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# Feasibility of nasal NO screening in healthy newborns

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## Abstract

**Background:** Nasal nitric oxide (nNO) measurement is recommended as a first line screening test for primary ciliary dyskinesia (PCD). While reliable velum- and non-velum-closure techniques exist for preschool children and older individuals, no data are available for neonates.

**Aims:** To determine feasibility of nNO screening and nNO concentration in healthy newborns in the first week of life.

**Methods:** Nasal NO was analyzed in tidal breathing during natural sleep using a CLD-88 sp NO analyzer (chemoluminescence sensor) and a NIOX MINO (electrochemical sensor). Test success and nNO concentration were determined and compared between the two devices.

**Results:** Nasal NO was measured in 62 healthy neonates within the first week of life. Feasibility of nNO measurement was 100% for at least one nostril and 85.5% for both nostrils using the chemoluminescence device, but significantly lower with the electrochemical device (85.5% and 53.2%; $p < .001$). Median nNO concentration was 38 ppb (interquartile range, 27–55; range, 9–100) with the ECOMEDICS device and 23 (15–33, 8–59) with the NIOX MINO ($p < .001$), with a trend towards higher values for older subjects. None of the subjects exceeded nNO levels of 100 ppb.

**Conclusion:** Measurement of nNO using a chemoluminescence device is highly feasible in newborns during natural sleep. However, nNO levels are considerably lower compared to the published data for older individuals and in the range of a PCD reference group of infants between 4 and 8 weeks of age, potentially resulting in a great overlap with subjects with PCD in this age group. Therefore, screening for PCD using nasal NO might not be useful in the first week of life. Upon clinical suspicion, other diagnostic tests such as high-speed video analysis of the cilia should be applied.

KEYWORDS

chemoluminescence, electrochemical sensor, nasal nitric oxide, newborns, primary ciliary dyskinesia, screening

**Abbreviations:** HSVA, high-speed video analysis of ciliary beating; IF, immunofluorescence labeling of ciliary proteins; nNO, nasal nitric oxide; PCD, primary ciliary dyskinesia; TEM, transmission electron microscopy.

## 1 | INTRODUCTION

Primary ciliary dyskinesia (PCD) is a rare, mostly autosomal recessive disease caused by a range of ultra-structural or functional defects in the respiratory cilia.[1–3] Clinical presentation of PCD can be apparent as early as during the neonatal period, with some patients experiencing airway symptoms from the time of birth. Symptoms may be caused by the retention of secretion due to reduced mucociliary clearance and range in severity from neonatal rhinorrhea and mild, transient tachypnoea to significant neonatal respiratory distress requiring prolonged respiratory assistance.[1,3,4] Persistent wet cough, recurrent chest infections leading to bronchiectasis, chronic rhinitis and recurrent otitis media with hearing impairment are common.[5] In approximately 50% of the cases organ laterality defects occur.[3,6]

Early diagnosis is important to avoid subjecting the patient to unnecessary diagnostic testing for differential diagnoses and is crucial for starting immediate and adequate therapy.[5] Although curative treatment for PCD is not available, early and consequent treatment helps patients to maintain lung function, prevents complications and improves quality of life. Furthermore, diagnosis allows genetic counseling for the family. For these reasons, a reliable screening test at neonatal age to detect children at risk would be desirable.

Measurement of nasal nitric oxide (nNO) is the recommended screening tool for PCD by the European Respiratory Society (ERS) and the American Thoracic Society (ATS) from the age of 5 years.[2,7] Nasal NO has been shown to have good sensitivity and specificity when testing for PCD, but is not accepted as a diagnostic tool on its own.[8] Nasal NO levels in patients with PCD are low (<77 nl/min when performed by exhalation against resistance), compared to normal values (mean, 287 nl/min; range, 125–867 nl/min), therefore nNO can be a useful test for PCD if performed correctly.[6] Another meta-analysis reported a mean ± SD nNO level of 19 ± 18.6 nl/min in PCD and 265 ± 118.9 nl/min in healthy controls.[9] As the output of nNO value depends on the device, study population and screening setting, to date no generally accepted cut-off values are available for nNO measurement performed via tidal breathing techniques. The measurements should be done using a chemoluminescence analyzer applying a velum-closure technique, for which the most data on reliability and validity exist.[1–3] If a chemoluminescence device is unavailable, devices with electrochemical sensors can be used.[2,10] When PCD is suspected clinically and/or as a result of nNO screening, confirmation of the diagnosis requires a combined approach, including high-speed video analysis of ciliary beating (HSVA), transmission electron microscopy (TEM), genotyping and immunofluorescence labeling of ciliary proteins (IF).[2,3] However, none of these methods can serve as a screening tool because of high costs and low availability outside of specialized centres.

Measurement by a chemoluminescence analyzer during velum closure is seen as the "gold standard" for nNO measurement but is obviously not feasible in young children, as velum closure needs the cooperation of the patient during breath hold or expiration against resistance.[11] Handheld devices with electrochemical sensors facilitate measurement for a broader application in the in- and outpatient setting, have been shown to be useful in clinical studies with good sensitivity and specificity and could potentially serve for widespread screening for PCD.[10,12] However, these devices have not been systematically tested and validated in newborns and infants.

In patients under the age of 5 years, measurements can be completed during tidal breathing with minimal cooperation, but may be less discriminative.[2,13] Previous studies have shown that nNO values differ significantly between the velum-closure technique and the tidal breathing method. In tidal breathing, contamination of nNO samples with lower airway gas leads to lower NO values. velum closure in resistor measurements samples mainly sinu-nasal NO leading to higher values than tidal breathing and has higher specificity and reproducibility.[11] While reliable velum-closure and non-velum-closure techniques are now available from the age of 2.5 years, there are no studies on the feasibility of these techniques for younger patients.[14] In addition, there are scarce data available on diagnostic cut-off values of nNO for children under two years of life.[15]

The aims of this study were to evaluate the feasibility of nasal NO measurement in healthy neonates within the first week of life, to determine nasal NO concentration in this specific age group and to compare measurements between a chemoluminescence device and an electrochemical sensor device.

## 2 | METHODS

### 2.1 | Study design

This was a multicentre, prospective, cross-sectional study in a healthy reference population. Measurements were performed at the Children's University Hospital Zurich and at the Cantonal Hospital Muensterlingen, Switzerland. The study was approved by the local ethics committee. The Declaration of Helsinki and the international rules for Good Clinical Practice were applied and written informed consent was obtained by all parents or legal guardians. Nasal NO measurements were performed under the terms of the guidelines of the ATS, ERS and PCD Foundation.[2,6,7]

### 2.2 | Subjects

Measurements were done in respiratory healthy neonates within the 1st week of life who were hospitalized at the maternity ward or neonatal ward after birth. Exclusion criteria were prematurity, neonatal infection or evidence for any inborn diseases. Patients requiring intensive care, oxygen or respiratory support were also excluded. A small clinical reference group of infants with PCD between the age of 4 and 8 weeks was also included. PCD had been confirmed in all subjects by high-speed video analysis of the cilia beating pattern, immunofluorescence staining of the ciliary proteins and/or genetic mutations analysis, according to international recommendations.[2,3,7]

### 2.3 | Equipment and sampling technique

Nasal NO measurements were performed using a CLD-88 sp NO analyzer (Eco Medics) and a NIOX MINO (Aerocrine). The CLD-88 device uses chemoluminescence for NO determination and provides real-time display of the NO curve on the screen at a sampling rate of 5.5 ml/s (0.30 L/min) with a lower detection limit of 0.06 ppb. Measurements were conducted in tidal breathing until the value stabilized around a virtual plateau, for a minimum of 5 s. Maximum stable peaks over a minimum of 5 s were averaged and recorded as nNO concentration for each nostril. The device was regularly calibrated according to the recommendation of the manufacturer. Ambient NO was captured at each measurement. The NIOX MINO makes use of an electrochemical sensor and the collected sample is buffered before analysis. The device represents the result as a screen output after approximately 45 s (sampling rate 5.0 ml/s equal 0.33 L/min), with a lower detection limit of 1.0 ppb. Only a complete, uninterrupted sampling throughout the required time leads to a successful test.[13]

### 2.4 | Nasal NO measurements

To achieve best possible standardization, obtain reliable values and facilitate measurements, nNO was assessed during tidal breathing in natural sleep. A complete nNO test consisted of two measurements. To control for possible functional or morphological differences, one measurements was performed in both nostrils. If results differed by more than 20%, a third measurement was performed at the nostril with the higher initial NO value. As unilateral nasal secretion such as remnants of the amniotic fluid may occur in neonates within the 1st days of life and can lead to false low NO values, the highest NO value was used for analysis. A synthetic olive with a central lumen was placed tightly in one nostril and connected with a tube to the NO analyzer. The other nostril remained unblocked. After placement of the olive, air was then withdrawn from the nostril by suction at the predefined sampling rate. All study subjects were measured with both analyzers in random order. Subjects with PCD received nNO testing only with the CLD-88 sp NO analyzer during their routine clinical diagnostic assessment, following the same protocol. Test success, nNO concentration in ppb, age, length, weight, sex, gestational age, and birth weight were recorded. NO production rate in nl/min were calculated as NO concentration multiplied with NO sampling rate for both devices.[2,6,7]

### 2.5 | Statistical analysis

It was calculated using STATA software (Version 15.1; StataCorporation) and SPSS 24 (IBM). Median and interquartile ranges were reported and nNO values of the different devices were compared using the Wilcoxon matched-pairs signed-rank test. nNO at different time points was compared with the Mann–Whitney test. To achieve normal distribution, nNO values were log-transformed and for regression analysis, results are shown as exponentiation coefficient with 95% confidence interval (CI). We studied the association between age at testing and nNO values with a univariable linear regression model. The agreement of the nNO measurements between the two devices was assessed graphically by the Bland–Altman method. With the Bland–Altman method, we calculated the upper and lower limits of agreement between the two nNO values measured with the different devices (mean difference ±1.96 SD of differences between devices).

## 3 | RESULTS

### 3.1 | Participants

In total, 62 healthy subjects (35 females, 56.5%) aged between 1 and 6 days of life were enrolled. Patient characteristics are depicted in Table 1. The clinical PCD reference group consisted of six individuals with confirmed PCD between the age of 4–8 weeks (Table 2).

### 3.2 | Feasibility and test success

For the chemoluminescence device, nNO measurements in at least one nostril were successful in all 62 neonates (100%) (Table 3). In

**TABLE 1** Clinical characteristics of the study participants

| | |
|---|---|
| Sex (female) | 35 (56.5%) |
| Gestational age at birth (weeks) | 39 (38, 40) (37–41) |
| Weight at birth (g) | 3320 (2960, 3700) (2050–5150) |
| Weight at measurement (g) | 3130 (2840, 3350) (1940–4900) |
| Length at measurement (cm) | 50 (48, 51) (40–54) |
| Age at measurement (days) | 3 (2, 3) (1–6) |

*Note*: Numbers are given as n (%) or median (interquartile range) (range).

**TABLE 2** Nasal NO values assessed by CLD-88 sp NO analyzer during a routine clinical assessment for infants with confirmed primary ciliary dyskinesia between 4 and 8 weeks of life

| Sex | Age (weeks) | nNO (ppb) | Median nNO (ppb) |
|---|---|---|---|
| F | 4 | 34 | 36 |
| F | 4 | 9 | |
| F | 5 | 38 | |
| M | 7 | 47 | |
| M | 8 | 22 | |
| M | 8 | 62 | |

Abbreviation: nNO,;.

**TABLE 3** Comparison of test success for nNO measurements using the chemoluminescence device (CLD-88 sp NO analyzer) and the electrochemical device (NIOX MINO)

| Test success (n, %) | CLD-88 sp NO analyzer | NIOX MINO | *p* |
|---|---|---|---|
| At least one nostril | 62 (100) | 53 (85.5) | <0.001 |
| Both nostrils | 53 (85.5) | 33 (53.2) | <0.001 |
| Failure due to technical problems | 0 | 6 (9.7) | NA |
| Failure due to awakening/agitation | 0 | 3 (4.8) | NA |
| Sampling time (s) | Individual (5–20) | Fixed (45) | NA |

*Note*: Numbers are given as *n* (%). Data were compared using $\chi^2$ and Mann–Whitney *U* test.

Abbreviations: NA, not assessed; nNO,;.

53 (85.5%) of the cases, nNO measurements in both nostrils was successful. A third measurement was performed in 13 of the 53 (24.5%) of the neonates. The highest NO value was used for analysis. Sampling duration was between 5 and 15 s, but never exceeded 20 s. No technical errors occurred, and measurements could also be completed if the subjects awakened during the measurement, as long as they remained breathing calmly. NO measurement was terminated once the subject started crying or became agitated, as this usually lead to a high risk of disconnecting the olive from the nostril.

For the electrochemical device, nNO measurements were successful in 53 cases (85.5%) (Table 3). Only 33 (53.2%) of the subjects had successful measurement for both nostrils. A third measurement was necessary in 13 individuals (39.4% of 33 subjects) when values for both nostrils differed >20%. Nasal NO results were not available in six children because of technical problems with the electrochemical device (continuous error messages due to interruption of the sampling flow as a result of nasal tidal breathing). In the remaining three subjects, nNO measurement was terminated due to the subject awakening with subsequent agitation. Consequently, success rate differed significantly between the two devices, with a higher success for the chemoluminescence device ($p < .001$).

### 3.3 | Nasal NO concentration in healthy newborns

Median nNO concentration for the chemoluminescence device was 38 ppb (interquartile range [IQR], 27, 55; range, 9–100) and thus considerably lower than the published reference values for older healthy children. Nasal NO did not exceed 100 ppb in any of the children; 42 subjects (67.7%) had NO levels below 50 ppb. Ambient air was at a constant stable level of 5.0 ppb (3.6, 6.2). The electrochemical sensor device showed even lower results with a median nNO concentration of 23 ppb (IQR, 15, 33.5; range, 8–59). All but one subject had values <50 ppb. NO concentration was significantly lower compared to the chemoluminescence device ($p < .001$; Figure 1A). The distribution of all nNO values for both devices is depicted in Figure 1B. For sampling rate corrected nNO production rate, differences between devices were comparable to nNO concentration. NO was 12.5 nl/min (9.9, 19.1; range, 2.9–33.0) for the chemoluminescence device and 6.9 nl/min (4.5, 9.9; range, 2.4–17.7) for the electrochemical sensor device. NO concentration was significantly lower for the electrochemical sensor device compared to the chemoluminescence device ($p < .001$).

Nasal NO measured by chemoluminescence device for subjects with PCD was in a similar range compared to the healthy neonates with 9–62 ppb and a median of 38 ppb, demonstrating a large overlap between healthy and affected individuals (Table 2).

We detected a high intra-subject variability in the nNO results measured with both devices. We calculated the difference in nNO values by subtracting the values obtained with the electrochemical sensor device from the values measured with the chemoluminescence device. Bland–Altman analysis revealed poor agreement between measurements performed with the two different testing devices. The upper and lower limits of 95% agreement between devices were 15 and −47 ppb. There was a high difference in nNO values measured with the two devices of 16 ppb in the mean (range −60 to +10). The majority of nNO values measured with the electrochemical sensor technique were lower ($n = 46$) and only a few were higher ($n = 6$) as compared to the chemoluminescence method (Figure 2A,B). Only one subject had the same nNO value measured on both devices. There were also relevant differences between the two testing devices when nNO was analyzed using the NO production rate after correction for sampling rate of the devices. The upper and lower limits of 95% agreement between the devices were 16 and −4 nl/min. The NO difference measured with the two devices was 6 nl/min in the mean (range, −3 to +20). The majority of nNO values measured with the electrochemical sensor technique were lower ($n = 50$) and only a few were higher ($n = 3$) as compared to the chemoluminescence method.

When nNO concentration was assessed during the first week of life by the CLD-88 sp NO analyzer, a trend towards higher values for older subjects was visible (Figure 3). In a univariable regression model, per one day increase in age, nNO (ppb) increased by (Coef., 8.87; 95% CI, 3.05–14.9, $p = .00$), and nNO (nl/min) by (Coef., 2.92; 95% CI, 1.01–0.84; $p = .004$). For the NIOX MINO device, a similar association was observed. In a univariable regression model, per one day increase in age, nNO (ppb) increased by (Coef., 4.1; 95% CI, −0.16–7.00; $p = .061$), and nNO (nl/min) by (Coef., 1.02; 95% CI, −0.5–2.10; $p = .061$).

## 4 | DISCUSSION

In this study on healthy neonates during natural sleep, nasal NO determination could successfully be performed in all subjects with a chemoluminescence device for at least one nostril and 85% for both nostrils. The success rate for a hand-held device with an

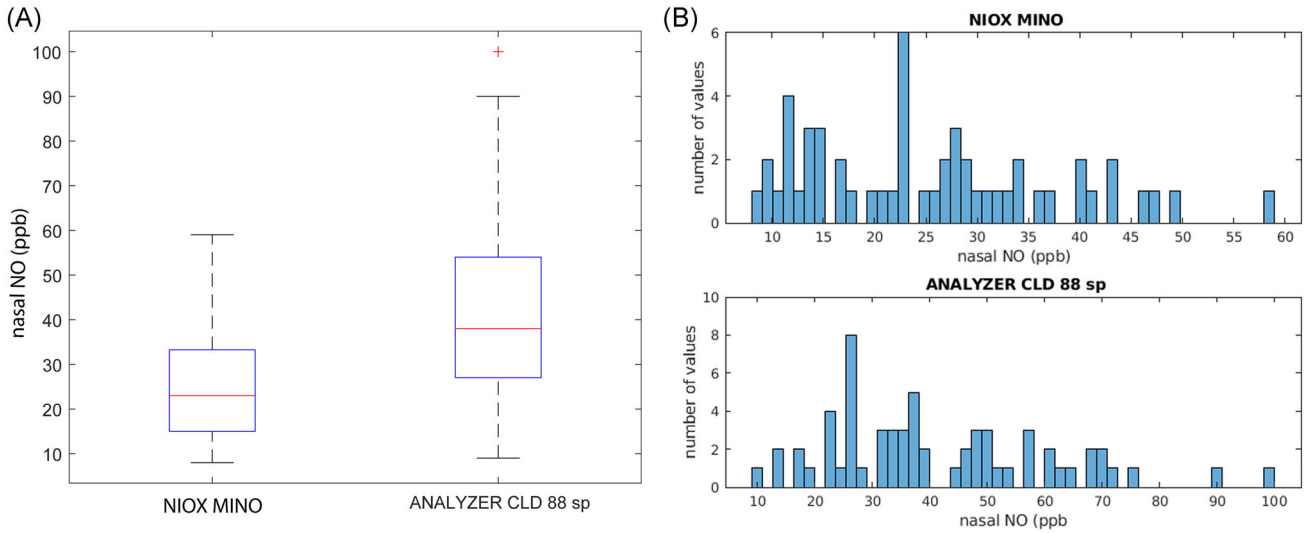


**FIGURE 1** (A) Comparison of nNO in newborns ≤7 days of age for the chemoluminescence device and the electrochemical sensor device ($p$ = <0.001) (Wilcoxon sign-rank test). (B) Distribution of nNO values of the entire study sample measured with the NIOX MINO and the CLD-88 sp NO analyzer [Color figure can be viewed at wileyonlinelibrary.com]

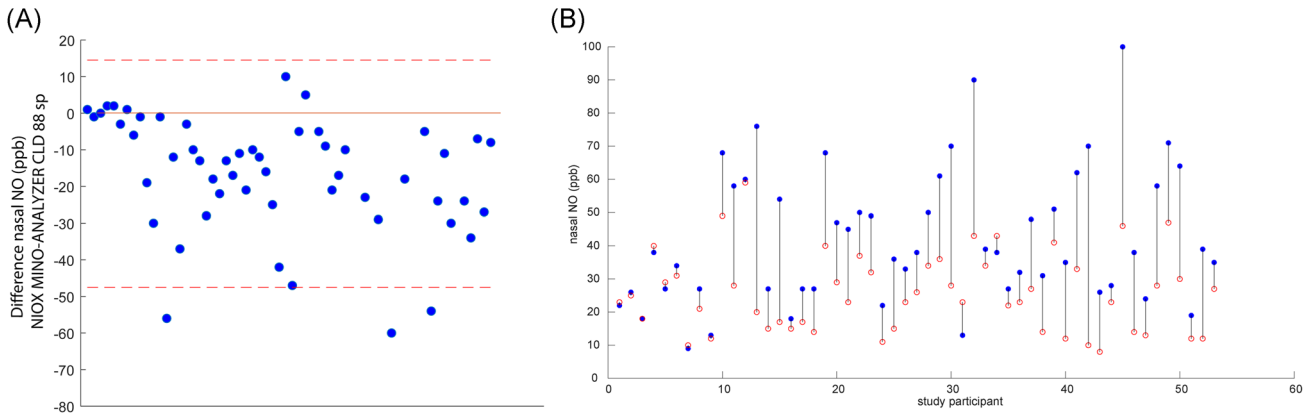


**FIGURE 2** (A) Bland-Altman plot showing the difference between nNO values measured with the CLD-88 sp NO analyzer or the NIOX MINO device. Upper and lower limits of 95% agreement are illustrated as dashed lines. The upper and lower limits of 95% agreement between devices were 16 and −47 ppb, respectively. (B) Distribution of nNO values measured with the CLD-88 sp NO analyzer or the NIOX MINO device. Closed blue circles show values measured with the CLD-88 sp NO analyzer, open red circles show values measured with NIOX MINO device. Paired measurements of each study participant are connected with a line [Color figure can be viewed at wileyonlinelibrary.com]

electrochemical sensor was significantly lower (85.5% and 53.2%). The median nNO concentration was below 100 ppb for all neonates and significantly lower when the electrochemical sensor device was used, with poor agreement between the two devices used. Nasal NO showed a trend towards higher values for older subjects, but still remained below 50 ppb for the majority of individuals. As nNO levels depend on sampling rate of the device used, we have calculated nasal NO production rate in addition to NO concentration for both methods, with comparable results.

For the application of nasal NO screening tools for PCD in young, non-cooperative children, standardized protocols, an increasing body of data on tidal breathing methods and the evaluation of threshold values are required. It is highly desirable that more research is done in this age group, so that age at diagnosis can be shifted from the current typical age of >3 years to an earlier age. The new handheld nNO devices are becoming widespread and have great potential because of their size and simple handling technique with minimal need for staff training. Since advanced methods of diagnostic testing for PCD such a HSVA, TEM and IF are only available in larger and experienced centres and genetic testing has still limited sensitivity to diagnose certain PCD variants, a simple test is desirable to achieve early PCD screening in young children including neonates and infants. Especially for smaller centres and pediatricians, more widespread use of the nNO measurements may occur as cheaper nNO analyzers become available. Developing an algorithm for the application of nNO devices in primary care to assess and interpret nNO

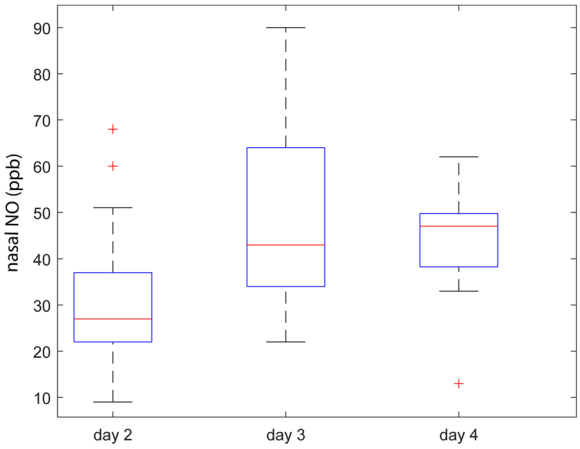


**FIGURE 3** Nasal NO concentration during the first week of life assessed by the CLD-88 sp NO analyzer. Median (IQR) nNO was 27 (22, 37) for day 2 ($n$ = 22 measurements), 43 (34, 64) for day 3 ($n$ = 26), and 47 (38, 50) for day 4 ($n$ = 11). nNO between day 2 and 3 differed significantly ($p$ = .001), but not between day 3 and 4 ($p$ = .777). Tested with the Mann–Whitney test. Measurements at days 1, 5, and 6 were excluded due to low case numbers. IQR, interquartile range [Color figure can be viewed at wileyonlinelibrary.com]

concentration and to determine when to repeat tests, when to refer the patient to a centre or when to rule out PCD, might be an important future goal.

This study assessed measurement of nNO in healthy newborns within the first week of life using a tidal breathing method during natural sleep. The measurements during spontaneous sleep and repeated measurements from both nostrils allowed some standardization to determine the maximum nasal NO concentration. Measurements proved feasible using both the NIOX MINO handheld device and the CLD-88 sp NO analyzer. However, the success rate was lower for the electrochemical sensor device and especially poor for this device in respect of yielding a result for both nostrils in the same individual. For the device with the electrochemical sensor, unpredictable technical errors occurred, most likely as a result of interruption of the sampling flow by tidal breathing via the nose. In addition, some neonates awakened with subsequent agitation during the rather long investigation time of 45 s, resulting in an interruption of the measurements. The fixed sampling time of 45 s is a result of the technical features of devices with electrochemical sensors, in which nNO analysis is completed in a collection chamber after a definite amount of air is aspired. This is different to chemoluminescence devices, which perform a "real-time" nNO analysis until a stable plateau of gas concentration is reached. The resulting, much shorter collection time of typically 5–15 s was an important factor for the test success rate of 100% in this age group of uncooperative neonates. As a possible limitation, the sampling time for both methods was below the 60 s for tidal breathing measurement as suggested by the PCD Foundation[6] due to technical reasons (electrochemical sensor device) and as a longer sampling time would have decreased feasibility of the chemoluminescence method. This might have resulted in non-maximal nNO values in some individuals.

Currently, nasal NO measurement is the recommended screening tool for PCD for children over the age of 5 years.[2,6,7] Nitric oxide is produced by the epithelial cells of the respiratory tract, with the paranasal sinuses as the main site of production, resulting in a high level of NO in sinus and nasal air. In patients with PCD, nNO values are significantly lower than in healthy individuals.[16] As the standardized measurement - exhalation against resistance—requires cooperation, the method is unavailable for younger, non-cooperative children. Mateos-Corral et al.[17] evaluated different non-velum-closure techniques (breath hold, tidal breathing mouth open, tidal breathing mouth closed and humming) to assess nNO in children from the age of 5 years with good reproducibility and discrimination between affected subjects and controls without PCD despite generally lower nNO values compared to velum-closure methods. In a previous study we showed that nNO determination is highly feasible in younger children from 2.5 years using a velum-closure method by tidal breathing via a resistance tube.[14] In infants below this age, nNO measurement is only possible in tidal breathing without velum closure.

Adams et al.[15] measured nNO in 42 healthy individuals within the 1st year of life. nNO values did not exceed 100 nl/min and depended on age, with lower values in younger individuals. The authors established a 95% prediction interval for normal nNO and demonstrated good discrimination in a small number of subjects with PCD in this age group. A more recent study also demonstrated feasibility of nNO measurement (with a success rate of >99%) and longitudinal increase in nNO over the 1st years of life.[18] Several healthy individuals had very low nNO levels indistinguishable from PCD, and the authors concluded that these subjects needed further diagnostic testing. However, in both studies no newborns below 8 days of age were included. To our knowledge, there is to date only one published case report on determination of nNO in a newborn with PCD.[19] This subject showed an nNO concentration of below 5 ppb on day four and kept a low value of 9.4 ppb on day 34, while six, healthy, term newborns with a median age of 14 days (range, 2–24) showed nNO levels in the range of 100–232 (median, 171.2 ppb). The authors suggest that nasal NO measurements may be used to diagnose PCD in newborns. However, further studies in newborns are so far lacking.

In our cohort of healthy neonates measured during the first week of life, nasal NO values were remarkably lower compared to published data for older individuals, despite the observed trends for slightly higher values in older newborns. Comparing the results of nNO determination of the healthy neonates to a small clinical group of slightly older individuals with confirmed PCD demonstrated nNO concentration in the same range for both groups with a similar median, and we could not distinguish the individuals as healthy or affected by their nasal NO values, None of the healthy individuals reached NO levels >100 ppb, and in most cases NO values remained below 50 ppb, in a range that has been shown to be strongly associated with PCD in older individuals. As the paranasal sinuses are not yet pneumatized in newborns, their very low nasal NO values can

mainly be explained as a result of this anatomical condition. In addition, the impossibility of applying velum-closure techniques in this age group might contribute to a generally lower nNO concentration. To date, no data have been published on the nasal NO concentration of newborns in the first week of life with PCD. Despite this, it can be assumed that the discriminative power of nasal NO in this age group to distinguish between affected and non-PCD individuals remains very weak. Nasal NO concentration was significantly lower when the device with the electrochemical sensor was used compared to the chemoluminescence "gold standard" device. The reason for this deviation in output is the different sampling method and analysis. Devices with electrochemical sensors calculate the mean NO over a fixed period of collection, and do not determine the maximum NO concentration in a stable plateau of the aspirated gas. While in older, healthy individuals with nasal NO values above 200 ppb, these technical differences between devices might be negligible in terms of clinical interpretation of the test results, it is problematic in younger individuals with generally lower NO levels. In this age group, nNO concentration is generally low, hampering the discriminative power to distinguish between individuals with PCD and healthy subjects and resulting in a larger overlap of NO values. As the electrochemical sensor device yields even considerably lower results compared to the chemoluminescence device, hand-held devices with electrochemical sensors might not be the first choice to screen for PCD in the neonatal and infant age.

A limitation of this study is the circumstance that we performed nasal NO measurement only in naturally sleeping children to standardize measurements in the best possible way. Although this is probably the approach of choice in hospitalized individuals, where time point of examination is rather flexible, it exceeds the possibilities of an outpatient clinic, where children are usually awake and the investigator's time is scarce. We have not assessed feasibility of nNO measurement of awake individuals in this study, to avoid confounders in respect of cooperation, and this is likely to influence the test results. Consequently, real-life studies in the outpatient setting might lead to a different outcome. Another limitation is the lack of a control group with diagnosed PCD in our study population,, despite we included a small reference group of slightly older individuals of 4–8 weeks with confirmed PCD. However, measuring nNO in neonates with PCD this is a difficult endeavor, as prevalence is low with an estimated 1:20.000 in Switzerland,[20] and individuals are mostly diagnosed at a later stage in life but not as early as the first week of life. Thus, larger populations of newborns with PCD to compare with healthy subjects in respect of their nNO levels are still lacking.

In conclusion, nNO determination is feasible in newborns during natural sleep in tidal breathing. Feasibility is significantly higher (up to 100%) when a chemoluminescence device is used compared to devices with electrochemical sensors. Nasal NO values in newborns were considerably lower compared to the published data for older individuals and did not succeed values of 50–100 ppb, but were in a similar range of individuals with confirmed PCD between 4 and 8 weeks of life, most likely resulting in a relevant overlap with subjects with PCD in this age group. Therefore, screening for PCD using nasal NO might not be useful in the first week of life because of its low discriminative power and can therefore currently not be recommended, though to date no data on nasal NO levels in newborns with PCD are available. It is be advisable to postpone PCD screening to a later age when discrimination between affected and healthy individuals is more robust. Upon clinical suspicion of PCD in a newborn child, a combination of other diagnostic methods such as HSVA of ciliary beating pattern, TEM of the structural defect, IF staining of the ciliary proteins and/or genetic testing should be applied, according to the current recommendations.[2,6,7]

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## AUTHOR CONTRIBUTIONS

**Flurina Buechel**: data curation (supporting); investigation (equal); resources (supporting); writing original draft (equal). **Jakob Usemann**: data curation (supporting); formal analysis (lead); methodology (supporting); resources (supporting); software (equal); supervision (supporting); validation (equal); visualization (equal); writing review & editing (equal). **Peter Salfeld**: conceptualization (supporting); data curation (supporting); investigation (equal); resources (supporting); supervision (supporting); writing original draft (supporting). **Alexander Moeller**: conceptualization (supporting); formal analysis (supporting); methodology (equal); resources (supporting); supervision (supporting); validation (supporting); writing original draft (equal); writing review & editing (equal). **Andreas Jung**: conceptualization (lead); data curation (lead); formal analysis (supporting); funding acquisition (lead); investigation (equal); methodology (equal); project administration (lead); resources (lead); software (supporting); supervision (lead); validation (lead); visualization (equal); writing original draft (lead); writing review & editing (lead).

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