0.94 (0.89–0.96)	14 (11–25)	9.0 (6.4–12)
T2	100–250	4	0.89 (0.84–0.93)	26 (18–56) ^{T1}	7.7 (6.0–16)	0.91 (0.89–0.95)	20 (14–39) ^{T1}	12 (5.6–16)
T3	150–250	3	0.84 (0.80–0.91)	52 (31–100) ^{T1, T2}	13 (7.5–24) ^{T1, T2}	0.88 (0.83–0.92)	35 (26–63) ^{T1, T2}	14 (7.8–30) ^{P1}
P-value*			<0.0001	<0.0001	<0.001	<0.005	<0.0001	<0.005

The data are shown as the median (inter-quartile range).

n: The number of expiratory flows, r: The Pearson's correlation coefficient, SE: The standard error of the estimate.

P1, P2, T1, T2: Significant differences ($P < 0.05$) indicated by the Dunn's post-test.

*P-value from the Friedman's test with the range for flows as a fixed effect.

^aLinear methods P and T applied in three ranges for expiratory flow.

$C_{\text{A}}\text{NO}$ obtained in asthmatics using method P and five flow rates (50–250 ml/sec) exceeded by 34% that of AR patients, while estimates using the three highest flow rates (150–200–250 ml/sec) generated a 26% lower median. For the median $J_{\text{aw}}\text{NO}$, the difference between asthmatics and AR patients reached 17% and

it further increased to 53% if the expiratory flows of 150–200–250 ml/sec were used in the estimation. The range of expiratory flows exerted the same influence on the between-group differences in the median $C_{\text{A}}\text{NO}$ and $J_{\text{aw}}\text{NO}$ values obtained using method T (data not shown).

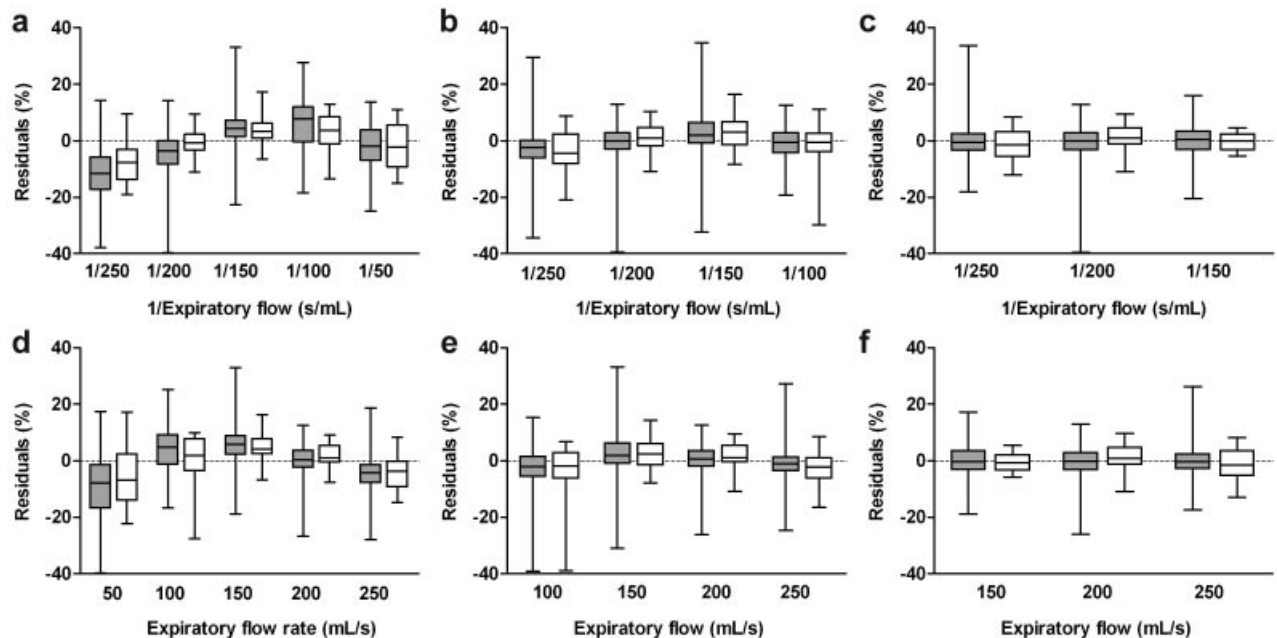


Fig. 3. The percent differences (y-residuals) between (a–c) the observed values of FE_{NO} and those predicted from the linear regression model P and (d–f) between the observed values of V_{NO} and those predicted from the model T in the three ranges for expiratory flows under comparison. In (a–e), the residuals displayed a bowed pattern and they were flow-dependent according to the results of the Friedman's test performed separately for the patients with atopic asthma (filled boxes, $P \leq 0.0001$) and allergic rhinitis (clear boxes, $P \leq 0.002$). When the lower limit of the range increased to 150 ml/sec (c,f) the differences between y-residuals became insignificant both in asthmatics ($P \geq 0.85$) and AR patients ($P \geq 0.12$).

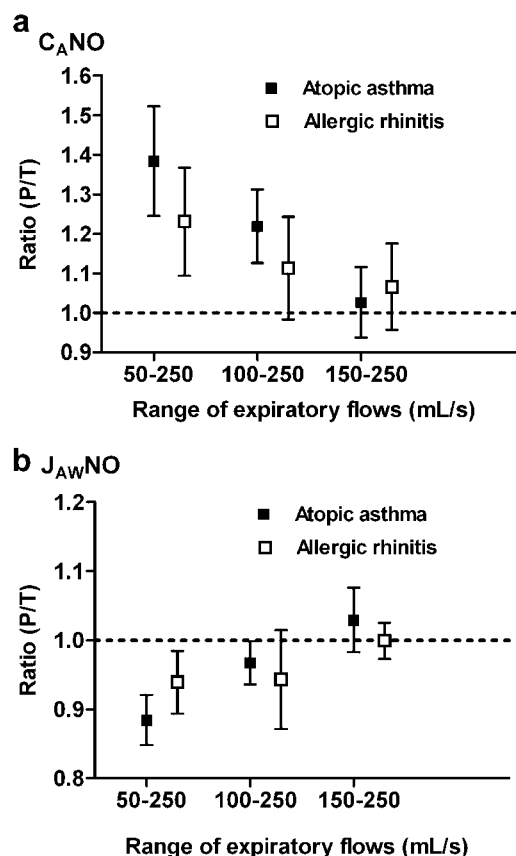


Fig. 4. Within-subject ratios (P/T) of the estimates of (a) $C_{A}NO$ and (b) $J_{aw}NO$ obtained with methods P and T in the three ranges for expiratory flow. The arithmetic means and 95%-confidence intervals for the means are shown. The analysis of variance for repeated measurements indicated a significant effect of the range for flows on the ratios of $C_{A}NO$ values in asthmatics ($P < 0.001$) and allergic rhinitis patients ($P < 0.05$) and on the ratios of $J_{aw}NO$ values in asthmatics ($P < 0.001$), while in allergic rhinitis patients, the range of flows had not significant effect on the ratio of $J_{aw}NO$ values ($P = 0.19$).

DISCUSSION

The present study evaluated two linear slope-intercept methods for estimation of NO-exchange parameters $C_{A}NO$ and $J_{aw}NO$ in school children and adolescents with AA and AR. For the first time, detailed testing of the condition of linearity was performed within an expiratory flow range of 50–250 ml/sec and the consequences of its violation were addressed. In both groups of patients and regardless of the modeling method, the condition of linearity was met only in the range of 150–250 ml/sec. Its violation at lower expiratory flows resulted in overestimation of $C_{A}NO$ and underestimation of $J_{aw}NO$, respectively. The values calculated in asthmatics in the full range of expiratory flows were on the average 2.8-fold higher ($C_{A}NO$) and 1.5-fold less

($J_{aw}NO$), respectively, than those obtained within the linear range. In AR patients, the magnitude of the difference was less both for $C_{A}NO$ (a 1.5-fold difference) and $J_{aw}NO$ (a 1.2-fold difference). As a result, the observed differences between AA and AR patients in the median values of the parameters under study were strongly influenced by the range of expiratory flows used for their estimation. Taken together, these findings clearly show that linear slope-intercept methods should not be applied outside the appropriate range for the expiratory flow.

The values of $C_{A}NO$ and $J_{aw}NO$ found in the present study agree well with those published previously. In a similar group of children and adolescents with well-controlled asthma receiving ICS, the means of $C_{A}NO$ and $J_{aw}NO$ were 5.5 ppb and 912 pl/sec when method T was applied in the range of 50–150 ml/sec.¹⁴ In a study by Paraskakis et al., children and adolescents performed exhalations at four rates between 50 and 260 ml/sec. After exclusion of the subjects in whom method T could not be used because of a serious violation of the linearity condition, the mean values of $C_{A}NO$ and $J_{aw}NO$ of the patients with well-controlled asthma were 2.3 ppb and 1,190 pl/sec, respectively.¹¹ Thus, the evidence from the literature also supports the conclusion that the range of expiratory flows used for modeling has a stronger influence on the estimates of $C_{A}NO$ than $J_{aw}NO$. Atopy and allergen exposure are major determinants of increased FE_{NO} levels which further augment in patients with asthma and decrease in response to ICS.^{22–24} Asthma was well controlled by ICS in our patients. In agreement with published studies, FE_{NO} values of asthmatics and AR patients overlapped to a large extent.^{22,25,26}

Other researchers were also confronted with the problem of departures from linearity while applying methods P and T. Mahut et al.^{16,27} suggested that for a valid calculation, the Pearson's correlation coefficient r of the relationship between V_{NO} and V should exceed its critical value for a given number of expiratory flows at $\alpha = 0.05$, i.e., 0.8 for six flows. The acceptance criterion was soft: Only the null hypothesis that the correlation coefficient equals zero had to be rejected. In another study, the condition of linearity was considered to be met when $r \geq 0.9$ in the linear regression of V_{NO} against four flows in the range of 30–200 ml/sec.²⁸

Our finding that deviations from linearity are greater in asthmatics than AR patients causing larger differences in the estimates of $C_{A}NO$ and $J_{aw}NO$ according to the particular range of flows is supported by reports of others. In a sample of schoolchildren, FE_{NO} was detected at four flows (30–50–100–300 ml/sec) and several linear as well as nonlinear modeling algorithms were used.²⁹ Quadratic regression models resulted in better fits to the data than did the methods P and T,

respectively. As an index of nonlinearity, the authors calculated the difference between regression slopes (SLPDIF) estimated by method T using the two lowest (30 and 50 ml/sec) and two highest (100 and 300 ml/sec) expiratory flows. The value of SLPDIF was positively correlated with FE_{NO50} and $J_{aw}NO$ being higher in children with asthma symptoms than in healthy children and children with asthma in the past.²⁹ Another large study with asthmatic and healthy children showed that the failure of linear method T to fit the data was associated with higher FE_{NO50} and lower FEV_1 .¹¹

Results of the methods P and T did not differ in the linear range of 150–250 ml/sec whereas departures from linearity at lower flows caused shifts between the alveolar and bronchial components of exhaled NO. In the case of $J_{aw}NO$, the differences between methods were relatively minor. However, much less agreement in $C_A NO$ values was found if the estimates were generated in the range of 50–250 ml/sec. The mean ratios P/T of the $C_A NO$ and $J_{aw}NO$ values calculated in the range of 100–250 ml/sec agree well with those found by Linn et al.²⁹ (1.14 and 0.96, respectively) in an unselected population of school children investigated in the range of 100–300 ml/sec.

Importantly, methods P and T showed similar performance as to the precision of estimates. The median SEs were small for $J_{aw}NO$ (<15%) regardless of the range of flows. The precision of $C_A NO$ estimates was less in all ranges. In the range of 150–250 ml/sec, the SEs of the estimates generated by both methods exceeded 50% in asthmatics and 30% in AR patients, respectively. The precision was poor despite the best fulfillment of the linearity condition indicated by the analysis of residuals and the r-values exceeding 0.84. The Pearson's correlation coefficient is widely used to examine the strength of a linear relationship between two variables but it can be misleading if used as a sole criterion for linearity. Besides the deviation from linearity, other factors influenced its value such as the precision of FE_{NO} and V measurements as well as the range of V (the variance of X). The increase in the lower limit of the range for flows resulted in improved linearity. At the same time, however, the correlation coefficient deteriorated. Presumably, small errors in V and FE_{NO} measurements exerted a concomitant negative influence that brought about deterioration in the precision of $C_A NO$ estimates at such relatively narrow interval of higher expiratory flows. The r-values were lower for method T in all ranges for expiratory flow, but deviation from linearity was similar to that of method P, as were the SEs of the estimates.

Results of this study support the view that linear modeling methods P and T require several expiratory flows ≥ 100 ml/sec in order to accurately estimate $C_A NO$ and $J_{aw}NO$ in schoolchildren and adolescents

with asthma and AR. The inclusion of 50 ml/sec caused departures from linearity in a majority of patients as evidenced by large shifts in $C_A NO$ as well as by the apparent flow-dependency of residuals. Data from previous studies with similar populations and the present study show that most patients are able to exhale at the controlled flow as high as 250–300 ml/sec.^{11,16} Modeling using only two expiratory flows is discouraged as verification of linearity is not possible. Instead, individual selection of the appropriate range of expiratory flows is recommended. The below described stepwise procedure for detecting nonlinearity and evaluating the quality of FE_{NO} measurements could replace the Pearson's correlation coefficient. In the first step, the plots of V_{NO} against V (method T) or FE_{NO} against $1/V$ (method P) should be visually inspected for nonlinearity. In the second step, linear regression analysis should be performed using all expiratory flows and should be repeated after the exclusion of the lowest one. This procedure gives the possibility to evaluate the decrease in $C_A NO$ caused by nonlinearity. If a large decrease is observed, the lowest expiratory flow should be excluded and the entire procedure repeated from the first step. A less stringent tolerance limit for the decrease (possibly 20%) is advisable because the precision of estimates is higher if the number and range of flows is at its possible maximum. Finally, the errors of duplicate readings of FE_{NO} (V_{NO}) at each measured level of V should be checked using the percent residuals and additional exhalations requested in case of a larger difference from the predicted value (>10%).

In the present study, the guidelines for FE_{NO} measurement were followed with one exception only. In order to limit the total number of exhalations, the stable plateau values of FE_{NO} from two successive measurements at each expiratory flow were allowed to differ by a maximum of 15% rather than 10%. The repeatability of the FE_{NO} measurement was good. The flow meter was calibrated on each study day and the control of expiratory flow was adequate. The limitation is that other groups were not studied, namely healthy children, steroid-naïve asthmatics, or patients with poor control of asthma despite therapy. The investigation was age-limited due to the fact that multiple-flow NO measurements are less feasible in children younger than six.²⁸ It is a drawback that subjects were evaluated on only one occasion and that short-term repeatability of $C_A NO$ and $J_{aw}NO$ measurements could not be assessed.

In conclusion, violation of the linearity condition at lower expiratory flows causes overestimation of $C_A NO$ and underestimation of $J_{aw}NO$ by linear methods P and T, shifts between the methods with regard to the partition of exhaled NO into alveolar ($P > T$) and bronchial ($T > P$) contributions as well as biased results of the comparison of the NO-exchange parameters between

asthmatics and AR patients, with $C_{A}NO$ being more sensitive to the above effects than $J_{aw}NO$. In the linear range, there are no differences between modeling methods P and T in the estimates of the parameters and their SEs. It is recommended that several expiratory flows ≥ 100 ml/sec are used to accurately estimate $C_{A}NO$ and $J_{aw}NO$ in children and adolescents with AA and AR. After its verification in future studies, the suggested stepwise procedure for detection of nonlinearity and evaluation of the quality of NO measurements should be used instead of the Pearson's correlation coefficient.

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