

A methodology for measurements of nasal nitric oxide in children under 5 yr

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Measurements of nasal nitric oxide (nNO) may give insight into respiratory conditions in children aged under 5 yr but no methodology has been described for this age-group. The present study aimed to establish the methodology and reproducibility for measuring nNO during tidal breathing in young children and to relate nNO to allergic conditions. Children and siblings aged under 5 yr attending hospital clinics were enrolled. On-line nNO measurements were obtained during tidal breathing using a chemiluminescence analyser. To establish our methodology, nNO was measured over 3, 5 or 10 s NO plateaus and nNO was also measured from left and right nostrils. nNO was then compared between children with and without allergic conditions. The reproducibility of nNO measurements over 24 h was studied in a separate group of children. Eighty-three children participated in the methodological part of the study and nNO was successfully measured in 57 (69%), mean (s.d.) age 3.4 (1.1) years, 14 with allergic conditions. Neither NO plateau duration nor choice of nostril influenced nNO values. The mean (s.d.) nNO for non-atopic children was 208 (103) parts per billion (ppb) and for atopic children was 284 (122), $p = 0.032$. Nasal NO values were not related to ambient NO, gender and passive smoke exposure; there was a non-significant trend for nNO to be positively related to age. Nasal NO measurements were reproducible in the 21 children tested, mean difference 9.6 ppb (limits of agreement-127, 146). We report a methodology for nNO measurement in young children. Further work is now required to establish the clinical utility of nNO in this age-group.

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Nasal nitric oxide (nNO) is elevated in children in association with allergic rhinitis (1) and reduced in association with cystic fibrosis (2–4), ciliary dyskinesia (5), sinusitis (6) and viral associated wheeze (7). Nasal NO values in children fall after the treatment of allergic rhinitis (1) and increase after treatment of sinusitis with antibiotics (6). Given that nNO measurements are reproducible in children (8) and that conditions including rhinitis, cystic fibrosis, sinusitis and viral associated wheeze are relatively common, measurements of nNO may be of clinical benefit in aiding diagnosis and management.

Upper and lower respiratory symptoms are particularly prevalent among children under 5 yr of age, but a method for measuring nNO in this age-group is not described.

International recommendations suggest that nNO is passively aspirated from the nostril whilst an individual exhales through the mouth against resistance (9), and this is to close the soft palate and reduce contamination with oral NO. Some studies involving older children have modified the international recommendations by asking subjects to hold their breath for between 10 and 25 s whilst nNO measurements are

taken (1, 10). Very young children and infants cannot exhale against resistance or hold their breath to command; in this age group a more appropriate technique will involve measuring nNO during tidal breathing. Previous studies have measured nNO during tidal breathing in school-age children (2, 11) and young infants (7, 12). The aims of the present study were to establish a methodology, report normal values and demonstrate reproducibility for nNO measurements made during tidal breathing in children aged between 6 months and 5 yr. Our hypothesis was that nNO can reliably be measured in children under 5 yr and is positively associated with allergic disease in this age group.

Methods

Protocol

Children attending hospital clinics were recruited. A respiratory questionnaire was completed. Three measurements of nNO were obtained from left and right nostril. nNO was measured over 3, 5 or 10 s plateaus. In a separate study population, three nNO measurements were taken from the right nostril at the same time of day on two successive days.

Eligibility

Children and siblings of children attending a hospital clinic were eligible if they were aged under 5 yr. Slightly older children were enrolled for the reproducibility study as we wished to relate nNO with lower airway NO (FE_{NO}) and FE_{NO} is not obtained in the majority of children aged <5 yr (13). There was no selection for asthma or allergic disease. Exclusion criteria included obvious nasal obstruction (nasal discharge and/or mouth breathing), upper or lower respiratory symptoms within the previous week or the use of inhaled corticosteroids. The study was approved by our institutional research ethics committee, parental consent and assent from the children (where relevant) was obtained.

Questionnaire

A respiratory questionnaire that has been developed by our group for use in NO studies was used (13). A history of allergic condition was defined as an affirmative response to the question: 'has your child ever had asthma, eczema or hay fever and do they still have this?'

nNO measurements

At the parent's discretion, the child was seated either on a chair or parent's knee. Once the child was comfortable with their surrounds and the investigator, a nasal olive with a central sampling canal (NIOX[®] Nasal I, paediatrics 06–1200) was gently placed against the nostril to achieve an air-tight seal, thus ensuring air could only leave the nostril via the central sampling channel within the olive. Older children were encouraged to breathe through their mouth. A chemiluminescence analyser (NIOX[®], Aerocrine, Solna Sweden) was used to aspirate air at 300 ml/min through the nasal olive; this flow was not verified. The analyser was calibrated every 14 days using certified 2000 parts per billion (ppb) calibration gas. Ambient NO was recorded. After randomization, three nNO measurements were taken from one nostril and the contralateral nostril. Nasal NO values and aspiration flow rates were observed on the computer screen by the operator in real-time to ensure that the air-tight seal was not broken (nNO values fall precipitously) or the sampling channel was inadvertently held against the nasal mucosa (flow falls precipitously). Air was aspirated over a total sampling period that included a measurement period (Fig. 1). The literature reports at least two different combinations of total sampling period and measurement period;

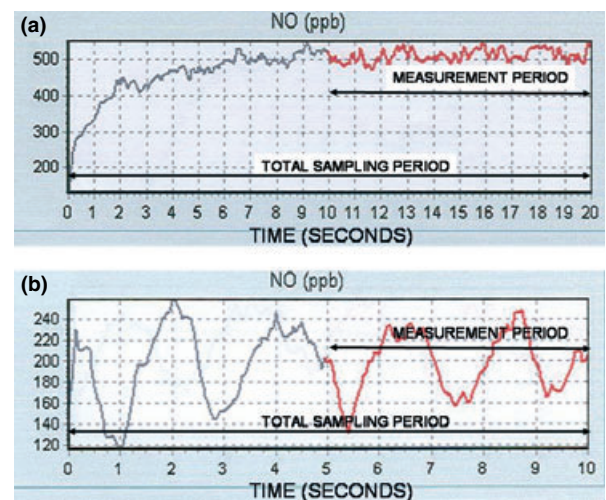


Fig. 1. Two graphical print outs showing measurements of nasal nitric oxide (NO, vertical axis) against time (horizontal axis). The total sampling period and measurement period are identified. In fig. 1a (technically acceptable), an NO plateau is reached after approximately 6 s and maintained until the end of the total sampling period. In fig. 1b (technically unacceptable), no NO plateau is reached and many more than two horizontal lines are crossed during the measurement period.

these include a 20-s total sampling period with 10 s measurement period (7) (abbreviated to 20/10) and a 10-s total sampling period with 3 s measurement period (10) (abbreviated to 10/3) and for this study, we adopted these plus an intermediate combination of a 10-s total sampling period with 5 s measurement period (abbreviated to 10/5). Termination of the measurement period and total sampling period always coincided. With the NIOX[®] software, nNO may be estimated more than once from the same dataset by adjusting the measurement period and we used this facility to study intrasubject reproducibility of nNO measurements over total sampling period and measurement periods of 10/5 and 10/3.

Quality control

The graphical display with the NIOX[®] analyser shows time (seconds) on the *x*-axis and NNO (ppb) on the *y*-axis (Fig. 1). The total number of broken horizontal lines remains roughly constant for individuals with high and low nNO values (Fig. 1). In real time, the display shows rising nNO values that reach a plateau, approximately parallel to one of the horizontal lines (Fig. 1a). An acceptable plateau is not achieved on every occasion (Fig. 1b). For the present study, an acceptable plateau was one where no more than one horizontal line was crossed during the measurement period (Fig. 1). Consistency for nNO values was defined as two nNO values within 5% of each other or three within 10% (9) and the mean of these consistent values was reported

Analysis

Comparisons between groups were analysed using Student's *t*-test. Paired nNO measurements were analysed using the method of Bland and Altman (14) and single-measures intraclass coefficient of variation. Standard statistical software was used for the analyses (SPSS, Chicago, Illinois, USA version 14.0). Significance was assumed at the 5% level.

Results

Study subject

There were 83 children recruited to establish the methodology and to study the relationship between nNO and allergic conditions, and of these 66 complied with nNO sampling, mean age 3.3 yr, 27 boys. A mean nNO was obtained from

at least one nostril in 57 children, mean (s.d.) age 3.4 (1.1) years, 25 boys, 14 with atopy (eight eczema, two asthma, two asthma and eczema, one asthma and hay fever, one eczema and hay fever). A mean nNO could not be obtained in nine compliant children because of either only one nNO value being obtained or ≤ 1 measurement satisfied quality control. The 57 children where a mean nNO was measured were older than the 26 children where nNO could not be measured [mean (s.d.) 3.4 (1.1) vs. 2.5 (1.1), $p = 0.002$]; there were no differences for gender or allergic condition. There was a trend that was not significant for a mean nNO value to be less likely obtained using total sampling period and measurement periods of 20/10 (61%, 22 successful measurement in 36 children) and more likely with 10/5 (75%, 15 in 20 children) or 10/3 (74%, 20 in 27 children), ($\chi^2_2 = 1.7$, $p = 0.43$). The median ambient NO value was 0.7 ppb (range 0–8.5), and there was no relationship between ambient NO and nNO.

nNO measurements – the influence of sampling from left and right nostrils and use of different total sampling period and measurement periods

A mean nNO value was obtained from the right nostril in 44 children, from the left nostril in 49 children and from both nostrils in 36 children. The mean difference between nNO measurements from left and right nostrils was 4 ppb (limits of agreement -63, 71), single measures ICC 0.94 [95% CI 0.89, 0.97]. The mean (s.d.) nNO values from the right nostril using different total sampling period and measurement periods were as follows: 20/10 238 (112) ppb, 10/5 265 (88) ppb and 10/3 172 (92), ANOVA trend test $p = 0.058$. The mean (s.d.) nNO values from the left nostril using corresponding time settings were as follows: 261 (124) ppb, 236 (99) ppb and 196 (108), ANOVA trend test $p = 0.212$. Among the nine children where nNO was calculated from the same dataset using total sampling period and measurement periods of 10/3 and 10/5, the mean difference between nNO from the left nostril was 1 ppb (limits of agreement -10, 7).

nNO measurements and other factors

Nasal nitric oxide values obtained in all children using different total sampling period and measurement periods were combined. There was a normal distribution of nNO, Fig. 2. Nasal NO from the left nostril was increased in the 14 atopic children, mean (s.d.) 284 ppb (122), com-

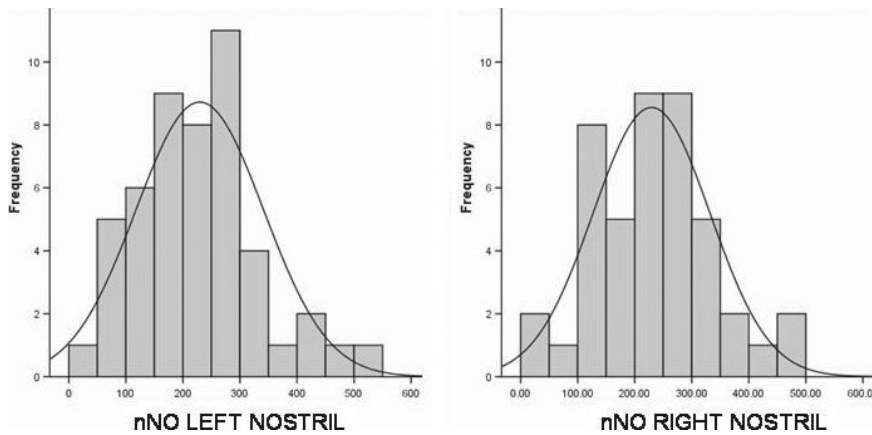


Fig. 2. Histograms for nasal nitric oxide (NNO) measurements in the left and right nostrils, normal curves are displayed for comparison in 43 children. The mean (s.d.) value for NNO from the left nostril was 230 ppb (112) and for the right nostril 229 ppb (103).

pared with the 35 non-atopic children, mean (s.d.) 208 (103) $p = 0.033$. Similarly, the mean (s.d.) nasal NO from the right nostril was 278 (86) in 11 atopic children and 213 (105) in 32 non-atopic children, $p = 0.072$. The remaining results are presented for nNO measurement taken from the left nostril only; the outcomes were comparable for nNO measurements at the right nostril: the mean (s.d.) nNO for the 22 boys was 252 (130) and 211 (92) in the 27 girls, $p = 0.203$; the mean (s.d.) nNO for 17 children with smoking parents was 232 ppb (136) and 229 (102) for 32 children with non-smoking parents, $p = 0.923$; there was a positive association that approached, but did not reach, significance between nNO and age, linear regression coefficient = 0.215, $p = 0.138$. A predictive equation for nNO was derived using linear regression:

$$\begin{aligned} \text{nNO (ppb)} \\ &= 159 + \text{age (years)} \times 15 \\ &+ \text{atopic condition (yes} = 1, \text{no} = 0) \times 67 \end{aligned}$$

Reproducibility of nNO

Measurements of nNO were obtained on successive days in 21 of 23 children studied, mean age (range) 6.1 (1.3, 10.3) years. The mean (s.d.) nNO on day 1 was 354 (139) ppb and on day 2 was 344 (136) ppb. The mean difference in paired nNO measurements was 10 ppb (limits of agreement -127, 146), Fig. 3. Paired FE_{NO} measurements were also made in the 12 older children (mean age 7.4 yr) where paired nNO measurements were made. The mean (s.d.) FE_{NO} on day 1 was 20 (30) ppb and on day 2 was 20 (29); FE_{NO} in one child, who has subsequently developed asthma symptoms, was 115 ppb. The mean difference in paired FE_{NO} measurements was

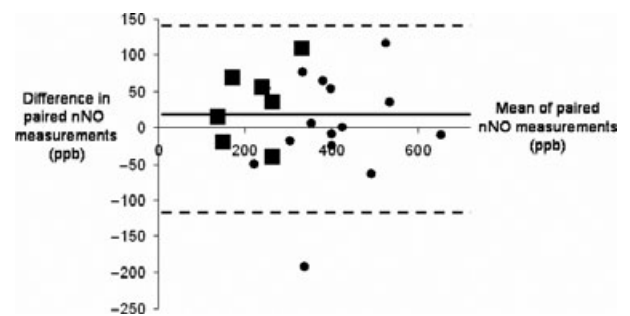


Fig. 3. A Bland-Altman plot comparing the mean and difference between paired nasal NO (nNO) values taken 24 h apart. The solid horizontal line corresponds with the mean difference and the broken horizontal lines correspond with the limits of agreement. The circles correspond with children aged 5 yr and over, the squares correspond with those aged under 5 yr.

0.2 ppb (limits of agreement -5.6, 6.0). Single measures intraclass correlation coefficient for nNO measurements was 0.88 [95% CI 0.71, 0.95] and for FE_{NO} was 0.99 [0.98, 1.00]. There was no correlation between nNO and FE_{NO} .

Discussion

This is the first study to measure nNO in children aged between 6 months and 5 yr. The major findings were that nNO could be measured in over two-thirds of the children, the nNO measurements were reproducible over a 24-h period and were elevated in association with allergic condition. Further evaluation of nNO measurements in young children with recurrent respiratory symptoms is required.

Methodological issues

The present study has adopted the methodology for nNO measurements previously used in young

infants and school-aged children for use in children aged between 6 months and 5 yr. We observed increased success in obtaining measurements with operator practice and thus the success rate we report is an underestimate of what we would expect from an experienced operator. The findings of the present study tend to support the use of a 10-s total sampling period with either a 3 or 5 s measurement period, a 20-s total sampling period was less well tolerated by the children, but not significantly so. We also report that the choice of nostril did not influence the result and this is consistent with studies in young infants (7), children (11) and adults (15). In future studies we will arbitrarily sample using total sampling and measurement periods of 10/3 and from the left nostril, unless this is obviously occluded.

Reproducibility

The reproducibility of nNO results was studied using two different strategies and values were found to be consistent; paired measurements of nNO measurements from left and right nostril and also from the same nostril at a 24-h interval were within the limits of agreement. A previous group has reported nNO values are reproducible in older children (8) and the present study extends this finding into the younger age group. We observed that reproducibility of FE_{NO} , as evidenced by intraclass correlation coefficient, was superior to than for nNO and this was not unexpected given the flow-dependent properties of NO can be mostly addressed by the single breath exhalation method for FE_{NO} but not for nNO during tidal breathing.

nNO values obtained

There are no comparable studies of nNO in children aged under 5 yr, however the mean nNO values obtained in the present study (230 ppb) are consistent with many studies that included older children. Baraldi et al. report mean nNO values of 186 and 216 ppb among two samples of healthy school children (1, 16). Using prediction equations from a Dutch population, where the potential confounder of atopic conditions is not included, the mean predicted nNO value (10) for the present study population was 355 ppb and higher than we observed. In contrast, other studies report a mean nNO values of 1024 ppb (4) and 1014 ppb (17) in healthy children. Differences in the methodologies used and also variations in the ages of populations studied may account for these differences in 'normal' values.

Relationship between nNO and other outcomes

The findings of the present study are consistent with studies of older children and this could indicate that the level of nNO production is already determined in early childhood. For example, we have observed an association between allergic conditions and elevated nNO, allergic rhinitis has previously been associated with increased nNO in school-aged children (1). In our study, most of the atopic children had eczema and not rhinitis and a study of adults has also found increased nNO among skin prick positive individuals (18), thus nNO may be elevated in all allergic conditions where rhinitis is not clinically apparent. Exposure to passive tobacco smoke was not associated with altered nNO in the present study and this confirms the report of Struben et al. (10). Ambient NO levels did not influence nNO results in our study and this is consistent with some studies in adults (15, 19) but not one in children (10); the latter study was undertaken in an environment with much higher ambient NO so we cannot be certain that with our method, nNO will not be influenced by higher ambient NO values. Finally in the present study, there was a trend for nNO values to be positively related to age and positive relationships are described elsewhere (2, 10). The consistency between the results of the present study and others assures us that our findings in this population, which is younger than previously studied, are reliable and our quality control robust, but confirmatory work is required.

Origins of nNO in children

The origin of nNO in children is poorly understood but data from studies of adults suggest that nitric oxide synthase (NOS) is expressed in the paranasal sinuses and is calcium independent and unaffected by topical steroids, in contrast NOS activity in the nasal mucosa is much less marked and is calcium dependent (20). In addition to NOS activity, other factors intrinsic to the nasal passages and paranasal sinuses are also likely to be important to nNO values and these include airspace narrowing and occlusion by mucosal oedema, increased nasal blood flow, increased nasal secretions and the relative ratio of airspace volume to epithelial surface area. This latter factor may account for the positive association between nNO and age where, as the paranasal sinuses enlarge nNO could be more influenced by the increase in NO production than the increase in airspace volume.

Strengths, weaknesses and limitations of the present study

The present study has a number of strengths and these include the rigorous methodology for collection and calculation of nNO and the reproducibility study. One weakness of our study is that we cannot exclude the possibility, indeed it is likely, that some younger children may have been breathing through their nose while nNO measurements were taken and this may have falsely lowered the results (3). A second weakness is that as the children were not exhaling against resistance, even while mouth breathing the soft palate will not have occluded the nasopharynx with resultant leak of nNO into the oropharynx and this again may explain our low nNO values using predictive equations from another study (10). The study has some limitations, for example a larger study population might have found a statistically significant relationship between age and nNO. Another limitation is that we pooled all the nNO results using different total sampling and measurement periods, although we found no difference between the measurements obtained, our results should be confirmed in a population where a single combination of total sampling and measurement periods are used. Subclinical nasal obstruction may have influenced the results and was not determined in this study, any influence is unlikely to have been substantial as the degree of obstruction required to reduce the relatively low aspiration rate would probably also result in symptoms. Finally, some children who participated in the reproducibility study were older than 5 yr and this was deliberately so to allow the reproducibility of nNO measurements to be compared against those for FE_{NO}, we have however shown that reproducibility for nNO was similar for those under 5 yr compared with those older (Fig. 3).

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