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A Novel Method of Measuring Fractional Exhaled Nitric Oxide in Tracheostomized Ventilator-Dependent Children

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BACKGROUND: The lower airway concentration of fractional exhaled nitric oxide (F_{ENO}) is unknown in children with chronic lung disease of infancy who have tracheostomy for long-term mechanical ventilation. We aimed to evaluate an online method of measuring F_{ENO} in a cohort of ventilator-dependent children with a tracheostomy and to explore the relationship between the peak F_{ENO} concentration (F_{ENO} peak) and the degree of respiratory support using the respiratory severity score. METHODS: We conducted a prospective cross-sectional study in 31 subjects who were receiving long-term respiratory support through a tracheostomy. We measured the F_{ENO} peak and F_{ENO} plateau concentration from the tip of the tracheostomy tube using a nitric oxide analyzer in subjects during a quiet state while being mechanically ventilated. We obtained 2 consecutive 2-min duration measurements from each subject. The F_{ENO} peak, exhaled NO output (equal to the F_{ENO} peak × minute ventilation), and pulmonary NO excretion (exhaled NO output/weight) were calculated and correlated with the respiratory severity score. RESULTS: The median F_{ENO} peak was 2.69 ppb, and the median F_{ENO} plateau was 1.57 ppb. The coefficients of repeatability between the 2 consecutive measurements for F_{ENO} peak and F_{ENO} plateau were 0.74 and 0.59, respectively. The intraclass coefficient between subjects within the cohort was 0.988 (95% CI 0.975–0.994, $P < .001$) for F_{ENO} peak and 0.991 (95% CI 0.982–0.996, $P < .001$) for F_{ENO} plateau. We found that the F_{ENO} peak was directly correlated with minute ventilation, but we did not find a direct relationship between the F_{ENO} peak concentration, exhaled NO output, or pulmonary NO excretion and respiratory severity score. CONCLUSIONS: F_{ENO} peak and plateau concentration can be measured online easily with a high degree of reliability and repeatability in infants and young children with a tracheostomy. F_{ENO} peak concentration from the lower airway is low and influenced by minute ventilation in children receiving mechanical ventilation. *Key words: tracheostomy; fractional exhaled nitric oxide; chronic lung disease of infancy.* [Respir Care 0;0(0):1–•. © 0 Daedalus Enterprises]

Introduction

Fractional exhaled nitric oxide (F_{ENO}) has been studied in many pulmonary diseases, including asthma, COPD, cystic fibrosis, primary ciliary dyskinesia, and pulmonary arterial hypertension.[1,2] In patients with asthma, F_{ENO} is now used as a biomarker of eosinophilic airway inflammation to diagnose, to monitor response and adherence to anti-inflammatory medications, and to predict upcoming exacerbations.[3] Studies measuring F_{ENO} in infants with respiratory distress syndrome, bronchopulmonary dyspla-

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sia (BPD), and chronic lung disease of infancy have yielded conflicting results.[4-8] The reasons for the variations in F_{ENO} include differences in patient population, timing of measurement in relation to the disease process and inhaled medication administration, use of different interface techniques, and other measuring conditions such as tidal breathing parameters and variable exhalation flow. In 2005, the American Thoracic Society and the European Respiratory Society jointly published recommendations[9] for standardized procedures for online and offline measurement of exhaled lower respiratory NO and nasal NO.

To our knowledge, F_{ENO} measurement in tracheostomized ventilator-dependent children with chronic lung disease of infancy has not been reported in the literature. A small study in adult subjects with a tracheostomy ($N = 5$) found that the baseline F_{ENO} was 4 times higher during oral exhalation compared with tracheal exhalation (16 ± 2 ppb vs 4.6 ± 0.8 ppb, $P < .05$), suggesting substantial contribution from the upper airways.[10] More recently, F_{ENO} was also found to be very low when measured from a tracheostomy tube in adult subjects ($N = 14$) after total laryngectomy (4 ppb, range 1–22) compared with healthy controls (21 ppb, range 9–41).[11] Contamination by nasal nitric oxide greatly affects the F_{ENO} level; therefore, it is essential to exclude the upper airways when determining F_{ENO}.

In this study, we evaluated a novel method of measuring F_{ENO} in a cohort of tracheostomized ventilator-dependent children with chronic lung disease of infancy. Second, we attempted to correlate the F_{ENO} concentration with demographic characteristics and degree of mechanical respiratory support at the time of measurement by calculating the respiratory severity score. We hypothesized that our method of measuring F_{ENO} would be feasible and reliable and that the F_{ENO} concentration measured from the tip of the tracheostomy tube would be low in ventilator-dependent children with chronic lung disease of infancy.

QUICK LOOK

Current knowledge

In patients with asthma, Fractional exhaled nitric oxide (F_{ENO}) is used as a biomarker of eosinophilic airway inflammation to diagnose, to monitor response and adherence to anti-inflammatory medications, and to predict upcoming exacerbations. Studies measuring F_{ENO} in infants with bronchopulmonary dysplasia and chronic lung disease of infancy have yielded conflicting results. Additionally, the lower airway concentration of F_{ENO} is unknown in children with a tracheostomy.

What this paper contributes to our knowledge

F_{ENO} concentration in ventilator-dependent children with chronic lung disease of infancy who have a tracheostomy was measured easily with a high degree of reliability and repeatability. The F_{ENO} concentration measured from the tip of the tracheostomy tube was low. F_{ENO} peak concentration from the lower airway was affected by minute ventilation in ventilator-dependent children with a tracheostomy.

Methods

Study Design and Subjects

We conducted a prospective cross-sectional study from February 1, 2013 to May 31, 2014 and enrolled 31 subjects from a cohort of infants with chronic lung disease of infancy who had tracheostomy for long-term mechanical ventilation. The diagnosis of chronic lung disease of infancy was based on the American Thoracic Society definition as a heterogeneous group of respiratory diseases that begin in the neonatal period, which includes BPD in premature infants and other chronic pulmonary conditions in term newborns that result from meconium aspiration, pneumonia, pulmonary hypoplasia, persistent pulmonary hypertension, congenital diaphragmatic hernia and tracheoesophageal fistula, congenital cardiac disease, and congenital neuromuscular disorders.[12] Those infants who remained hospitalized were recruited while they were being cared for in the ICU, whereas those infants who were discharged home were recruited during their clinic visit to the infant home ventilator clinic. Our reported rate of BPD is 45% for infants with a birthweight of <1,500 g. The rate of tracheostomy in our patients with severe BPD is higher because of the inherent population selection bias due to the fact that we are a level IV regional referral hospital where ventilator-dependent infants are being transferred for evaluation for tracheostomy and chronic home ventilation. To be included, subjects had to be ventilator-dependent through a tracheostomy tube and clinically stable on a laptop ventilator, LTV series 950 or 1150 (CareFusion, San Diego, California). Those patients whose ventilator dependence was due to abnormal control of breathing (eg, central hypoventilation syndrome, severe post-hypoxic ischemic encephalopathy) or peripheral neuromuscular disorders (eg, spinal muscular atrophy) were excluded. Additionally, those infants with a diagnosis of cystic fibrosis or primary ciliary dyskinesia confirmed by genetic testing as documented in the electronic medical records were also excluded. Eligible infants who had received escalated respiratory support within 48 h before F_{ENO} measurement as well as those who had received inhaled nitric oxide within the previous 48 h were likewise excluded. Our study was approved by the institutional review board

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at Children's Mercy Hospitals and Clinics, and written informed consent was obtained from each family.

F_{ENO} Measurement

We measured F_{ENO} concentration using the online Sievers nitric oxide analyzer (NOA 280i, GE Analytical Instruments, Boulder, Colorado). The NOA 280i was calibrated according to Sievers' guidelines with 45-ppm nitric oxide gas (Ikaria Therapeutics, Clinton, New Jersey) and NO-free medical-grade air (Airgas, Merriam, Kansas) passed through a zero gas filter (GE Analytical Instruments). Sievers' NOA 280i breath software (version 3.21) facilitated data collection and processing. Sievers' NOA specifications for gas samples had sensitivity = 0.5 ppb (range 0.5 ppb to 500 ppm) and repeatability = ±5%.[13] A 20-cm-long NO sampling catheter (GE Analytical Instruments) was connected to the NOA 280i intake line. The expired gas from the subject reached the NOA 280i via this sampling catheter, which was inserted snugly through the suction catheter opening in the adapter to a predetermined length in the tracheostomy tube. Expired gas from the subject was directed to the analyzer at a constant flow of 40 mL/s. Ambient air was recorded before measuring F_{ENO} in each subject. There was no correlation between environmental NO and F_{ENO} peak ($r = .29$, $P = .20$).

Before and during F_{ENO} measurements, subjects maintained respiratory support from their ventilator, inhaling NO-free medical-grade air containing <1 ppb of NO. The F_{IO_2} was maintained constant through both measurements. To avoid NO contributions from nasal and oral cavities, F_{ENO} was measured at the tip of the tracheostomy tube with the cuff inflated around the tracheostomy tube. The measurement was taken while the subject was awake and quiet according to American Thoracic Society recommendations. The measurement was performed ≥6 h after treatment with a β_2 agonist and/or a corticosteroid inhaler, to avoid airway caliber changes. F_{ENO} peak and F_{ENO} plateau concentrations were measured 32 times/s over 2 min, and the mean peak and mean plateau values were calculated by the Sievers software program. The F_{ENO} unit was expressed in ppb. Two serial measurements were taken for each subject with several minutes (2–10 min) of washout period between measurements. The exhaled NO output was calculated from the product of F_{ENO} peak and the minute ventilation measured directly from the ventilator. The pulmonary NO excretion was calculated using the formula, pulmonary NO excretion = exhaled NO output/weight.

At the time of sampling, we recorded relevant demographic information, respiratory support (mean airway pressure), F_{IO_2}, end-tidal carbon dioxide, and exhaled minute ventilation. The respiratory severity score was calculated as mean airway pressure multiplied by F_{IO_2}.

Statistical Analysis

Data analysis was done using SPSS 20 (IBM Corp, Armonk, New York) and SAS 9.4 (SAS Institute, Cary, North Carolina). Quantitative data were expressed with mean and SD or median and interquartile range. Categorical variables were reported as *n* (%). We compared 2 consecutive measurements of F_{ENO} levels to determine the reliability and repeatability of our method. The reliability and repeatability of the F_{ENO} measurements were expressed as coefficient of repeatability, intraclass correlation coefficient, and the Bland-Altman limits of agreement. The relationships between F_{ENO} peak, exhaled NO output, pulmonary NO excretion, and respiratory severity score as well as the demographic and respiratory variables were examined using the Spearman correlation coefficient. A significance level of $P < .05$ was used for all tests.

Results

Among 36 eligible patients, 31 participated in the study. One parent declined, and 4 patients in state custody did not participate, because their legally authorized representatives declined to provide permission. The subjects were enrolled between February 2013 and May 2014. All subjects were awake and quiet during the 2 consecutive measurements except for one subject. The F_{ENO} concentration from this subject, who was calm during the first sampling but crying during the second, showed very disparate values; therefore, these data were excluded from the data analysis. Of the 30 subjects whose data were included in the analysis, 60% (18 of 30) were white, 23% (7 of 30) were black, and 17% (5 of 30) were Hispanic or Asian. The ratio of males to females was 1:1. The median gestational age at birth was 30 weeks (interquartile range, 24.75–37.00 weeks), and the median birthweight was 1.38 kg (interquartile range, 0.70–2.76). All 30 subjects were broadly categorized to have chronic lung disease of infancy. Two thirds (67%) had a primary diagnosis of severe BPD, and half of them had co-existing bronchomalacia. The diagnosis of bronchomalacia was based on bronchoscopic findings performed by an ear-nose-throat specialist and/or by tracheobronchography performed by interventional radiologists.[14] Associated comorbidities that were not the reason for their ventilator dependence included congenital cardiac (17%), neurologic (7%, in the form of post hemorrhagic hydrocephalus, periventricular leukomalacia, and cerebral palsy), genetic (7%), and other congenital anomalies (13%). All subjects had tracheostomy performed for chronic assisted ventilation and had been ventilator-dependent for a minimum of 2 months before the study. The median age at

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tracheostomy was 3 months for the cohort. The median age at the time of study enrollment (F_{ENO} measurement) was 10.5 months, whereas the median weight was 7.96 kg. All subjects were treated chronically with inhaled corticosteroid, 61% were receiving twice daily β_2 agonist bronchodilator, and 16% were receiving phosphodiesterase-5 inhibitor (sildenafil) for pulmonary hypertension. Demographic information and ventilator support at the time of F_{ENO} measurement are presented in Table 1. We found that the median F_{ENO} peak was 2.69 ppb (interquartile range, 2.05–4.00) and the median F_{ENO} plateau was 1.57 ppb (interquartile range, 1.07–2.77), as shown in Table 2.

Table 1. Demographic Characteristics and Respiratory Support at the Time of the Study

Characteristics	Values
Gestational age at birth, median (IQR) weeks	30 (24.75–37.00)
Birthweight, median (IQR) kg	1.38 (0.70–2.76)
Male, %	50
Caucasian, %	60
BPD, %	67
Age at tracheostomy, median (IQR) months	3 (2)
Age at F_{ENO} measurement, median (IQR) months	10.5 (7.00–15.75)
Weight at F_{ENO} measurement, median (IQR) kg	7.96 (5.70–10.08)
Height at F_{ENO} measurement, median (IQR) m	0.68 (0.59–0.78)
Inhaled corticosteroids, % daily use	100
Inhaled β_2 agonist, % daily use	61
Phosphodiesterase-5 inhibitor, % daily use	16
$\bar{P}_{aw}$, median (IQR) cm H_2O	14.0 (10.00–15.25)
F_{IO_2}, median (IQR)	0.23 (0.21–0.30)
P_{ETCO_2}, median (IQR) mm Hg	40 (37–44)
$\dot{V}_E$, median (IQR) L/min	3.31 (2.81–3.89)
RSS, median (IQR)	3.13 (2.10–4.50)

$N = 30$.
IQR = interquartile range
BPD = bronchopulmonary dysplasia
F_{ENO} = fraction of exhaled nitric oxide
$\bar{P}_{aw}$ = mean airway pressure
P_{ETCO_2} = end-tidal carbon dioxide
$\dot{V}_E$ = minute ventilation
RSS = respiratory severity score

The individual F_{ENO} concentrations at first and second sampling, for both F_{ENO} peak and F_{ENO} plateau, are presented in Appendix 1 (see the supplementary materials at http://www.rcjournal.com).

We determined that the coefficient of repeatability for F_{ENO} peak and F_{ENO} plateau were 0.74 and 0.59, respectively. Repeatability was further determined by using the intraclass correlation coefficient, which was 0.988 (95% CI 0.975–0.994, $P < .001$) for F_{ENO} peak and 0.991 (95% CI 0.982–0.996, $P < .001$) for F_{ENO} plateau. In addition, we generated Bland-Altman plots showing the 95% limits of agreement between the 2 sets of measurements for both F_{ENO} peak and F_{ENO} plateau, as shown in Figure 1.

Each of the demographic and respiratory variables was correlated with F_{ENO} concentration. We found a direct correlation only between F_{ENO} concentration (F_{ENO} peak and F_{ENO} plateau) and minute ventilation, as shown in Figure 2. The maximum minute ventilation was 6.3 L/min. Upon review of this particular subject, this measurement

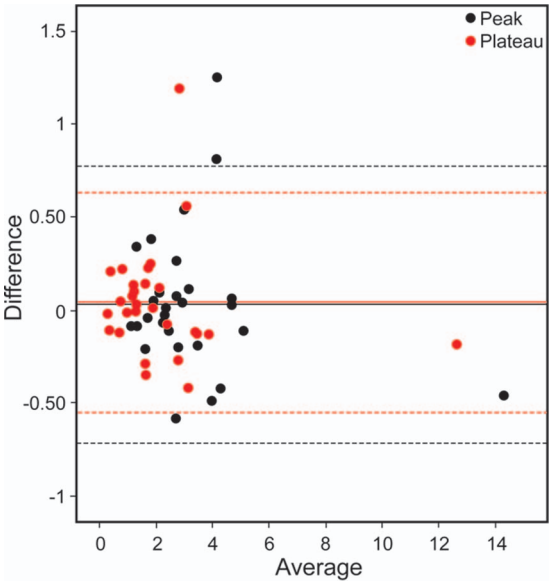

Fig. 1. Bland-Altman plot analysis for the repeatability of fractional exhaled nitric oxide (F_{ENO} peak and plateau) measurements. Dashed lines show the 95% limits of agreement.

Table 2. Fraction of Exhaled Nitric Oxide Peak and Plateau Concentrations for All Subjects

Parameters	F_{ENO} Peak (1)	F_{ENO} Peak (2)	F_{ENO} Peak (Average of 2 Serial Measurements)	F_{ENO} Plateau (1)	F_{ENO} Plateau (2)	F_{ENO} Plateau (Average of 2 Serial Measurements)
Median	2.53	2.62	2.69	1.44	1.51	1.57
IQR	2.10–3.79	2.00–3.59	2.05–4.00	1.10–2.69	1.04–2.47	1.07–2.77
Minimum to maximum	1.05–14.04	1.12–14.50	1.10–14.27	0.29–12.51	0.28–12.69	0.31–12.60

$N = 30$.
F_{ENO} = fraction of exhaled nitric oxide
IQR = interquartile range

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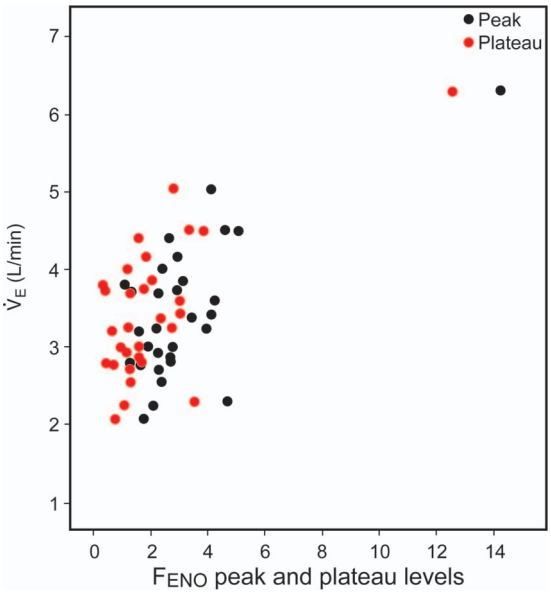

Fig. 2. Correlation between fractional exhaled nitric oxide (F_{ENO} peak and F_{ENO} plateau) and minute ventilation ($\dot{V}_E$). The Spearman coefficient of correlation was 0.453, $P = .01$.

Table 3. Spearman's Rho for Fraction of Exhaled Nitric Oxide Peak, Exhaled Nitric Oxide Output, and Pulmonary Nitric Oxide Excretion Versus Respiratory Severity Score

Relationships	Correlation Coefficient	*P* (2-tailed)
F_{ENO} peak versus RSS	0.182	.34
Exhaled nitric oxide output versus RSS	0.115	.55
Pulmonary NO excretion versus RSS	0.255	.17

F_{ENO} peak = fraction of exhaled nitric oxide peak concentration
RSS- respiratory severity score = mean airway pressure multiplied by fraction of inspired oxygen (F_{IO_2})
Exhaled nitric oxide output = fraction of exhaled nitric oxide peak concentration × minute ventilation
Pulmonary NO excretion = exhaled nitric oxide output/weight

was found to be consistent and accurate. This same subject was also found to have the highest F_{ENO} peak and plateau values. Table 3 shows the correlation between F_{ENO} peak concentration, exhaled NO output, and pulmonary NO excretion versus respiratory severity score using the Spearman correlation coefficients and the corresponding *P* values, suggesting no direct relationship between F_{ENO} peak concentration, exhaled NO output, or pulmonary NO excretion and respiratory severity score.

Discussion

We present a novel method of measuring the concentration of F_{ENO} in a cohort of infants and children with a tracheostomy who were receiving long-term ventilatory support for chronic lung disease of infancy, the majority of whom had severe BPD. We measured the F_{ENO} concentration online from the tip of the tracheostomy tube in a closed circuit, with the tracheostomy cuff inflated at the time of measurement to prevent air from leaking around the tube. This method effectively eliminated any potential contribution of nasal exhaled NO. Additionally, NO-free air was provided through the closed circuit to eliminate environmental contamination. These measurements were performed with a fast-response chemiluminescent NOA that sampled NO concentrations continuously at a constant flow of 40 mL/s. The NOA 280i measured NO accurately to 1 ppb and had a 90% response time of 400 ms.

We obtained 2 consecutive measurements while subjects were awake, quiet, and breathing spontaneously while receiving assisted ventilation (mechanical breaths plus spontaneous breaths with pressure support). Comparison of 2 consecutive F_{ENO} concentrations, for both F_{ENO} peak and F_{ENO} plateau, showed a very high degree of agreement and repeatability. Theoretically, the repeatability of measurements refers to the variation in repeat measurements made on the same subject under identical conditions.[15] We used the same instrument, the same method, and the same observers for all measurements. The measurements were made over a short period of time, over which the underlying value can be considered to be constant. Our measurements were separated by several minutes (2–10 min) of washout time between the 2 samplings. We estimated that this period of time was enough to allow for any potential variation in conditions during the measurement. Our repeat measurements had minimal mean differences on the paired sample *t* test. In other words, the measurements we obtained had low variability with very little error that can be ascribed to the measurement process itself.

The repeatability of F_{ENO} concentration as measured by our method was assessed in 2 ways: first, by comparing the F_{ENO} concentrations between the subjects within the group using the intraclass correlation coefficient, and second, by using Bland-Altman analysis. We found that 99% of the variability in our measurements was estimated to be due to genuine differences in the F_{ENO} peak and F_{ENO} plateau, with the remaining 1% attributable to errors in the measurement process. These intraclass coefficients support very convincingly that the F_{ENO} peak and plateau concentrations as measured by our method in this cohort of infants with a tracheostomy were extremely reliable, with almost zero measurement errors. The Bland-Altman analysis was done by plotting the difference between the 2 measurements on the y axis and the average of the measurements on the x axis. The 95% limits of agreement, as shown by the dotted lines, are narrow, confirming the high degree of repeatability of F_{ENO} measurements using our method.

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We performed our measurements during the daytime hours, either in the morning or in the afternoon, on the same day. A study that measured F_{ENO} in adults and children that used the NIOX system showed that the F_{ENO} concentrations were free from diurnal and day-to-day variation.[16] We obtained 2 samples over a 2-min period and used a computer software program to calculate the F_{ENO} concentration, eliminating the potential effect of single breath-to-breath variation in F_{ENO} levels.

We reported both plateau and peak F_{ENO} concentrations. Traditionally, when measurements are done at the mouth level, plateau concentrations must be reported to avoid exhaled NO contamination from the upper airway. Due to an infant's short expiratory times during tidal breathing, the Sievers nitric oxide analyzer (NOA 280i, GE Analytical Instruments, Boulder, Colorado) calculates the "plateau" as the average NO value detected between one half and seven eighths of the duration of expiration. Because tracheal levels are free from contamination of exhaled NO produced in the upper airway, we suggest that both peak and plateau concentrations should be reported when measurements are taken below the vocal cords (tracheostomy or at the end of the endotracheal tube). This will allow comparison of exhaled NO concentrations measured by devices that may calculate plateau concentrations in a different way. It is notable that there were 3 subjects (see Appendix 1, subjects 1, 2, and 18) in our cohort with consistently low F_{ENO} plateau concentrations below the threshold of detection for the device (<0.5 ppb), and yet their F_{ENO} peak concentrations were consistently >1.0 ppb.

The final concentration of exhaled NO is dependent on 3 main factors: (1) respiratory epithelial endogenous NO production and secretion onto the lower airways; (2) reabsorption of NO into the capillaries perfusing the lower airways; and (3) lower airway air exchange resulting in removal of NO present in the airway lumen.[17] Without taking into account upper-airway NO production, the balance among these 3 variables determines lower airway exhaled NO level. This explains why an increase in air flow can significantly decrease and a breath-hold can significantly increase exhaled NO concentrations.[18]

In this cohort, the measured F_{ENO} concentrations are lower than previously reported in the literature.[5-8] We speculate that our method of measuring exhaled NO at the tracheostomy tip eliminated the contribution of NO from the upper respiratory tract. Additionally, all of our subjects were receiving inhaled corticosteroids, and studies in children with asthma who are treated with inhaled corticosteroid have shown significantly lower exhaled NO concentrations, reflecting decreased bronchial eosinophilic inflammation. [19] Furthermore, a significant proportion of subjects in our cohort have established BPD, and it has been reported that exhaled NO levels are low in this population.[20]

We explored the correlation between the F_{ENO} peak concentration, exhaled NO output, and pulmonary NO excretion and the respiratory severity score in our cohort of infants receiving assisted mechanical ventilation. Respiratory severity score is the product of mean airway pressure and F_{IO_2}. It is a practical tool to assess respiratory failure in later neonatal life, because usually at that time, there are no indwelling arterial lines to determine steady state oxygenation. The respiratory severity score therefore represents a steady state assessment and thus is a useful tool for the clinician to assess the severity of respiratory failure.[21] We did not find direct correlation between the F_{ENO} peak, exhaled NO output, and pulmonary NO excretion and the respiratory severity score. Additionally, the respiratory severity score has been used to categorize the degree of illness of premature infants at risk for developing BPD, and the results indicated that a respiratory severity score of >3.5 was associated with a higher incidence of death or BPD.[22] We found that the median respiratory severity score in our cohort was <3.5, suggesting a stable established form of chronic respiratory failure.

In this study, higher F_{ENO} peak levels were associated with larger minute ventilation. When we tried to clarify the effect of breathing frequency or tidal volume with this association, neither breathing frequency nor tidal volume alone correlated with peak F_{ENO} concentration, but the product of both (minute ventilation = breathing frequency $\times$ tidal volume) had a significant direct association.

Our study has both strengths and limitations. We were able to measure the F_{ENO} concentration in this cohort of ventilator-dependent children free of possible contamination from the upper airway and from the environment. Our method of measurement was easy, with a high degree of reliability and repeatability. We acknowledge that we did not stabilize the expiratory flow during measurement, since this would have required the use of additional equipment and adjustment of the ventilator settings during tidal breathing measurement. Although the patient population of our cohort was relatively homogeneous, the range of ages when the measurements were performed was relatively wide, which may suggest different stages of a chronic disease process. Finally, in our study, we performed a cross-sectional NO measurement at one point in time only, and our sample size was relatively small. Future studies of a larger patient population with longitudinal measurements may help to elucidate the significance of F_{ENO} concentration in ventilator-dependent infants and children with chronic lung disease of infancy. We speculate that in infants and children with chronic lung disease of infancy who are ventilator-dependent and are being treated chronically with inhaled corticosteroids, serial F_{ENO} levels may be used to individualize therapy.

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Conclusions

Measurement of F_{ENO} concentration in children with chronic lung disease of infancy, who have a tracheostomy and are ventilator-dependent, is feasible and easy to perform with a high degree of reliability and repeatability. The F_{ENO} concentration measured from the tip of the tracheostomy tube is low. Our study suggests that in addition to maintaining a stable flow, having stable minute ventilation is important when measuring F_{ENO} concentration in children receiving mechanical ventilation.

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