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BRIEF COMMUNICATION

Between-occasion repeatability of fractional exhaled nitric oxide measurements in children

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ABSTRACT

The aim of the study was to assess short-term repeatability of measurements of fractional exhaled nitric oxide (F_ENO) and its correlates in children in the 6- to 9-year age bracket participating in a respiratory epidemiological survey. F_ENO was measured in two sessions one week apart in 101 children. Participants were divided into three **groups:** asymptomatic (n = 76); symptomatic (n = 14); and asthma (n = 11). Absolute and relative differences between the measurements, as well as concordance correlation coefficients, were used in order to assess repeatability. The two F_ENO measurements were strongly correlated (0.98). Although intragroup comparisons of the two measurements were not significantly different (p = 0.2), intergroup comparisons were. F_ENO measurements are reproducible in children in epidemiological settings.

Keywords: Nitric oxide; Exhalation; Asthma.

The measurement of fractional exhaled nitric oxide (F_ENO) is recognized as a useful method in the clinical assessment and management of respiratory disease, including asthma.[1-3] Little is known about its role in respiratory epidemiological studies, although F_ENO is recommended as a supplemental outcome for observational studies.[3] Inclusion of F_ENO in population-based studies on pediatric asthma could be helpful in the characterization of asthma phenotypes and chronic respiratory symptoms in children. This type of application requires that the test is standardized and that its repeatability is known and acceptable. Validation studies on F_ENO measurement have shown a very small intra-measurement variability as well as diurnal variation that—in children—is likely to range from 1 ppb to 2 ppb, on average.[4,5] Little is known about the repeatability of F_ENO measurement assessed on independent occasions in healthy, symptomatic, and asthmatic children examined in the field setting. Against this background, we performed a study on the short-term variability of F_ENO levels in a sample of elementary school children participating in a respiratory epidemiological survey. The objectives of the study were to assess the repeatability of F_ENO measured in that group of children on two occasions (one week apart) and to analyze its anthropometric and respiratory correlates. The study was performed in the voivodship of Silesia, Poland.

The subjects were 104 elementary school children, between 6 and 9 years of age, randomly selected in the town of Tychy. Respiratory symptoms were assessed using the translated version of the International Study of Asthma and Allergies in Childhood questionnaire,[6] which was completed by the parents. Informed consent was obtained from the parents or legal guardians of all participants included in the study. The study protocol was approved by the Research Ethics Committee of the Medical University of Silesia (Protocol no. KNW/0022/KB1/37/I/14).

All measurements were obtained in local schools. Anthropometric variables (age, height, and body mass) were measured before F_ENO and spirometry tests. F_ENO was measured with the child in a sitting position using a specific device (NIOX MINO®; Aerocrine, Solna, Sweden). The test consisted of a maximum of five attempts until one acceptable measurement was obtained. The second F_ENO measurement was performed one week after the first examination. Spirometric variables, including FVC, FEV_1, FEV_1/FVC ratio, $FEF_{25\%}$, $FEF_{50\%}$, and $FEF_{75\%}$, were obtained in accordance with the American Thoracic Society/European Respiratory Society recommendations, with the use of a spirometer (EasyOne®; ndd Medizintechnik AG, Zurich, Switzerland), being expressed in absolute values. Spirometry was performed after F_ENO measurement.

Statistical analyses were performed with the Statistical Analysis System, version 9.2 (SAS Institute Inc., Cary, NC, USA). The difference in FeNO levels between the two occasions was determined by subtracting the second measurement from the first one. The mean absolute value was calculated as the mean of the individual differences between the first and second measurements. The mean relative value was obtained by the following formula: first measurement − second measurement, expressed as %. Statistical significance of between-group differences in quantitative variables was assessed by the nonparametric Kruskal-Wallis test, and that of within-group differences was assessed by the Wilcoxon paired signed-rank test. Repeatability of F_ENO measurements was assessed by calculating the concordance correlation coefficient (r_{CC}). Statistical significance of between-group differences in qualitative variables was assessed by the chi-square test or Fisher's exact test. McNemar's test and Cohen's kappa

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test were used in order to assess the agreement of two qualitative results of F_ENO levels (cut-off point of 35 ppb). The correlates of within-subject variability of F_ENO were assessed using linear regression analysis, the relative F_ENO difference being used as the dependent variable. Simple and multivariate models were used in order to examine effects of gender, age, body mass index, respiratory status, and lung function variables. Interpretation of the results was based on the $p < 0.05$ criterion.

All subjects included in the study were examined in their schools on a Monday morning, and tests were applied after instructions. Two children were unable to perform the tests, and one child was unable to perform repeated F_ENO measurements. As a result, the analyses involved data obtained from 101 children (boys, 63%). The sample was divided into three groups: asymptomatic—no physician-diagnosed asthma, bronchitis, allergic diseases, or asthma symptoms (n = 76); symptomatic—no physician-diagnosed asthma but presenting with symptoms of wheezing (unrelated to having a cold) or dyspnea in the last year (n = 14); and asthma—physician-diagnosed asthma (n = 11). No significant differences were found among the groups regarding mean age (7.1 ± 0.7 years), height (128.3 ± 7.3 cm), or weight (26.7 ± 6.4 kg).

Table 1 shows the mean results of F_ENO obtained from the two measurements separately, as well as its between-occasion variability. In the sample as a whole, the mean absolute value was 1.4 ppb (11.7%). Intragroup comparisons between the two measurements were not significantly different (p = 0.2) and were strongly correlated (r_{CC} = 0.98; 95% CI: 0.98-0.99). The occurrence of F_ENO levels > 35 ppb showed very good repeatability (no discordant pairs). However, intergroup comparisons were significantly different regarding F_ENO levels (Tables 1 and 2). The mean values were the lowest in the asymptomatic group and the highest in the asthma group. The variability among the groups was similar regarding the mean absolute values (0.9-1.8 ppb) and the mean relative values (7.5-12.8%). No statistically significant differences were found in the means between the two measurements in the asymptomatic, symptomatic, and asthma groups (p = 0.6; p = 0.5; and p = 0.7, respectively). The groups showed strong correlations between the measurements: asymptomatic group (r_{CC} = 0.96; 95% CI: 0.94-0.97); symptomatic group (r_{CC} = 0.99; 95% CI: 0.99-0.99); and asthma group (r_{CC} = 0.99; 95% CI: 0.98-0.99). Table 2 shows the measurements divided into different ranges of F_ENO levels for the sample as a whole and for each group. F_ENO levels > 35 ppb were 100% reproducible on both measurements in each group (no discordant pairs). The correlates of within-subject variability of F_ENO levels were assessed using linear regression analysis,

Table 1. Results of two measurements of fractional exhaled nitric oxide and differences in between-occasion measurements in children by their respiratory status (quantitative variables).[a]

Variable		Total sample	Group			p*
			Asymptomatic	Symptomatic	Asthma	
		(N = 101)	(n = 76)	(n = 14)	(n = 11)	
F_ENO, ppb	Measurement 1	15.1 ± 13.5 [10 (7)]	12.6 ± 7.9 [10 (5.5)]	17.3 ± 19.7 [9 (17)]	29.1 ± 24.4 [21 (40)]	0.08
	Measurement 2	15.6 ± 14.0 [10 (8)]	13.0 ± 8.1 [10 (7)]	17.7 ± 19.5 [10 (14)]	30.6 ± 25.8 [23 (41)]	0.03
Between-occasion measurement difference	Mean absolute value, ppb	1.4 ± 1.5	1.4 ± 1.5	0.9 ± 0.9	1.8 ± 1.8	0.4
	Mean relative value, %	11.7 ± 14.2 [9.0 (18.1)]	12.8 ± 15.5 [10.0 (19.5)]	8.8 ± 10.0 [8.0 (11.1)]	7.5 ± 6.8 [7.0 (6.7)]	0.5
	Mean difference	−0.50 ± 2.01	−0.39 ± 2.08	−0.35 ± 1.27	−1.45 ± 2.02	0.1

F_ENO: fractional exhaled nitric oxide. [a]Values expressed as mean ± SD or [median (Interquartile range)]. *Kruskal-Wallis test.

Table 2. Results of two measurements of fractional exhaled nitric oxide and differences in between-occasion measurements in children by their respiratory status (qualitative variables).[a]

Variable		Total sample	Group			p*
			Asymptomatic	Symptomatic	Asthma	
		(N = 101)	(n = 76)	(n = 14)	(n = 11)	
Measurement 1, range in ppb	0-19	81	66	10	5	0.006
	20-35	14	8	3	3	
	> 35	6	2	1	3	
Measurement 2, range in ppb	0-19	82	67	10	5	0.004
	20-35	13	7	3	3	
	> 35	6	2	1	3	
Relative difference > 10%		44	37	5	2	0.1
Relative difference > 20%		19	15	3	1	0.8

[a]Values expressed as n. *Chi-square test or Fisher's exact test.

the relative difference being used as the dependent variable. Neither simple nor multivariate models showed any association of the relative difference with gender, age, height, body mass index, respiratory status, or spirometric variables.

Our findings showed very good repeatability of F_ENO measurements in our sample. Moreover, the repeatability was found to be equally good in healthy children and in children with chronic respiratory symptoms or in children with asthma, a finding that corroborates the evidence concerning diurnal variation observed in healthy and asthmatic children.[4,5] In our study, short-term variability of F_ENO levels measured over one week was independent of demographic or lung function variables. Between-test differences in F_ENO levels could be confounded by the contents of fat, antioxidants, and nitrates in food or by physical exercise.[3,7] Exposure to outdoor air pollution was also found to increase short-term variability of F_ENO levels.[8] We did had control for the aforementioned factors, and our study was performed under conditions that are common in respiratory surveys in children.

The results support a view that F_ENO as measured by portable devices is a well-accepted noninvasive method for the assessment of eosinophilic airway inflammation in respiratory epidemiology. Another interesting finding of our study is a convincing between-group gradient of F_ENO levels that reflects the respiratory status of children as identified by a questionnaire, a relationship that seems to add to the reliability of our measurements.

Few studies have addressed the issue of repeatability of F_ENO measurements in children. However, the published evidence is mostly pertinent to diurnal variation; recent studies reporting FENO repeatability in young subjects have primarily examined small groups of children (especially children with asthma) in a hospital setting with F_ENO measurements obtained during one visit.[4,5,9,10] Although our study involved a relatively small group of subjects, it is distinct because of its real-epidemiology protocol and because of the fact that the measurements were made in two different sessions, one week apart. The results of our study show that F_ENO measurement is stable under epidemiological conditions, corroborating the slight day-to-day variations found in another study.[11]

In conclusion, our findings demonstrate that F_ENO measurements performed with a portable device in a field setting are highly reproducible and seem to support a view that F_ENO measurement is a valuable tool in respiratory health surveys in children and, perhaps, in asthma screening programs for that age group.

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