

Head-to-head comparison of single-breath and tidal-breath exhaled nitric oxide measurements

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BACKGROUND: Exhaled nitric oxide (eNO) is an endogenous gas involved in airway pathophysiology and is determined in orally exhaled air by various techniques. However, traditional single-breath technique (eNO_{SB}) requires active cooperation and is not always easily practicable (especially in young children); simpler techniques including tidal breathing measurements (eNO_{TB}) are not standardized. The aim of this study was to evaluate the possible correlation and correspondence between eNO_{SB} and eNO_{TB} and the impact of potential confounders in children with chronic adenotonsillar disease.

METHODS: Eighty-six children (mean age 8.7 ± 3.2 y) underwent eNO assessment by means of eNO_{SB} and eNO_{TB}. The correlation among eNO_{TB}, eNO_{SB}, and other potential confounders (i.e., gender, age, weight, height, BMI, and passive smoking exposure) were studied.

RESULTS: The analyses showed a poor correspondence between eNO_{SB} and eNO_{TB}, with the latter underestimating ($P < 0.001$) mean eNO values: 6.4 parts per billion (ppb) (95% confidence interval (CI): 8.4–11.4 ppb) vs. 9.8 ppb (95% CI: 5.6–7.3 ppb). A greater correlation was found between eNO_{SB} and eNO_{TB} in children younger than 6 y. Only eNO_{SB} and age predicted eNO_{TB} ($R^2 = 43.6\%$).

CONCLUSION: eNO_{TB} is not a good predictor of eNO_{SB} in children. Constant-flow eNO_{SB} is the technique of choice for eNO assessment in young children.

Nitric oxide (NO) is an endogenous gas involved in airway pathophysiology and is produced in the upper and lower airways as a result of the NO synthase (NOS) catalysis of L-arginine. It can be determined in orally exhaled and nasal air by means of various noninvasive techniques (1,2).

Orally exhaled NO (eNO) is mainly involved in important phlogistic pathways in the lower airways and can be considered a marker of inflammation in asthmatic patients (3). Increased eNO levels have been found in patients with allergic rhinitis (4) and upper respiratory tract viral infections (5) and reduced eNO levels have been found in patients with cystic fibrosis and primary ciliary dyskinesia (6). Furthermore, NO pathways are thought to be involved in the pathogenesis of chronic adenoidal disease because high nasal NO levels have been found in

children with adenoidal hypertrophy (7), whereas contrasting results have been found in children with chronic tonsillar disease (8,9).

The assessment of eNO is not used in routine pediatric practice because it is not always easily practicable to follow the international guidelines (1,2). Traditional eNO measurement of oral exhalation against resistance (the single-breath technique, eNO_{SB}) is considered the standard procedure by the American Thoracic Society (2) because it has several advantages: a constant flow is maintained, which is useful because eNO is a flow-dependent variable (10); exhalation from total lung capacity is important because the degree of lung expansion affects eNO values measured at the same flow rate (10); and contamination with nasal NO is avoided because of the induced velum closure when exhaling against resistance (10).

However, correct eNO_{SB} performance requires the active cooperation of patients, which is not always easily achieved, especially in the case of young children (1). For this reason, simpler but less standardized techniques have been introduced in an attempt to overcome the poor compliance of children, including tidal-breath eNO measurements (eNO_{TB}) with or without flow control, and offline reservoir sampling (1,11–13). Unfortunately, these do not provide accurate data because they have some methodological drawbacks, such as the use of uncontrolled expiratory flow and nasal NO contamination (1). Furthermore, previous studies (11–13) of eNO levels assessed by means of eNO_{SB} and eNO_{TB} with or without the use of sampling bags in infants or children with bronchopulmonary disease have yielded conflicting results.

The aim of this study was to evaluate the possible correlation and correspondence between eNO_{SB} and eNO_{TB} measurements and the impact of potential confounders in children with chronic adenotonsillar disease.

RESULTS

As shown in **Figure 1**, 184 children were considered eligible for enrollment, but the final analyses were based on the measurements of 86 children (53.5% males; mean age 8.7 ± 3.2 y) whose main characteristics are summarized in **Table 1**.

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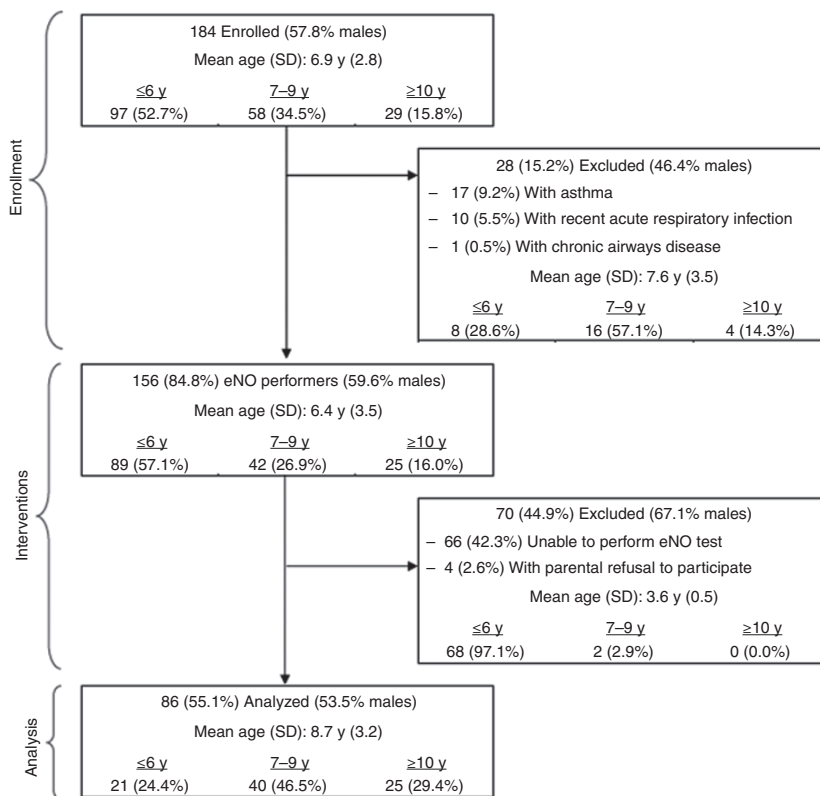


Figure 1. Study flowchart. eNO, exhaled nitric oxide.

Table 1. Main characteristics of the 86 pediatric patients

Variable	n	%
Gender		
Male	46	53.5
Female	40	46.5
Age (y)		
≤6	21	24.4
7-9	40	46.5
≥10	25	29.1
Passive smoking exposure		
No	68	79.1
Yes	18	20.9
	Median	Q ₁ -Q ₃
Weight (kg)	27	21.3-39.0
Height (cm)	130	116.0-142.0
BMI		
< -1 SD	25	29.1
-1 SD to +1 SD	40	46.5
> +1 SD	21	24.4
	Mean	CI _{95%}
eNO _{SB}	9.8	5.6-7.3
eNO _{TB}	6.4	8.4-11.4

CI_{95%}, 95% confidence interval; eNO_{SB}, exhaled nasal nitric oxide measured with single-breath technique; eNO_{TB}, exhaled nasal nitric oxide measured with tidal breathing technique; Q1, first quartile; Q3, third quartile.

Although the coefficient of variation was the same for both techniques (33.4%), the geometric mean of eNO_{TB} was significantly less than that of eNO_{SB}: 6.4 ppb (95% confidence interval: (CI) 8.4-11.4) vs. 9.8 ppb (95% CI: 5.6-7.3) (*P* < 0.001). The underestimates of the eNO_{TB} values in comparison with the eNO_{SB} values are shown in Figure 2: 76% of the eNO_{TB} values were under the bisectrix indicating perfect correspondence.

Only eNO_{SB} and age were predictors of eNO_{TB}; the *R*² of 43.6% suggests that the model explains slightly less than half of the variability of eNO_{TB}. The scatter plots of the values predicted by the final equations, ln(eNO_{TB}) = f[age, ln(eNO_{SB})] vs. ln(eNO_{SB}) and residuals vs. ln(eNO_{SB}), are shown in Figure 3 and confirm the underestimated eNO_{TB} values. They also show that the correlation between eNO_{SB} and eNO_{TB} values was higher in children younger than 6 y and that the discrepancy between the two techniques increased with age (Figure 4).

However, the differences between eNO_{SB} and eNO_{TB} led to the misclassification of normal and pathological values in every age class. When eNO was assessed using the tidal breathing technique, ~50% of the patients whose eNO_{SB} values were in the normal range would have been considered pathological cases, and 19% of those whose eNO_{SB} values were out of the normal range would have been considered normal. As a matter of fact, correct responses occurred in 55.8% of cases when the population was considered as a whole; when considered by age class, correct responses occurred in 52.4% of the children aged 4-7 y, in 52.5% of those aged 8-13 y, and in 64.0% of those aged 14-17 y.

DISCUSSION

The assessment of eNO levels could be very useful in clinical practice for diagnostic purposes and for monitoring respiratory diseases such as airway inflammation (3,6,14), adenoidal disease (7), and rhino-sinusitis (15). The international guidelines recommend eNO_{SB} as the “gold standard” technique (1) on the grounds of its accuracy and reproducibility (10). However, the active cooperation required to achieve the lasting expiratory force necessary to perform the test correctly makes it impossible in some patients, especially preschool children (16), as was found in ~45% of our enrolled patients who had a mean age of 3.6 ± 0.5 y.

Practical difficulties in assessing eNO_{SB} in pediatric populations have been previously reported by other investigators (11,17), including Jöbssis *et al.* (11) who found that 29% of 101 children with a mean age of 11.7 y were unable to perform the test. These difficulties have led to the introduction of simpler

but less standardized means of assessing eNO, including eNO_{TB} measurements (11,13), but these have the disadvantages of variable expiratory flow and the impossibility of excluding nasal NO (1,18).

We therefore evaluated the correlation and correspondence between eNO_{TB} and eNO_{SB} values in children, also considering the other variables known to influence eNO levels, such as gender, age, height, weight, BMI, and passive smoking exposure (2), in order to assess the possibility of using eNO_{TB} in pediatric clinical practice.

As expected (on the basis of the fact that both techniques evaluate eNO levels), our findings showed a certain correlation (good correlation occurred when eNO_{SB} and eNO_{TB} systematically varied together) between eNO_{SB} and eNO_{TB} in 86 children with chronic adenotonsillar disease (70% of whom were younger than 9 y).

However, our results showed that a good correspondence (good correspondence occurred when eNO_{SB} and eNO_{TB} gave the same response) between the eNO measurements was obtained neither when the population was analyzed as a whole nor when it was analyzed by age class.

In comparison with eNO_{SB}, eNO_{TB} systematically underestimated eNO values, as also shown by the statistically significant difference in their mean values of 9.8 and 6.4 ppb, respectively. Furthermore, the underestimated eNO_{TB} values seem to be more pronounced at higher values (see Figure 3), which is in line with the findings of Franklin *et al.* (12). The systematic underestimation of eNO values by eNO_{TB} is not easily interpreted and should be considered cautiously. Contamination with nasal NO, which is increased in children with adenoidal disease (7), should theoretically increase rather than decrease the eNO levels measured during tidal breathing. However, it must be remembered that eNO levels vary with flow (10), and the multiple respirations without flow control during eNO_{TB} lead to considerable differences between the levels determined by the two techniques.

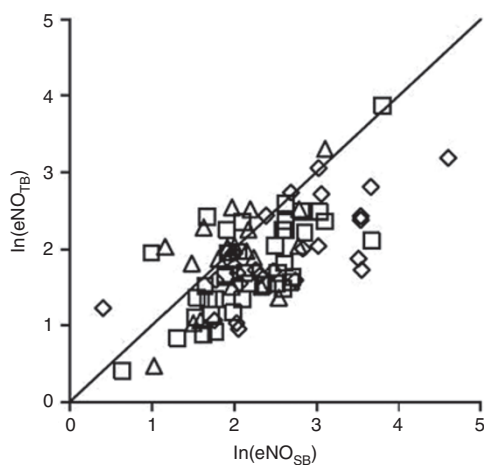


Figure 2. Relationship between logarithm of exhaled nasal nitric oxide (eNO) measured with tidal-breath (TB) and single-breath (SB) techniques. Triangles, children ≤6 y; squares, children 7–9 y; diamonds, children ≥10 y.

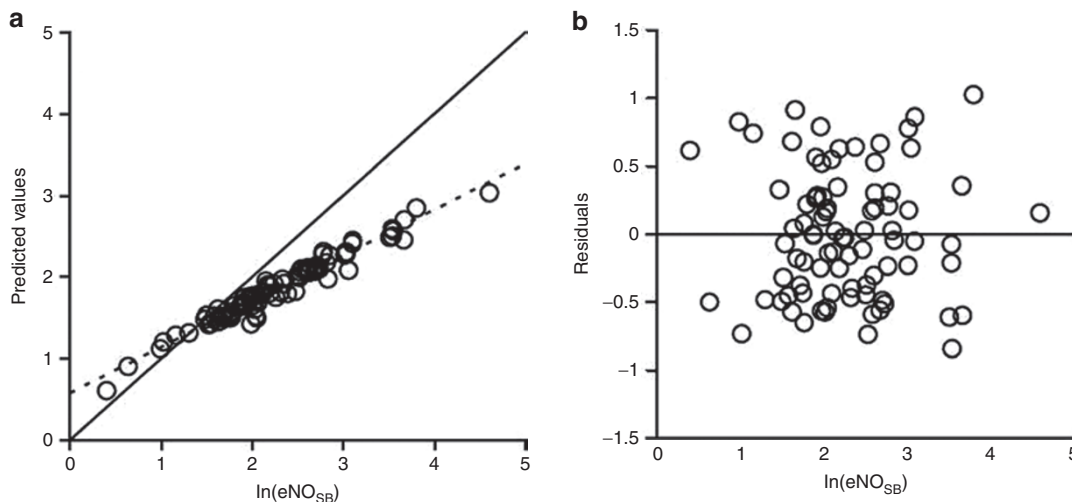


Figure 3. Relationship between predicted eNO_{TB} and observed eNO_{SB} values. (a) Scatter plots of the values predicted by the final equation $\ln(eNO_{TB}) = f(\text{age}, \ln(eNO_{SB}))$ vs. $\ln(eNO_{SB})$. (b) Scatter plots of the residuals from the final equation $\ln(eNO_{TB}) = f(\text{age}, \ln(eNO_{SB}))$ vs. $\ln(eNO_{SB})$. eNO, exhaled nitric oxide; SB, single breath; TB, tidal breath.

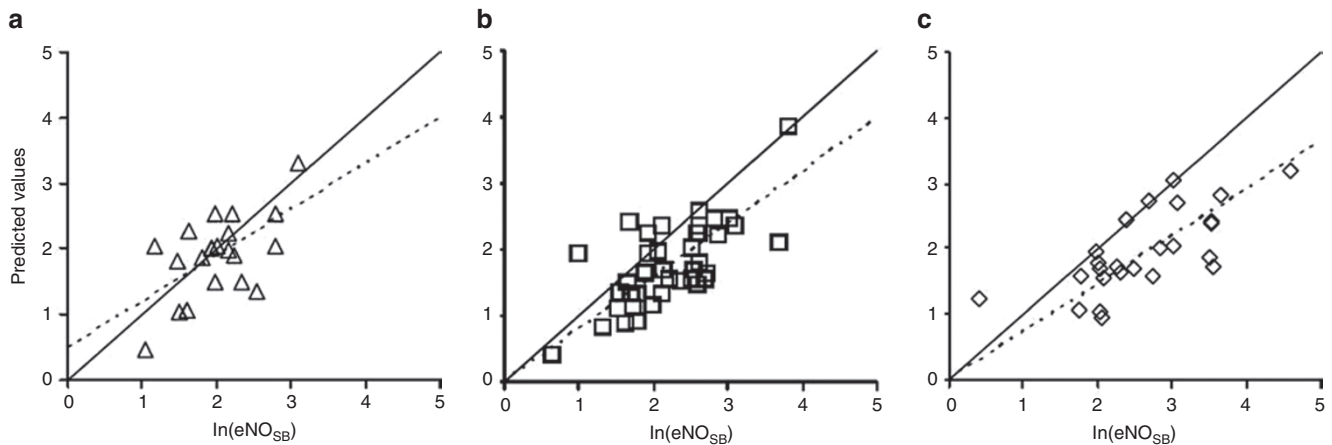


Figure 4. Scatter plots of the values predicted by the final equation $\ln(eNO_{TB}) = f(\text{age}, \ln(eNO_{SB}))$ vs. $\ln(eNO_{SB})$. (a) Triangles, children ≤ 6 y ($n = 21$). $\ln(eNO_{TB}) = 0.93 \times \ln(eNO_{SB})$ ($CI_{90\%}(\beta) = (0.86-1.00)$). (b) Squares, children 7–9 y ($n = 40$). $\ln(eNO_{TB}) = 0.79 \times \ln(eNO_{SB})$ ($CI_{90\%}(\beta) = (0.75-0.83)$). (c) Diamonds, children ≥ 10 y ($n = 25$). $\ln(eNO_{TB}) = 0.73 \times \ln(eNO_{SB})$ ($CI_{90\%}(\beta) = (0.68-0.77)$). Line, bisector; dotted line, interpolating straight line. $CI_{90\%}$, 90% confidence interval; eNO, exhaled nitric oxide; SB, single breath; TB, tidal breath.

Our results add to the debate concerning the conflicting results from previous studies comparing eNO levels as measured by means of eNO_{SB} and eNO_{TB} in pediatric patients with lower airway diseases (11,19). Jöbbs *et al.* (11) and Franklin *et al.* (12) found that agreement between eNO_{SB} and eNO_{TB} was poor in asthmatic children and in infants with recurrent wheeze, recurrent cough, or no respiratory symptoms. Our findings confirm this in pediatric patients with chronic upper airway disease. To the best of our knowledge, only Daniel *et al.* (13) did not find any significant difference between eNO_{SB} and eNO_{TB} (using a facemask) in a small case series of 11 children aged 8–12 y.

Among all the variables assessed, only age significantly changed the correlation between eNO_{SB} and eNO_{TB} . In particular, the greatest discrepancy between the eNO_{SB} and eNO_{TB} values was observed in children older than 6 y, whose eNO_{SB} values were generally higher than their eNO_{TB} values (Figure 4). It can be speculated that nasal contamination during eNO_{TB} is more significant in younger children because they are more frequently affected by adenoidal disease (20), which is responsible for increased nasal NO levels (7). This may explain the reduced difference between eNO_{SB} and eNO_{TB} values in children younger than 6 y.

In conclusion, our results showed that although there was a certain correlation between eNO_{SB} and eNO_{TB} , eNO_{TB} cannot be considered a good predictor of eNO_{SB} in children with chronic adenotonsillar disease on the basis of its poor correspondence even when it was adjusted for age. On the basis of our results, and given the dependence of eNO levels on expiratory flow, constant-flow eNO_{SB} should be considered the technique of choice for eNO assessment in young children with chronic upper airway disease, and clinicians should question the reliability of eNO_{TB} .

METHODS

Patients and Setting

The study involved 184 consecutive children aged 3–17 y, who attended the Otorhinolaryngological Unit of the University of Milan's Department of Clinical Sciences and Community Health between January 2008 and May 2009 because of chronic adenoidal and/or tonsillar disease. Chronic

adenoiditis was defined as adenoidal hypertrophy (assessed by means of flexible nasopharyngeal endoscopy) with ongoing nasopharyngeal inflammation/infection (i.e., at least three episodes of acute adenoiditis requiring antibiotic therapy in a period of 6 mo or at least four episodes in the previous year) (21), persistent mucous secretion on the adenoidal surface, and/or persistent otitis media with effusion (persistent middle ear effusion for at least 3 mo, without any sign of concomitant acute inflammation) (22), and/or recurrent acute otitis media (i.e., a history of at least three documented episodes in the previous 6 mo or at least four in the previous year) (23). Chronic tonsillitis was defined as severe recurrent tonsillar infections (at least seven episodes of acute tonsillitis in the previous year, at least five episodes per year for 2 consecutive years, or at least three episodes per year for at least 3 consecutive years) (24).

Exclusion criteria were as follows: an inability to perform eNO_{SB} or eNO_{TB} ; allergic asthma or chronic airway diseases (25); acute respiratory infections during the previous 30 d and/or the administration of systemic antibiotics and/or steroids or other medications affecting the airways (e.g., inhaled steroids, nasal steroids, or decongestants) during the previous month; systemic diseases (e.g., cystic fibrosis, primary ciliary dyskinesia, and primary or secondary immunodeficiency) (21); and parental refusal to participate.

The protocol was approved by the University of Milan's Ethics Committee, and written informed consent was obtained from the children's parents or their legal guardians.

Intervention

Gender, age, weight (W), height (H), BMI (computed as $W(\text{kg})/H^2(\text{m}^2)$), and passive smoking exposure (defined as whether parental figures such as mother, father, stepfather, or mother's partner, and stepmother or father's partner smoked in the home every day or sometimes) (19) were recorded for each patient, and the children underwent eNO_{SB} and eNO_{TB} determinations by means of a dedicated chemiluminescence analyzer (CDL 88 sp, Ecomedics, Dürnten, Suisse). At the parent's discretion, the child was comfortably seated on a chair or on the parent's knee. The child was encouraged to breathe only through his/her mouth and to breathe quietly for some minutes so as to acclimatize. The eNO_{TB} measurement was made first using an online method of tidal breathing: the child was asked to perform oral tidal breathing continuously through a disposable mouthpiece connected to the CDL 88 sp by means of a two-way valve. The exercise was repeated three times separated by at least 30 s of rest. The recorded eNO_{TB} level, expressed in parts per billion (ppb), was the geometric mean of the three values obtained.

eNO_{SB} measurement was made using an online, constant flow method in accordance with the international guidelines (1,2). The child was encouraged to inhale NO-free air (<5 ppb) orally for 2–3 s to total lung capacity from a disposable mouthpiece connected to the analyzer by means of a two-way valve and then to exhale orally for >4 s without

holding the breath at a constant flow rate (50 ml/s) through an expiratory resistance, targeting a mouth pressure of 10 cm H₂O in order to close the velum and avoid nasal contamination. The test ended with another oral inhalation through the CDL 88 sp. The children were helped to maintain a constant expiratory flow rate by means of a real-time visual aid, and eNO_{SB} was computed when an eNO_{SB} plateau of >2 s could be identified during exhalation. The eNO_{SB} level was recorded after three exhalations were repeated after intervals of >30 s. The analyzer was calibrated every 2 wk using certified 2,000 ppb calibration gas.

The correspondence between the eNO_{SB} and eNO_{TB} levels classifying patients as normal or pathological was assessed on the basis of published normal values for age classes, i.e., 5–8 ppb in children aged 4–7 y; 8–16 ppb in children aged 8–13 y; and 10–13 ppb in children aged 14–17 y (26–30).

Statistical Analysis

Because the eNO_{SB} and eNO_{TB} values were not normally distributed, log-transformed data were used for the analyses. The eNO_{SB} and eNO_{TB} data are given as geometric mean values with 95% CIs; the height and weight data as median values and interquartile ranges; and the other variables as frequencies (absolute numbers and percentages).

Multivariable linear regression models were used to examine the correlation among eNO_{TB}, eNO_{SB}, and the other potential confounders such as gender, age (continuous), height (continuous), weight (continuous), BMI, and passive smoking exposure. The goodness of fit of the models was evaluated using the coefficient of determination (R²). A *P* value of <0.10 was considered statistically significant. Data processing and all of the statistical analyses were performed by using the SAS software (version 9.1, SAS Institute, Cary, North Carolina).

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