Normal values of offline exhaled and nasal nitric oxide in healthy children and teens using chemiluminescence

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Abstract
Nitric oxide (NO) can be used to detect respiratory or ciliary diseases. Fractional exhaled nitric oxide (FeNO) measurement can reflect ongoing eosinophilic airway inflammation and has a diagnostic utility as a test for asthma screening and follow-up while nasal nitric oxide (nNO) is a valuable screening tool for the diagnosis of primary ciliary dyskinesia. The possibility of collecting airway gas samples in an offline manner offers the advantage to extend these measures and improve the screening and management of these diseases, but normal values from healthy children and teens remain sparse. Methods. Samples were consecutively collected using the offline method for eNO and nNO chemiluminescence measurement in 88 and 31 healthy children and teens, respectively. Offline eNO measurement was also performed in 30 consecutive children with naïve asthma and/or respiratory allergy. Results. The normal offline eNO value was determined by the following regression equation: $-8.206 + 0.176 \times \text{height}$. The upper limit of the norm for the offline eNO value was 27.4 parts per billion (ppb). A separate analysis was performed in children, pre-teens and teens, for which offline eNO was 13.6 ± 4.7 ppb, 16.3 ± 13.7 ppb and 20.0 ± 7.2 ppb, respectively. The optimal cut-off value of the offline eNO to predict asthma or respiratory allergies was 23.3 ppb, with a sensitivity and specificity of 77% and 91%, respectively. Mean offline nNO was determined at 660 ppb with the lower limit of the norm at 197 ppb. Conclusion. The use of offline eNO and nNO normal values should favour the widespread screening of respiratory diseases in children of school age in their usual environment.

Introduction
Numerous reports highlight the clinical usefulness of nitric oxide (NO) in the diagnosis of respiratory or ciliary diseases, such as asthma [1–4] or primary ciliary dyskinesia (PCD) [5, 6]. Fractional exhaled nitric oxide (FeNO) is increased in patients with asthma, whereas nasal nitric oxide (nNO) is decreased in PCD patients [7–9]. Abnormal nNO levels can also be found in other respiratory disorders such as cystic fibrosis, bronchiectasis, chronic sinusitis, and panbronchiolitis [10–12]. In asthma and PCD, NO measurement is widely used as a screening tool. FeNO measurement can complete the diagnosis of the majority of conventional tests recommended in international clinical guidelines for the diagnosis of asthma, such as peak flow recordings and spirometry [4, 13]. Moreover it is a time-efficient and resource-efficient screening tool for airway hyperresponsiveness and exercise-induced bronchoconstriction [14–16], as well as a sensitive and non-invasive marker of inflammation that reacts rapidly in response to treatments [17] or exacerbations [18, 19]. In PCD symptomatic patients, even if ultra-structural analysis of the cilia remains the reference test, nNO measurements complete the diagnosis and could be helpful to decide on the need for further testing [8, 20].
Online or offline methods can be used to measure FeNO and nNO and both can be used in children [21]. The offline collection technique has the advantage of allowing remote collection (e.g., in clinics, emergency rooms or outside hospitals, at school or in other institutions) with centralized use of a single analyser that greatly expands the potential use of this measurement and enables mass screening sessions of respiratory or ciliary diseases. Standard values of FeNO [22–24] and nNO [25–27] are available for healthy children using the online method. However, there is a paucity of offline reference values in healthy children according to the recommendations of current international guidelines.

In this study, our aim was to determine normal values with the offline method in healthy children of school age. We measured offline eNO and nNO with two devices used for the offline measurement of NO, following the recommendations of international guidelines [28, 29]. A particular regard was dedicated to the methodology of measurements and the use of standardized collection techniques.

Methods

Subjects
Children aged 6 to 16 that were referred to the paediatric outpatient’s department of the Centre Hospitalier Universitaire of Nantes, France, were eligible for the study. The inclusion criteria for ‘Healthy children’ were children without asthma or allergy based on a medical diagnosis at the enrollment time, including asthmatic and/or atopic symptoms in history and respiratory tests. These children were excluded from the normal values analysis. We also excluded children with prematurity and/or respiratory distress syndrome at birth, recurrent childhood respiratory and/or otorhinolaryngological disorders, recent (six weeks prior to the study) respiratory tract infection and/or otorhinolaryngological disorders, and other disease diagnosed by the physician that could interfere with the results. Children with asthma and/or one or several respiratory allergies (e.g. allergic rhinitis, dust mites or dust allergy, animal dander allergy, and in general any common aeroallergens) were included in a separate group named ‘Asthmatic and/or atopic children’. None of the children were undergoing anti-inflammatory or anti-histaminic treatments. Furthermore, they were non-smoking and none of them had eaten, drunk or had strenuous physical exercise 2 h before NO measurements, as recommended by international guidelines [22, 28, 29].

Online and offline values were also measured in a group of healthy adult subjects, to assess for the quality control of measurements and samples. Repeatability of measurements was controlled on all samples available in our study and a few Mylar bags were analysed to test the stability of NO concentration. The study complied with the recommendations of the Declaration of Helsinki. According to French legislation about biomedical non-interventional research, this study did not require competent authorities’ authorization nor ethics committee approval. However, all participants received information in both oral and written forms, and parents and children gave their consent.

Study design
This study was prospectively carried out in our institution from May to August 2012 specifically to provide normal values for offline e/nNO. Samples were consecutively collected in the morning in Mylar bags whereas measurements were subsequently performed 4 h apart. Ambient NO was measured each day during sessions, as well as the ATMO index in Nantes (Air Pays de la Loire, Nantes, France), which is based on four pollutants of major concern in Europe (ozone, nitrogen dioxide, sulphur dioxide, particulate matter) and done on a scale from 1 (very low pollution) to 10 (very high pollution). Exhaled and nasal NO measurements were performed according to the instructions [21, 28]. Samples were collected in air-tight Mylar bags which were immediately sealed and kept at room temperature before their measurements.

Offline eNO and nNO collections
Samples were obtained using the FeNO offline collection kit (ECO MEDICS AG, Duernnet, Switzerland). The procedure to collect an offline eNO sample avoids sample contamination by ambient NO and collection of dead space gas, using portioning of the expiratory sample and a NO-scrubbing filter in the aspiratory limb of the collection system. Samples were collected with the single breath technique. Children inhaled orally to total lung capacity (TLC) and immediately performed a deep slow exhalation with a constant expiratory flow rate of 50 ml s⁻¹, without breath holding and against expiratory resistance higher than 5 mm H₂O, to ensure that the soft palate was closed against the nasal cavity, thus preventing contamination of eNO with nNO [29].

Offline collections of nNO were obtained at a constant aspiration flow rate of 300 ml min⁻¹ through a NIOX® nasal olive (Aerocrine AB, Solna, Sweden) inserted in the dominant nostril and linked to a suction pump (THOMAS, Gardner Denver product, USA). A Mylar bag was fixed to the collection system downstream of the pump. Samplings were collected with the transnasal flow in series technique. A nasal olive was inserted in one of the nostrils of the subject, completely occluding it to avoid ambient air sampling, whereas the contralateral nostril was left open. After 5 s of tidal breathing, the child was asked to take a deep inspiration and hold it for 20 s under careful monitoring from the technician. The aspiration from the first 5 s of breath-hold time was withdrawn, after which the sample was collected in the bag for measurements. The
critical manoeuvre for measuring nNO with the aspiration method is to ensure that the velopharynx remains closed, thus avoiding contamination of lower-airway NO [28–30]. Adopting a breath-hold manoeuvre with a closed glottis allows velopharyngeal closure to be achieved in children as young as 5 years of age [31].

Online FeNO and nNO collection

Online FeNO samples were collected using an online nitric oxide monitor (NIOX® Flex; Aerocrine AB, Solna, Sweden), with the clinical mode view of the NIOX® software. Fractions were obtained following the same procedure as previously described for offline eNO collection. Online nNO collections were also obtained using the NIOX® system, with the nasal mode view of the NIOX® system and following the same procedure as previously described for offline nNO collection. The same nostril was closed with the nasal olive for all nNO collections (i.e. online and offline collections) in the same subject.

NO measurement

Fractions of NO were subsequently measured twice in the same bag by chemiluminescence using the online NO monitor (NIOX® Flex; Aerocrine AB, Solna, Sweden). Stability of NO samples in Mylar bags was tested, measuring NO concentration every 3 h, up to 48 h after the collection. The offline mode view of the NIOX® system was used for real-time NO fractions measurement. According to the guidance of the manufacturer, an automatic measurement began after 5 s: the NO profile showed a rapid rise to a steady NO plateau, at which eNO or nNO concentrations were calculated. The air was sampled with a flow of 300 ml min⁻¹. NO was expressed as parts per billion (ppb). The analyser was calibrated at least every 14 days using two certified NO calibration gases with guaranteed stability (201 ppb for eNO and 2011 ppb for nNO; Air Liquide Santé France, AIR LIQUIDE, Paris, France) and according to the recommendations of the manufacturer.

Statistical analysis

Demographic and clinical data were presented as means and standard deviation (SD) for continuous variables. Pearson correlation coefficients, t-tests and linear multiple regression were used to examine the relationships between offline exhaled or nasal NO and factors possibly affecting NO values, and to estimate these values with the predictor variables in our healthy paediatric population. A receiver–operator characteristic (ROC) curve, which allows a graphical representation of sensitivity and specificity, was plotted in order to detect the best cut-off value which differentiates healthy children from children with asthma and/or respiratory allergy in our population. Bland–Altman analysis was used to visualize the repeatability between online and offline exhaled and nasal NO collection techniques [32]. The same statistical method was applied when analysing the repeatability of measurements with the NIOX® Flex (Aerocrine AB, Solna, Sweden). The stability of NO in Mylar bags was analysed by ANOVA and the post-hoc Holm–Sidak test. A p-value of less than 0.05 was considered significant. Statistical analyses were performed using the commercially available SPSS software (version 16.0; SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

All participants were able to provide technically acceptable manoeuvres for exhaled and nasal NO measurements, resulting in 118 children for analysis (figure 1). Of these, 88 were healthy subjects who had been enrolled for eNO measurement. The nNO measurement was realized in 31 of them. We also studied 30 children with asthma and/or respiratory allergy. The characteristics of the patients are detailed in table 1.

Normal values of offline eNO

For offline exhaled measurements, the average time between collection and measurement of eNO fractions was 4:40 ± 1:00 h and the mean ATMO index mean was 4.0 ± 0.1. Ambient NO was less than 5 ppb during eNO fractions collections. Five healthy children were second-hand smokers, with a last exposure at least the evening before collections and four of the children with asthma and/or respiratory allergy were second-hand smokers, with a last exposure at least 3 h before collections. In the whole healthy population (n = 88) (figure 2), significant positive correlations were observed between offline eNO and height (r = 0.424, p < 0.001), weight (r = 0.418, p < 0.001), body surface (r = 0.412, p < 0.001), age (r = 0.391, p < 0.001) and body mass index (BMI) (r = 0.226, p < 0.05). Gender was not a significant variable (t = −0.509, p = 0.612). A linear multiple regression was performed in this paediatric population, where height was the best predictor of offline eNO value among the independent variables. The following linear regression equation in this population was: Offline eNO = −8.206 + 0.176 + height (R² = 0.180). Mean offline eNO was 17.0 ± 7.0 ppb with a median output of 15.6 ppb and standard error of estimate (SEE) was 6.3 ppb. The limits of agreement in our healthy paediatric population were: upper limit of the norm (ULN) = 27.4 ppb and lower limit of the norm (LLN) = 6.6 ppb.

As height was a good predictor of eNO value, a separate analysis was also performed in children aged [6; 9] years, in pre-teens aged [9; 12] years and in adolescents aged [12; 16] years. Moreover, these groups are identified categories of interest for clinicians, because of likely similarities in their physiology and
size. The characteristics and eNO values of the subgroups are detailed in table 2.

The 30 subjects with asthma and/or respiratory allergy had a mean offline eNO of 38.9 ± 23.8 ppb. The best eNO value for diagnosis of asthma and/or respiratory allergy was 23.3 ppb from the ROC curve, with an AUC of 0.832. This value provided 77% sensitivity and 91% specificity (figure 3).

Normal values of offline nNO
For the 31 healthy subjects, the average time between collection and measurement of offline nNO fractions was 4:20 ± 1:50 h and the ATMO index mean was 4.3 ± 0.8. Ambient NO was less than 5 ppb during nNO fractions collections. None of the children were second-hand smokers.

Results are presented in table 3 and figure 4. The limits of agreement in our healthy paediatric population were: ULN = 1123 ppb and LLN = 197 ppb.

In these healthy population, no significant correlation was shown between offline nNO and height (r = 0.208, p = 0.061), age (r = 0.241, p = 0.191), body surface (r = 0.234, p = 0.205), weight (r = 0.208, p = 0.261) or BMI (r = −0.062, p = 0.741). Gender was not a significant variable (t = −0.647, p = 0.523).

Comparisons between online and offline methods, and reproducibility and stability of NO measurements
Analysis of the measurements repeatability with the NIOX® Flex (Aerocrine) (n = 164), study of the NO stability in Mylar bags (n = 7) and comparisons between online and offline collection methods for eNO (n = 27) and nNO (n = 32) are provided in the supplementary data (figures (A)–(D) available at stacks.iop.org/JBR/11/036008/mmedia).

Discussion
In the present study, reference ranges of eNO and of nNO obtained with the offline collection method in a group of healthy children aged 6 to 16 were defined according to current international recommendations [29]. Normal values of these biomarkers and limits of agreement allow clinicians to assure that individual measured values follow the expected range for healthy children of the same age or, if not, to give valuable information that helps clinicians to refine their diagnoses and medical treatments. Furthermore, these reference values obtained offline allow during the detection of abnormal values of eNO or nNO in mass screening sessions of children considered healthy, who had thus no reason to have a medical visit or treatment prior to NO measurements. Exhaled NO may not only act as a screening tool for asthma or atopy, but could also prevent under-diagnosis and under-treatment of exercise-induced bronchoconstriction in atopic asthmatic school children [33].

An important finding of the current study was the determination of limits of agreement of offline nNO. To our knowledge, this is the first report on offline nNO normal levels in a French paediatric population aged 6 to 16. In the majority of the clinical studies on online nNO, normal values were determined using a different approach, comparing the values from children suffering from different diseases to healthy controls [3, 9, 22, 31, 34–37]. Here, the determination of normal values was the primary outcome and every child was included after a thorough examination to

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**Figure 1.** Flow chart of the study. eNO: exhaled NO; nNO: nasal NO.
ensure that he/she was healthy. Apart from the offline method, one study developed a promising portable NO analyser for screening in primary ciliary dyskinesia, but no reference values were specified [38]. Few studies intended to establish normal nNO values in a school-aged population, but they were designed for online values [25–27]. Mean values of nNO reported in our study were different than those reported in these ones. This could be explained by the use of different sampling flow air, subjects having held their breath or blown into an apparatus during measurements, researchers having recorded or omitting to record the ambient NO level, and the use of different analysers and methods. Therefore, direct comparisons with the present study remain difficult. Leigh et al have successfully attempted to standardize methodology to study online nNO across six collaborating sites with different analysers [26]. Nevertheless, a recent study highlighted that nNO values and thus a useful threshold might largely differ according to online or offline methods [39]. They also recommended that more attention needs to be paid to the method of collecting samples and to factors influencing nNO output such as ambient NO and transnasal flow sampling; all parameters that were controlled in our study.

Our study does not corroborate that height, weight, BMI but not sex influenced nNO levels [27]. This could be explained by the development of the facial skeleton, especially the volume of maxillary

### Table 1. Demographic characteristics of the children.

|                  | Healthy children for offline eNO | Healthy children for offline nNO | Asthmatic and/or atopic children for offline eNO |
|------------------|----------------------------------|---------------------------------|-----------------------------------------------|
| n                | 88                               | 31                              | 30                                            |
| Sex ratio, F/M   | 33/55                            | 14/17                           | 13/17                                         |
| Age, yrs        | 10.8 ± 2.9                       | 11.5 ± 3.1                      | 11.1 ± 2.0                                    |
| Height, cm³     | 143.7 ± 16.9                     | 150.1 ± 19.0                    | 144.7 ± 11.6                                  |
| Weight, kg³     | 36.9 ± 11.7                      | 40.2 ± 13.7                     | 37.2 ± 8.9                                    |
| BMI³            | 17.4 ± 2.4                       | 17.2 ± 2.2                      | 17.5 ± 2.0                                    |
| Body surface, m²| 1.20 ± 0.26                      | 1.29 ± 0.32                     | 1.22 ± 0.20                                   |

* Data are reported as mean ± SD.
BMI: body mass index.

### Table 2. Demographic characteristics and eNO value of children’s subgroups.

| Age at time of test | [6; 9] | [9; 12] | [12; 16] |
|---------------------|--------|---------|----------|
| n                   | 27     | 24      | 37       |
| Sex ratio, F/M      | 8/19   | 10/14   | 15/22    |
| Age, yrs            | 7.3 ± 0.8 | 10.3 ± 0.8 | 13.8 ± 1.2 |
| Height, cm³         | 126.2 ± 7.1 | 138.3 ± 6.2 | 160.0 ± 10.2 |
| Weight, kg³         | 25.3 ± 3.5 | 33.6 ± 4.9 | 47.4 ± 9.2 |
| BMI³                | 15.8 ± 1.5 | 17.6 ± 2.2 | 18.4 ± 2.5 |
| Body surface, m²    | 0.93 ± 0.09 | 1.1 ± 0.11 | 1.4 ± 0.2 |

**Offline eNO, ppb**

- Mean: 13.6 ± 16.3 ± 20.0
- SD: 4.7 ± 7.0 ± 7.2
- Median: 11.9 ± 13.7 ± 15.6
- LLN: 4.2 ± 2.3 ± 5.6
- ULN: 23 ± 30.3 ± 34.4
- Min: 6.4 ± 9.2 ± 9.3
- Max: 28.9 ± 43.4 ± 40.6

* Data are reported as mean ± SD.
BMI: body mass index.
sinuses, that increases the surface of the airway epithelium. However, while Struben et al found a positive correlation between online nNO and age [27], this factor was not correlated with offline nNO in our population. The limits of agreement of online nNO were not determined in these studies, unlike our study. For infants less than 1 year old, nNO reference values were even established based on 42 measures [40].

The present study is also a report on offline eNO normal levels and limits of agreement in school-aged children. Mean values of offline eNO reported in this study were higher than those reported by Jöbsis et al in 211 children aged 5 to 16 [42]. Jöbsis et al notably determined mean offline eNO at 6.4 ± 0.4 ppb for a collection technique with breath holding, without a nose clip and with ambient NO < 10 ppb. Nevertheless, sampling flow rate was not controlled, sample contaminations by ambient NO were not avoided using an NO-scrubbing filter to aspire NO-free air, and collected samples contained dead space gas. Limits of agreements, and particularly the ULN, were not determined [23]. In North African children, Rouatbi et al determined mean of offline eNO at 5.0 ± 2.9 ppb and the ULN at 5.4 ppb. They concluded that an eNO value greater than 17.0 ppb may be considered abnormal in North African and Arab children. Thus, the variability of NO values could have methodological issues, or be due to ethnic differences. It would also be important to clarify what environmental issues, such as the nature of pollutants, and epidemiological issues, such as infectious diseases and atopy in childhood, influence these biomarkers. These points may explain the ULN value of 27.4 ppb that is slightly higher in our study. Even though this study is not the first to report offline nNO results, we here have paid great attention to the method of collecting samples and to factors influencing nNO output such as ambient NO and transnasal flow sampling. Moreover, we have detailed values for three groups of interest for clinicians, i.e. children, pre-teens and teens.

As several previous studies reported, eNO was correlated with age, height, weight, BMI, and body surface but not with sex [29, 41, 43, 44]. Several paediatric studies [23, 45] have reported no significant relationship between eNO and BMI, and Santamaria et al [46] found that a positive correlation between eNO and BMI in healthy children was explained by age.

Table 3. Demographic characteristics and nNO values of the children.

| Characteristics       | Value          |
|-----------------------|----------------|
| n                     | 31             |
| Sex ratio, F/M        | 14/17          |
| Age, yrs             | 11.5 ± 3.1     |
| Height, cm           | 150.1 ± 19.0   |
| Weight, kg           | 40.2 ± 13.7    |
| BMI                  | 17.2 ± 2.2     |
| Body surface         | 1.29 ± 0.32    |
| Offline nNO, ppb      |                |
| Mean                 | 659.7          |
| SD                   | 231.5          |
| Median               | 652.7          |
| LLN                  | 196.7          |
| ULN                  | 1122.7         |
| Min                  | 307            |
| Max                  | 1330           |

* Data are reported as mean ± SD.

BMI: body mass index; LLN: lower limit of the norm; ULN: upper limit of the norm.
A multivariable linear regression model for eNO values in healthy children was estimated in our study, although the adjusted $R^2$ suggests a very moderate fitting of the model to data. We determined that height was the best determinant of FeNO in healthy children, as Malmberg et al previously reported \[44\].

Both atopy and asthma were identified as independent risk factors associated with high FeNO \[47–49\]. Regarding diagnosis of asthma or respiratory allergy, at a cut-off point of 23.3 ppb eNO offers the best predictive value for a suspicion of asthma or respiratory allergy with a sensitivity of 77% and specificity of 91%. Different optimal thresholds to diagnose asthma have been reported, as summarized by Majid et al \[50\]. Interestingly, in studies about the management of childhood asthma in children aged 6 to 18 \[51\], Fritsch et al found a clinically relevant threshold of online FeNO with best sensitivity and specificity for predicting asthma exacerbations at 22.9 ppb. Woo et al \[49\] suggested a threshold at 22 ppb in children aged 8 to 16 and Jerzynska et al \[47\] worked with a cut-off point of FeNO at 23 ppb. Even in pre-school children aged 1 to 5 years old, a 95th percentile of online eNO has been established at 22.6 ppb \[52\]. Of note, the ATS advised that eNO values between 20 and 35 ppb in children should be interpreted cautiously and with reference to the clinical context as well as accounting for persistent or high allergen exposure as a factor associated with higher levels of eNO \[33\].

One can notice that several studies mention values of online or offline eNO or nNO, although measurement techniques vary considerably, making their interpretation difficult. Standardized protocols and cut-off data should be developed rigorously according to international guidelines. Confounding factors such as age, ambient NO or atopic symptoms have also not always been taken into account, and it is still not clear whether the use of different chemiluminescence analysers could influence the results.

Our study suggests one measurement per bag is enough to determine NO concentration using the NIOX® Flex system (Aerocrine AB, Solna, Sweden). Comparisons of online versus offline eNO values show good agreement between collection techniques, just as between online and offline nNO collection techniques, as previously described \[30, 43, 54\]. In addition, the current study corroborates the results of some previous studies assessing the stability of NO over time in Mylar bags, showing that NO values remain stable for at least 6 h after collection when stored at room temperature \[41, 54–56\]. As in Silkoff et al \[30\], we reported eNO to increase compared with baseline values after 24 h, which is enough in clinical practice using the offline collection method.

This study determined reference values according to current international guidelines \[29\], with special attention dedicated to the use of standardized methods. We systematically took into account factors possibly affecting NO levels and potential confounders such as ambient NO, lifetime tobacco exposure, ATMO index level or current medication. We also studied the stability of NO samples in Mylar bags and the repeatability of measurements with the NO analyser.

The limitations of this study are the male predominance observed in our healthy population for the study of offline eNO, the small number of healthy subjects for the study of offline nNO and the inter-subject variability in nNO output in the 31 healthy children. In the present study, we screened children for inclusion based on medical examination and questionnaires without a physical exam, nor biomarkers of allergy, spirometry or provocation tests to confirm the health status of the studied children. This could have led to categorizing some children as healthy by error. However, allergic disease is not detected by physical examination with good sensitivity, as Struben et al noted \[27\], and children were not included who were in any doubt about their health status. Another point should be questioned concerning cooperation of the youngest children. The constant flow single breath technique requires cooperation, and offline nNO was measured during aspiration flow of 300 ml min\(^{-1}\) from one nostril during a 20 s breath hold, which seems quite long for the youngest children. However,
NO collections were well-tolerated by all of the children, who were able to provide technically acceptable manoeuvres for NO measurements and for which none of them needed more than three attempts.

In conclusion, we determined normal values and limits of the norms for eNO and nNO in healthy children aged 6 to 16 and in subgroups composed of children, pre-teens and adolescents, using the offline collection method. When offline eNO values increase or offline nNO values fall outside these reference ranges, this may indicate an abnormal status that justifies confirmation and further investigation by a physician. Given the importance of eNO as a surrogate of eosinophilic airway inflammation and nNO as a valuable biomarker for the diagnosis of PCD, our data could be useful in clinical practice, enabling the screening of large paediatric populations, especially for epidemiological purposes.

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Conflict of interest

None of the authors have any potential, perceived or real conflict of interest or financial arrangement (includes involvement with any organization with a direct financial, intellectual or other interest in the subject of the manuscript).

References

[1] Alving K, Weitzberg E and Lundberg J M 1993 Increased amount of nitric oxide in exhaled air of asthmatics Eur. Respir. J. 6 1368–70 (PMID 7907065)
[2] Byrnes C A, Dinarevic S, Shinebourne E A, Barnes P J and Bush A 1997 Exhaled nitric oxide measurements in normal and asthmatic children Pediatr. Pulmonol. 24 312–8
[3] Lundberg J O, Nordvall S L, Weitzberg E, Kollberg H and Alving K 1996 Exhaled nitric oxide in paediatric asthma and cystic fibrosis Arch. Dis. Child. 75 325–6
[4] Nelson B V et al 1997 Exhaled nitric oxide as a marker for childhood asthma J. Pediatr. 130 423–7
[5] Jackson C L et al 2016 Accuracy of diagnostic testing in primary ciliary dyskinesia Eur. Respir. J. 47 837–48
[6] Shapiro A J et al 2016 Diagnosis, monitoring, and treatment of primary ciliary dyskinesia: PCD foundation consensus recommendations based on state of the art review Pediatr. Pulmonol. 51 113–52
[7] Bush A et al 1998 Primary ciliary dyskinesia: diagnosis and standards of care Eur. Respir. J. 12 982–8
[8] Karadag B, James A J, Gultekin E, Wilson N M and Bush A 1999 Nasal and lower airway levels of nitric oxide in children with primary ciliary dyskinesia Eur. Respir. J. 13 1402–5
[9] Lundberg J O, Weitzberg E, Nordvall S L, Kuylenstierna R, Lundberg J M and Alving K 1994 Primarily nasal origin of exhaled nitric oxide and absence in Kartagener’s syndrome Eur. Respir. J. 7 1501–4
[10] Arnal J F et al 1999 Nasal nitric oxide concentration in paranasal sinus inflammatory diseases Eur. Respir. J. 13 307–12
[11] Nakano H et al 2000 Reduced nasal nitric oxide in diffuse panbronchiolitis Am. J. Respir. Crit. Care Med. 162 2218–20
[12] Robroeks C M et al 2008 Biomarkers in exhaled breath condensate indicate presence and severity of cystic fibrosis in children Pediatr. Allergy Immunol. 19 652–9
[13] Smith A D et al 2004 Diagnosing asthma: comparisons between exhaled nitric oxide measurements and conventional tests Am. J. Respir. Crit. Care Med. 169 473–8
[14] Berkman N, Aitai A, Breuer R, Bardach E, Springer C and Godfrey S 2005 Exhaled nitric oxide in the diagnosis of asthma: comparison with bronchial provocation tests Thorax 60 383–8
[15] de Meer G, van Amsterdam J G, Janssen N A, Meijer E, Steerenberg P A and Brummelkamp B 2005 Exhaled nitric oxide predicts airway hyper-responsiveness to hypertonic saline in children that wheeze Allergy 60 499–504
[16] Lex C, Dynek S, Heying R, Kovacevic A, Kramm C M and Schuster A 2007 Value of surrogate tests to predict exercise-induced bronchoconstriction in atopic childhood asthma Pediatr. Pulmonol. 42 235–30
[17] Yates D H, Khairitow N A, Robbins R A, Thomas P S and Barnes P J 1995 Effect of a nitric oxide synthase inhibitor and a glucocorticosteroid on exhaled nitric oxide Am. J. Respir. Crit. Care Med. 152 892–6
[18] Jones S L et al 2001 The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control Am. J. Respir. Crit. Care Med. 164 738–43
[19] van der Valk R J, Baraldi E, Stern G, Frey U and de Jongsteg J C 2012 Daily exhaled nitric oxide measurements and asthma exacerbations in children Allergy 67 265–71
[20] Beucher J, Chambellan A, Segaln J and Deneuville E 2011 Primary ciliary dyskinesia: a retrospective review of clinical and paraclinical data Rev. Mal. Respir. 28 856–63
[21] Baraldi E and de Jongsteg J C 2002 European Respiratory Society/American Thoracic Society task F. Measurement of exhaled nitric oxide in children, 2001 Eur. Respir. J. 20 223–37
[22] Baraldi E, Azzolin N M, Cracco A and Zachello F 1999 Reference values of exhaled nitric oxide for healthy children 6–15 years old Pediatr. Pulmonol. 27 54–8
[23] Buchvald F et al 2005 Measurements of exhaled nitric oxide in healthy subjects age 4 to 17 years J. Allergy Clin. Immunol. 115 1130–6
[24] Kovesi T, Kulka R and Dales R 2008 Exhaled nitric oxide concentration is affected by age, height, and race in healthy 9- to 12-year-old children Chest 133 169–75
[25] Daya H et al 2002 Nasal nitric oxide in children: a novel measurement technique and normal values Laryngoscope 112 1831–5
[26] Leigh M W et al 2013 Standardizing nasal nitric oxide measurement as a test for primary ciliary dyskinesia Ann. Am. Thorac. Soc. 10 574–81
[27] Struben V M et al 2005 Nasal NO: normal values in children age 6 through to 17 years Eur. Respir. J. 26 453–7
[28] American Thoracic Society 1999 Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999 Am. J. Respir. Crit. Care Med. 160 2104–17
[29] American Thoracic Society 2005 ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide Am. J. Respir. Crit. Care Med. 171 912–30
[30] Silkoff P E, Stevens A, Pak J, Bucher-Bartelson B and Martin R J 1999 A method for the standardized offline collection of exhaled nitric oxide Chest 116 754–9
[31] Balfour-Lynn I M, Laverty A and Dinwiddie R 1996 Reduced upper airway nitric oxide in cystic fibrosis Arch. Dis. Child. 75 319–22
[32] Bland JM and Altman DG 1986 Statistical methods for assessing agreement between two methods of clinical measurement *Lancet* 1 307–10

[33] Grzelweski T et al 2012 Fractional exhaled nitric oxide (FeNO) may predict exercise-induced bronchoconstriction (EIB) in schoolchildren with atopic asthma *Nitric Oxide* 27 82–7

[34] Corbelli R, Bringolf-Isler B, Amacher A, Sasse B, Spycher M and Hammer J 2004 Nasal nitric oxide measurements to screen children for primary ciliary dyskinesia *Chest* 126 1054–9

[35] Horvath I et al 2003 Comparison of exhaled and nasal nitric oxide and exhaled carbon monoxide levels in bronchiectatic patients with and without ciliary dyskinesia *Thorax* 58 68–72

[36] Narang I, Ersu R, Wilson NM and Bush A 2002 Nitric oxide in chronic airway inflammation in children: diagnostic use and pathophysiological significance *Thorax* 57 586–9

[37] Piacentini GL et al 2008 Nasal nitric oxide for early diagnosis of primary ciliary dyskinesia: practical issues in children *Respir. Med.* 102 541–7

[38] Harris A et al 2014 Validation of a portable nitric oxide analyzer for screening in primary ciliary dyskinasias *BMC Pulm. Med.* 14 18

[39] Beydon N et al 2015 Technical and practical issues for tidal breathing measurements of nasal nitric oxide in children *Pediatr. Pulmonol.* 50 1374–82

[40] Adams PS, Tian X, Zahid M, Khalifa O, Leatherbury L and Lo CW 2015 Establishing normative nasal nitric oxide values in infants *Respir. Med.* 109 1126–30

[41] Jobus Q, Schellekens S L, Kroesbergen A, Hop WC and de Jonge JC 2001 Off-line sampling of exhaled air for nitrogen oxide measurement in children: methodological aspects *Eur. Respir. J.* 17 898–903

[42] Rouatbi S, Aldqouda A, Ben Mdella S and Ben Saad H 2013 Fraction of exhaled nitric oxide (FeNO) norms in healthy North African children 5–16 years old *Pediatr. Pulmonol.* 48 981–95

[43] Kissoon N, Duckworth L J, Blake K V, Murphy S P, Taylor CL and Silkoff PE 2000 FeNO(1): relationship to exhalation rates and online versus bag collection in healthy adolescents *Am. J. Respir. Crit. Care Med.* 162 539–45

[44] Malmberg LP et al 2006 Exhaled nitric oxide in healthy nonatopic school-age children: determinants and height-adjusted reference values *Pediatr. Pulmonol.* 41 635–42

[45] Kharitonov SA, Goni F, Kelly C, Meah S and Barnes PJ 2003 Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children *Eur. Respir. J.* 21 433–8

[46] Santamaria F, Montella S, De Stefano S, Speri F, Barbarano F and Valerio G 2005 Relationship between exhaled nitric oxide and body mass index in children and adolescents *J. Allergy Clin. Immunol.* 116 1163–4 author reply 4–5

[47] Jerzynska J et al 2014 Predictive value of fractional nitric oxide in asthma diagnosis–subgroup analyses *Nitric Oxide* 40 87–91

[48] Kovesi T and Dales R 2008 Exhaled nitric oxide and respiratory symptoms in a community sample of school aged children *Pediatr. Pulmonol.* 43 1198–205

[49] Woo SL, Lee JH, Kim H, Kang JW, Sun Y H and Hahn Y S 2012 Utility of fractional exhaled nitric oxide (Fe(NO) measurements in diagnosing asthma *Respir. Med.* 106 1103–9

[50] Majid H and Kao C 2010 Utility of exhaled nitric oxide in the diagnosis and management of asthma *Curr. Opin. Pulm. Med.* 16 42–7

[51] Fritsch M et al 2006 Exhaled nitric oxide in the management of childhood asthma: a prospective 6-months study *Pediatr. Pulmonol.* 41 835–62

[52] van der Heijden HH, Brouwer ML, Hoekstra F, van der Pol P and Merkus PJ 2014 Reference values of exhaled nitric oxide in healthy children 1–5 years using off-line tidal breathing *Pediatr. Pulmonol.* 49 291–5

[53] Dweik RA et al 2011 An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FeNO) for clinical applications *Am. J. Respir. Crit. Care Med.* 184 602–15

[54] Schiller B, Hammer J, Barben J and Trachsel D 2009 Comparability of a hand-held nitric oxide analyser with online and offline chemiluminescence-based nitric oxide measurement *Pediatr. Allergy Immunol.* 20 679–85

[55] Bodini A, Pijnenburg M W, Boner AL and de Jongste JC 2003 Exhaled nitric oxide in mylar balloons: influence of storage time, humidity and temperature *Mediators Inflamm.* 12 47–9

[56] Linn W, Avila M and Gong H Jr Exhaled nitric oxide: sources of error in offline measurement *Arch. Environ. Health* 2004 59 385–91