Nasal nitric oxide measurement variability to establish a standard for reliable results

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To the Editor:

Nasal nitric oxide (nNO) measurement is a first-line test used to increase the post-measurement probability of primary ciliary dyskinesia (PCD) in subjects with symptoms consistent with this diagnosis [1]. The accuracy of nNO measurement is essential since it will orientate the work-up towards tests that are usually highly specialised and sometimes invasive. Accuracy of biological measurements relies on the technical and on the biological variability. While the accuracy of NO analysers is known better for chemiluminescence devices (*e.g.* <1 ppb with 1% linearity from 0.1 to 5000 ppb for CLD 88 (Eco Medics, Duernten, Switzerland)) than for widely used electrochemical devices (*e.g.* ±5 ppb for values <50 ppb and 10% for values >50 ppb for Niox Vero (Circassia, Oxford, UK)) [2], little is known on the biological variability of nNO measurements, except for increased nNO output variability in adults with rhinitis compared with healthy subjects and the positive effect of training on the level of nNO taken during expiration against a resistance (nNO-ER) in children [3, 4]. The PCD Foundation Clinical Center Network and Genetic Disorders of Mucociliary Clearance Consortium recommend sampling both nostrils for nNO-ER and to perform at least two measurements per nostril with the aim of obtaining a 10% repeatability [4]. For measurements performed during tidal breathing (nNO-TB) it is recommended to record peak values within 10% and aim for inter-nostril repeatability of 10% [4].

Using, in most cases, the coefficient of variation (CV) of three measurements to assess the variability of nNO in children and adults, small studies ($n \leqslant 50$) reported intra-nostril CV during breath hold (BH) (CV 3.8%, 9% and 12.5%) [5–7], ER (CV 5%) [7] or TB (CV 9.9% and 11%) [7, 8], and inter-nostril CV of nNO-BH (CV 10%) [9, 10]. Two large studies conducted in children and adults established nNO-ER CV at 6.9% (interquartile range 4.1–14.5%) for six nNO-ER measurements performed three in each nostril (n=226) [11], and 10.4% (SD 14.2%) for three measurements performed in same nostril (n=282) [12]. The latter study also established the CV (SD) of nNO-BH at 6.7 (8.8)% and of nNO-TB at 12.3 (15)%. In line with these results, we previously found an inter-nostril repeatability up to 10% more frequently for nNO-ER (93%) and nNO-BH (78%) than for nNO-TB (57% for the mean of five peaks (TB5p), and 68% for the mean of a 10 s period (TB10s)) [13], possibly because of the constantly variable nasal flow associated with the permanent open velum during TB. To date, the frequency of peaks within 10% on nNO-TB traces has never been evaluated.

Since many factors can influence the value of nNO, such as the size and the local airflow aerodynamic of the nostrils, or the degree of velum closure, and because, in large studies, the CV or the 10% repeatability of nNO measurements depended on the methods of measurement, we sought to evaluate in a large paediatric population tested in routine practice the relevance of the 10% repeatability criterion. We studied values obtained using different methods of measurement (two methods with velum closure, *i.e.* ER and BH, and two TB methods, *i.e.* TB5p and TB10s, the latter to mimic electrochemical sampling). We also evaluated relationships between nNO repeatability and age (different size of nostrils and ability to close the velum) or level of nNO (more difficulty in reaching % repeatability at low nNO values).

We retrieved, retrospectively, from our database of children referred for nNO measurement between 2009 and 2022, all nNO results checked for trace quality by the author (N.B.). Each child could contribute for one or more visits, and for nNO-ER and/or nNO-BH and/or nNO-TB measurements on one (if repeated) or both nostrils.

Shareable abstract (@ERSpublications)
A repeatability of 10% for NO measurements obtained with the velum closed in the same or both nostrils is relevant, while measurements taken during tidal breathing should aim for a repeatability of 20% and 30%, respectively https://bit.ly/3sMnug6

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TABLE 1 Proportions of repeatable nasal nitric oxide (nNO) measurements according to different criteria of repeatability and between-peak variability in the 409 study children

	Subjects, n	Repeatability of nNO measurements		
		⩽10%	⩽20%	⩽30%
Inter-nostril				
nNO-ER	271	208 (76.8%)		
nNO-BH	227	157 (69.2%)		
nNO-TB5p	395	138 (34.9%)		267 (67.6%)
nNO-TB10s	337	107 (31.8%)		226 (67.1%)
Intra-nostril				
nNO-ER	237	171 (72.2%)		
nNO-BH	141	105 (74.5%)		
nNO-TB5p	140	78 (55.7%)	137 (78.6%)	
nNO-TB10s	96	51 (53.5%)	69 (71.9%)	
		Intra-measurement variability of five peaks during TB		
nNO-TB5p CV	157	135 (86%)	148 (94.3%)	
nNO-TB5p Min%Max	157	105 (66.9%)	137 (87.3%)	

nNO: nasal nitric oxide; ER: expiration against a resistance; BH: breath hold; TB5p: tidal breathing, mean of five peaks; TB10s: tidal breathing, mean of a 10 s period; CV: coefficient of variation; Min%Max: minimum peak as a percentage of the maximum peak.

nNO was measured using a chemiluminescence NO analyser (NIOX Flex (Aerocrine, Solna, Sweden) until 2014, then a CLD 88 analyser (Eco Medics AG), with a sampling flow of 0.3 $L \cdot min^{-1}$ and 0.33 $L \cdot min^{-1}$, respectively) in subjects without an obstructed nose (inspection of the ventilation with one nostril obstructed alternatively).

Most of the data showed a non-Gaussian distribution. Results are medians (25^{th}; 75^{th} percentiles) and numbers (%). nNO results are displayed in concentration (ppb). The inter- or intra-nostril repeatability is the difference between two measurements expressed as percentage of the maximal inter- or intra-nostril value, respectively. The nNO-TB between-peak variability is the CV of five peaks and the minimum peak as a percentage of the maximum peak (Min%Max). We used non-parametric tests to study correlations (Spearman correlation) and to compare values (Mann–Whitney, Kruskal–Wallis, Friedman and Wilcoxon matched-pairs sign rank tests), and the Chi-squared or Fisher's exact test to compare proportions of children (GraphPad Prism version 6.01; San Diego, CA, USA). Families were informed of the possible retrospective use of their children's nNO results and gave consent electronically.

We included 409 children, 238 (58.2%) males, who had repeated nNO measurements in both nostrils and/or in the same nostril using the same method (ER, BH or TB) and who contributed 550 visits (186 children had one visit, 148 had two visits, 216 had three visits or more) at a median age of 7.9 (5.7; 11.5) years. We assessed the inter- and intra-nostril repeatability using, respectively, 271 and 237 nNO-ER measurements, 227 and 141 nNO-BH measurements, 395 and 140 nNO-TB5p measurements, and 337 and 96 nNO-TB10s measurements. Children tested with the TB method were significantly younger than children tested with ER or BH methods (6.3 (4.2; 9.6), 8.6 (6.4; 12.3), and 9.9 (7.4; 13.2) years, respectively; $p<0.0001$). Median nNO-ER, nNO-BH, nNO-TB5p and nNO-TB10s were 325.0 (76.2; 652.6), 280.0 (87.4; 596.0), 162.4 (44.0; 354.2) and 120.4 (32.6; 287.3) ppb, respectively.

Inter-nostril repeatability was significantly different among the four methods of measurements (nNO-ER 5.1 (2.0; 10.0)%, nNO-BH 5.6 (2.3; 13.4)%, nNO-TB5p 17.1 (7.2; 37.4)%, and nNO-TB10s 19.4 (7.8; 36.4)%; $p<0.0001$) with no difference within the two velum closure methods ($p=0.21$) or the two TB methods ($p=0.55$). There was no significant correlation between the inter-nostril repeatability and age or maximal nNO value for all methods (all r between −0.12 and 0.08, all $p>0.08$), except for a poor but significant correlation between inter-nostril nNO-TB variability and age (nNO-TB5p, $r=0.11$, $p=0.025$ and nNO-TB10s, $r=0.15$, $p=0.005$).

Intra-nostril repeatability was different according to the method of measurement (nNO-ER: 5.7 (2.6; 11.8)%, nNO-BH: 5.6 (1.8; 11.0)%, nNO-TB5p 9.6 (3.4; 19.2)%, nNO-TB10s 10.0 (4.0; 22.6)%, $p<0.0001$), without any difference within the two velum closure methods or the two TB methods ($p=0.33$

and p=0.47, respectively). There was no significant correlation between the intra-nostril repeatability and age or maximal nNO value for all methods (all r between −0.13 and 0.05, all p>0.13) except for poor but significant correlations between intra-nostril nNO-TB5p repeatability and age (r=0.23, p=0.007) or maximal value (r=0.17, p=0.042).

The distributions of nNO-ER, nNO-BH and nNO-TB repeatability, and the between-peak variability of nNO-TB according to different criteria are shown in table 1.

This study showed that velum closure methods had better within-occasion repeatability than TB methods, while both velum closure methods and both TB methods had similar repeatability. The proportion of children with nNO inter-nostril repeatability lower than 10% was slightly lower than that previously published [12], but acceptable for velum closure methods. Conversely, 30% inter-nostril repeatability for nNO-TB measurements would be necessary to achieve proportions of successful measurements similar to those of nNO with velum closure. Results were similar for intra-nostril repeatability, with 20% repeatability of nNO-TB needed to achieve similar repeatable measurement frequency as velum closure methods (table 1). However, between a third and a quarter of repeated measurements failed the repeatability criterion, in favour of testing both nostrils and twice each.

In conclusion, in a large cohort of patients with a wide range of nNO values, we confirmed that an inter-measurement repeatability of 10% is acceptable for inter- or intra-nostril nNO-ER or nNO-BH measurements. These results cannot be extended to nNO-TB (except for between peaks variability), which would require 30% inter-nostril repeatability or 20% intra-nostril repeatability to achieve similar frequencies of repeatable measurements. However, since these repeatability criteria are not met in about a quarter of cases, a second measurement on the same side or on the other side can deviate significantly from the first, in favour of sampling both nostrils and at least twice each, in order to record the best value. This standard could change the interpretation of nNO in children with low/borderline results on a single measure.

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