Lung (2019) 197:209–216
https://doi.org/10.1007/s00408-019-00202-x

AIRWAY BIOLOGY

Proof of Concept: Very Rapid Tidal Breathing Nasal Nitric Oxide Sampling Discriminates Primary Ciliary Dyskinesia from Healthy Subjects

Mathias G. Holgersen[1] · **June K. Marthin**[1] · **Kim G. Nielsen**[1]

Received: 17 October 2018 / Accepted: 6 February 2019 / Published online: 14 February 2019

Abstract

Introduction Nasal nitric oxide (nNO) is extremely low in individuals with primary ciliary dyskinesia (PCD) and is recommended as part of early workup. We investigated whether tidal breathing sampling for a few seconds was as discriminative between PCD and healthy controls (HC) as conventional tidal breathing sampling (cTB-nNO) for 20–30 s.

Methods We performed very rapid sampling of tidal breathing (vrTB-nNO) for 2, 4 and 6 s, respectively. Vacuum sampling with applied negative pressure (vrTB-nNO_{vac}; negative pressure was applied by pinching the sampling tube) for < 2 s resulted in enhanced suction of nasal air during measurement. Feasibility, success rate, discriminatory capacity, repeatability and agreement were assessed for all four sampling modalities.

Results We included 13 patients with PCD, median (IQR) age of 21.8 (12.2–27.7) years and 17 HC, 25.3 (14.5–33.4) years. Measurements were highly feasible (96.7% success rate). Measured NO values with vrTB-nNO modalities differed significantly from TB-nNO measurements (HC: $p < 0.001$, PCD: $p < 0.05$). All modalities showed excellent discrimination. The vacuum method gave remarkably high values of nNO in both groups (1865 vs. 86 ppb), but retained excellent discrimination. vrTB-nNO_{4sec}, vrTB-nNO_{6sec} and vrTB-nNO_{vac} showed identical specificity to cTB-nNO (all: 1.0, 95% CI 0.77–1.0).

Conclusion vrTB-nNO sampling requires only a few seconds of probe-in-nose time, is feasible, and provides excellent discrimination between PCD and HC. Rapid TB-nNO sampling needs standardisation and further investigations in infants, young children and patients referred for PCD workup.

Keywords Primary ciliary dyskinesia · Nasal nitric oxide · Rare lung diseases

Introduction

Primary ciliary dyskinesia (PCD), a rare heterogeneous genetic disorder in which functionally defective motile cilia lead to recurrent airway infections, causes irreversible lung damage over time. In PCD patients, lung function deteriorates with age; subnormal lung function and structural damage are not uncommon, even among children [1–4]. Early diagnosis is critical for prompt interventions to decelerate lung damage.

Symptoms of PCD are often non-specific, though in recent years tools for aiding referrals have been made [5]. Diagnosis is often delayed despite recent advances; median age at diagnosis in Europe is 5.3–6 years [6, 7]. Measurement of nasal nitric oxide (nNO) is an inexpensive, non-invasive and highly specific test used as part of the diagnostic workup of PCD in cooperative patients [8, 9]. Significantly reduced nNO level is a known feature of PCD. NO is an

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00408-019-00202-x) contains supplementary material, which is available to authorized users.

✉ Kim G. Nielsen
Kim.G.Nielsen@regionh.dk

Mathias G. Holgersen
Mathias.geldermann.holgersen.01@regionh.dk

June K. Marthin
June.Kehlet.Marthin.01@regionh.dk

[1] Danish PCD & chILD Centre, CF Centre Copenhagen, Paediatric Pulmonary Service, Department of Paediatrics and Adolescent Medicine, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

endogenous gas synthesized in the respiratory epithelium, with concentrations significantly higher in the upper airways than in the lower airways. To avoid dilution from the lower airways, methods that provide velum closure (VC) are recommended; however, such methods require cooperation and are generally only considered in children > 5 years of age [8, 10–13].

nNO is low in patients with PCD compared with healthy subjects and disease controls, regardless of VC or non-closure sampling [14]. Diagnosis is a complex multi-step process; additional diagnostic workup includes genetic testing, live ciliary assessment by High Speed Video Microscopy (HSVM), and/or transmission electron microscopy (TEM) analysis of the nasal epithelium. Because such tests are more expensive, invasive and time consuming, nNO is often an early test prior to confirmatory diagnostic workup [8, 9].

In patients unable to cooperate to VC, conventional tidal breathing sampling (cTB-nNO) is an alternative sampling method with comparable ability to discriminate PCD from healthy although cTB-nNO values are generally lower than VC nNO [10, 11, 15, 16].

We have observed how nNO values in patients with PCD reach maximal plateau levels within a very short time, regardless of whether VC nNO or cTB-nNO sampling is used. Thus, we hypothesized that substantially reducing sampling time would not compromise separation of PCD from HC.

Methods

Aim

Shortening probe-in-nose time may increase acceptance of measurements in uncooperative patients. We aimed to study the potential of very rapid nNO measurement during tidal breathing (vrTB-nNO) to satisfactorily discriminate patients with PCD from healthy controls (HC).

Design

This study was a single-centre, single-visit, proof of concept, method comparison study testing multiple vrTB-nNO measurement modalities in subjects with PCD and non-smoking, age-matched HC.

Participants' Characteristics

Inclusion Criteria

All subjects were anatomically able to perform NO measurements from both nostrils.

Confirmed PCD diagnosis was based on clinical phenotype and diagnostics by ≥ 1 of the following: nasal biopsy/scraping with ultrastructural defects compatible with PCD hallmarks, genetic test positive for bi-allelic mutation in a known PCD-causing gene, positive immunofluorescence testing or multiple separate occasions of low nNO together with abnormal ciliary motility on high-speed video microscopy.

Exclusion Criteria

No subjects were current or recent smokers (defined as < 6 months since quitting) or had acute respiratory symptoms and/or signs of upper or lower respiratory tract infection within the last 2 weeks. No HC had asthma, airway-related or immune problems. Subjects with radial spoke head (RSPH) mutations were not excluded, although this mutation might result in normal nNO levels [17, 18].

Setting for nNO Measurements

nNO was measured using the stationary CLD88sp F_ENO chemiluminescence analyser (ECO MEDICS® AG, Duernten, Switzerland) at a sampling rate of 0.33 L/min. Ambient NO was recorded before each measurement. NO zero calibrations using a DENOX 88 module to generate continuous NO-free airflow were performed before each subject inclusion. Flow calibrations were performed daily using a 100-mL calibration syringe (Hans Rudolph Inc.™, Shawnee, USA). To ensure the accuracy of NO measurements, SPAN calibrations were performed monthly using a calibration gas at 2 ppm (Linde AG™, Munich, Germany). All measurements were made using the offline setting.

nNO Sampling Modalities

We compared conventional for 30–45-s TB-nNO (cTB-nNO) measurements during tidal mouth breathing with very rapid modalities (vrTB-$nNO_{2,4,6sec}$) with sampling spans of 2, 4 and 6 s, respectively. We also introduced a novel very rapid sampling modality (vrTB-nNO_{vac}; sampling span < 2 s); in this procedure, pinching the sample tube increased the sample flow rate upon release. Demonstrational screen-recordings of all modalities are available in the online supplementary material.

cTB-nNO Measurement

Full-length cTB-nNO measurements were performed using a nasal sampling tube. Subjects were instructed to breathe normally with an open mouth. The sampling tube was gently inserted into the nostril and transnasal air was sampled continuously for 30 s (constant flow 0.33 L/min).

vrTB-nNO$_{2,4,6sec}$ Measurement

For vrTB-nNO$_{2,4,6sec}$ modalities, measurements were started on the apparatus before nasal sampling tube insertion. Once started, a live wave-form of ambient NO concentration appeared on-screen following a timeline. The sampling tube was inserted into a nostril at the timeline 3-s mark. Sampling continued for 2, 4 or 6 s; the sampling tube was removed to end the measurement.

vrTB-nNO$_{vac}$ Measurement

The nasal sampling tube was inserted into a nostril before starting vrTB-nNO$_{vac}$ measurement. Pinching the sample tube between the thumb and index finger resulted in a build-up of negative pressure. Measurement was started on the apparatus when NO concentration was zero (indicating a complete block of airflow) and with release of the pinching at the timeline 3-s mark. This entails a high inspirational flow through the tube, resulting in an abnormally high concentration peak, after which the measurement was ended.

Tracing Analysis

cTB-nNO Measurement

Once a stable plateau was reached, maximal values from the three highest concentration peaks were averaged to estimate the concluded nNO value.

vrTB-nNO$_{2,4,6sec}$ Measurement

Due to the short time frame of sampling, only one to two concentration peaks appeared. The concluded nNO value was estimated as the maximal value reached during each measurement.

vrTB-nNO$_{vac}$ Measurements

The maximal value reached was used as the concluded TB-nNO estimate.

TB-nNO Reporting

The measurement modalities were performed three times, and the mean of all three concluded nNO concentration values was used and reported in parts per billion (ppb).

Statistical Considerations

Statistical analyses were performed using SPSS version 22. Discriminatory capacity was assessed with receiver operating characteristic (ROC) curves to estimate each test's sensitivity and specificity. Cut-offs were set at 100% sensitivity with the highest possible specificity. 95% Confidence intervals for specificity and sensitivity were calculated using a clinical research calculator [19]. The Shapiro Wilks test was used to test for normal data distribution, with rejection of normality if $p < 0.05$. Statistically significant differences between nNO levels obtained with cTB-nNO and vrTB-nNO$_{2,4,6sec}$ were assessed using a paired sample *t* test and the Wilcoxon signed rank test for non-parametric data. Bland–Altman (difference) plots were used to assess agreement and average bias between measurement modalities for both groups. cTB-nNO was considered the reference method, and therefore, used as the best estimate of the true nNO concentration and plotted on the *x* axis [20]. Differences that were not normally distributed were transformed to percent difference to achieve normality and preserve easy interpretation as opposed to logarithmic transformation. To assess the presence of any proportional bias, simple linear regression was performed with the aim of rejecting the null hypothesis that the coefficient (β) for cTB-nNO equals 0. Based on experience with TB-nNO measurements [11, 21], including approximately 10 subjects per group would ensure the hypothesized difference between patients with PCD and HC was detected with > 99% power. A *p* value of < 0.05 was considered significant.

Results

Patient Characteristics

We included 13 patients with PCD [10 males, 3 females; median age 21.8 years (IQR 12.2–27.7; range 9.0–65.0)] and 17 age-matched HC [7 males, 10 females; median age 25.3 years (IQR 14.5–33.4; range 4.8–56.9)]. PCD characteristics are presented in Table 1. One subject had mutations in RSPH9; however, nNO levels were low and substantially below cut-off (cTB-nNO: 29.3).

vrTB-nNO Values in PCD Patients and HC

Dispersion of nNO measurements are presented in Fig. 1. Median (range) HC/PCD values (all in ppb) were as follows: vrTB-nNO$_{2sec}$, 372 (141–553)/19 (3–179); vrTB-nNO$_{4sec}$, 496 (240–652)/23 (3–90); vrTB-nNO$_{6sec}$, 530 (335–824)/30 (4–108); vrTB-nNO$_{vac}$, 1865 (556–3804)/86(8–335). Ambient NO was rarely > 5 ppb (1.65 nL/min) and never > 10 ppb (3.3 nL/min).

Feasibility

Three successful measurements for all modalities were achieved for 29/30 subjects with five total tries maximum

Table 1 Characteristics of patients with PCD

Median age (IQR)	21.8 (12.2–27.7)
Sex, male/female	10/3
Situs inversus, yes/no	6/5
Median age at diagnosis (years) (IQR)	9.5 (1.0;22.0)
Median cTB-nNO at diagnosis (ppb) (IQR)	25.6 (11.0;38.6)
Mutation site, *n* (%)	
DNAH5	2 (15.4)
DNAH11	1 (7.7)
CCDC39	1 (7.7)
CCDC40	1 (7.7)
CCDC151	1 (7.7)
RSPH9	1 (7.7)
HYDIN	1 (7.7)
NA	5 (38.5)
Abnormal IF site, *n* (%)	
ODA	4 (30.8)
N-DRC & IDA	4 (30.8)
RSP	1 (7.7)
None	1 (7.7)
NA	3 (23.1)
TEM defect, *n* (%)	
ODA	4 (30.8)
Transposition	2 (15.4)
Radial spoke	2 (15.4)
CP	1 (7.7)
Hydin	1 (7.7)
NA	3 (23.1)
HSVM result, *n* (%)	
Low frequency, asynchronous	9 (69.2)
Immotile	4 (30.8)

per modality. In one case (PCD, age 13) we successfully obtained only two acceptable vrTB-nNO$_{6sec}$ measurements; therefore, one measurement is missing, and the success rate was 96.7%.

Discriminatory Capacity

With vrTB-nNO$_{2,4,6sec}$, PCD nNO values were consistently low regardless of probe time (Fig. 1). Results from ROC-analysis are presented in Table 2. All test modalities showed excellent discrimination between PCD and HC. Both groups showed considerably higher nNO levels with vrTB-nNO$_{vac}$, but all subjects were still correctly identified with cut-off set at 446 ppb.

Repeatability and Agreement

Differences between individual triplet measurements are visualized in Fig. 2. Intra-measurement variations were considerable; coefficients of variation (CV) ranged from 0.01 to 0.84 (Supplementary figure available online) but decreased with increasing measurement time. cTB-nNO had a median CV of 0.11; median CV for vrTB-nNO$_{2,4,6sec}$ were 0.21, 0.17 and 0.16, respectively. vrTB-nNO$_{vac}$ had a median CV of 0.21.

Using the vrTB-nNO$_{2,4,6sec}$ modalities, nNO levels among HC differed significantly from the reference method ($p < 0.001$). The same was true among PCD patients ($p < 0.05$), except for vrTB-nNO$_{2sec}$ ($p = 0.507$).

Bland–Altman plots are presented in Fig. 3. For both groups, limits of agreement, defined as mean ± 1.96 SD, were narrower with increased measurement time, although at best, they were too wide to be acceptable for precision measurements. In the PCD group, proportional bias was present for vrTB-nNO$_{2,4sec}$ ($p < 0.05$, $\beta = 0.739$ and -0.379); curiously, one was positive and the other negative. In the control group all modalities showed proportional bias ($p < 0.001$, $\beta = -0.927$; -0.868 and -0.722 for 2, 4 and 6 s, respectively). As is evident in Fig. 3, all control group measurements showed a systematic bias: differences in measured nNO grew with increasing concentration. With few exceptions, nNO levels obtained from the vrTB-nNO modalities were lower than values obtained using the cTB-nNO method.

Discussion

We introduced novel methods of very rapid TB-nNO measurement tested in older children and adults. A few seconds of TB-nNO sampling exhibited excellent discrimination between PCD and HC. Sampling for 4 and 6 s as well as vacuum sampling showed 100% sensitivity and specificity. Sampling for 2 s showed some overlap between PCD and HC, but still fair and significant discrimination.

Agreement between vrTB-nNO and cTB-nNO measurements was generally poor, and vrTB-nNO reduced the difference in mean ppb between PCD and HC. Our results suggest that, given their very low nNO levels in general, PCD patients are rapidly outperformed by HC nNO; sampling for only a few seconds is sufficient for significant discrimination with high sensitivity and specificity.

One current limitation of nNO measurements is the inability to rigorously test uncooperative children [8]. Guidelines recommend nNO measurements using the VC technique [8, 12] or sampling manoeuvres involving exhalation for > 20 s and a steady NO signal plateau for 3–10 s during sampling [13]. However, these methods usually exclude children < 5 years of age, an important drawback because early PCD diagnosis is warranted. Although cTB-nNO is equally discriminant for PCD [10, 11] with stationary chemiluminescence or a handheld electrochemical analyser [21] and

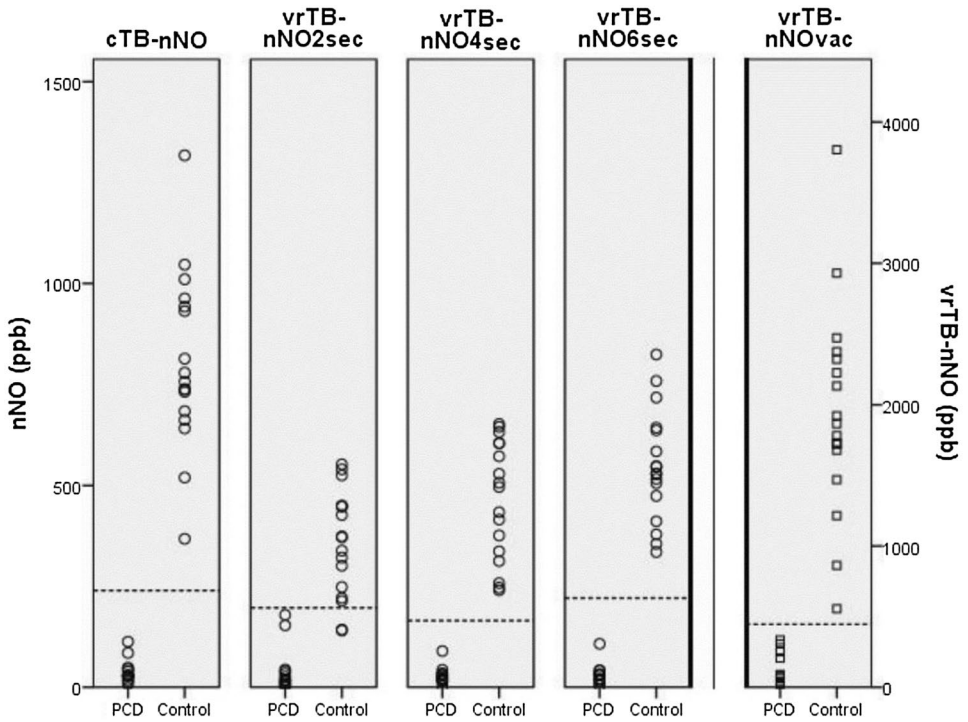

Fig. 1 Overview of nNO measurements

Table 2 ROC analysis of measurement modalities

Parameter (95% CI)	cTB-nNO	vrTB-nNO_{2sec}	vrTB-nNO_{4sec}	vrTB-nNO_{6sec}	vrTB-nNO_{vac}
Area	1.0 (1.0–1.0)	0.98 (0.95–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)
Cut-off (ppb)	240	197	165	221	446
Sensitivity	1.0 (0.72–1.0)	1.0 (0.72–1.0)	1.0 (0.72–1.0)	1.0 (0.72–1.0)	1.0 (0.72–1.0)
Specificity	1.0 (0.77–1.0)	0.88 (0.62–0.98)	1.0 (0.77–1.0)	1.0 (0.77–1.0)	1.0 (0.77–1.0)
PPV	1.0 (0.72–1.0)	0.87 (0.58–0.98)	1.0 (0.72–1.0)	1.0 (0.72–1.0)	1.0 (0.72–1.0)
NPV	1.0 (0.77–1.0)	1.0 (0.75–1.0)	1.0 (0.77–1.0)	1.0 (0.77–1.0)	1.0 (0.76–1.0)

possible in infants and young children, measurements are not readily performed and often take several attempts. Currently, nNO has a lesser role in younger children, mainly due to the lack of reference standards and the inability of these children to cooperate. There are no definitive cut-off values for young children, and age-specific nNO distributions are warranted in this age group. The use of vrTB-nNO with reduced probe-in-nose time may enable broader use of nNO measurement in infants and young children and facilitate further research in this age group.

This study aimed to provide a proof of concept for vrTB-nNO measurements to discriminate between PCD and HC. The main target group for such a test is infants and young children, who are non-cooperative for VC nNO measurements and require an alternative. We did not include this age group. Further studies addressing feasibility, reference values and discriminatory capacity in infants and young children are warranted.

Sample size was small, but fulfilled the number needed to achieve separation with high confidence. Including more subjects in a future study of vrTB-nNO will enable improved determination of specificity. Discrimination was convincing despite the limited number of subjects, supporting nNO as a strong discriminator for PCD, whether measured by VC or TB technique and whether TB-nNO sampling was very rapid or more prolonged. Although nNO measurements alone are insufficient to diagnose PCD, nNO is a valuable non-invasive test with immediate results. Other diagnostic procedures such as genetic testing and TEM analysis are more expensive, invasive, time-consuming and flawed tests with estimated sensitivities of 70% [8]. Negative results from these tests should still raise suspicion of PCD in patients with a strong clinical phenotype, leaving nNO to potentially detect such missed cases. Our results suggest that even shortening the duration of nNO measurements to an extreme can provide valuable

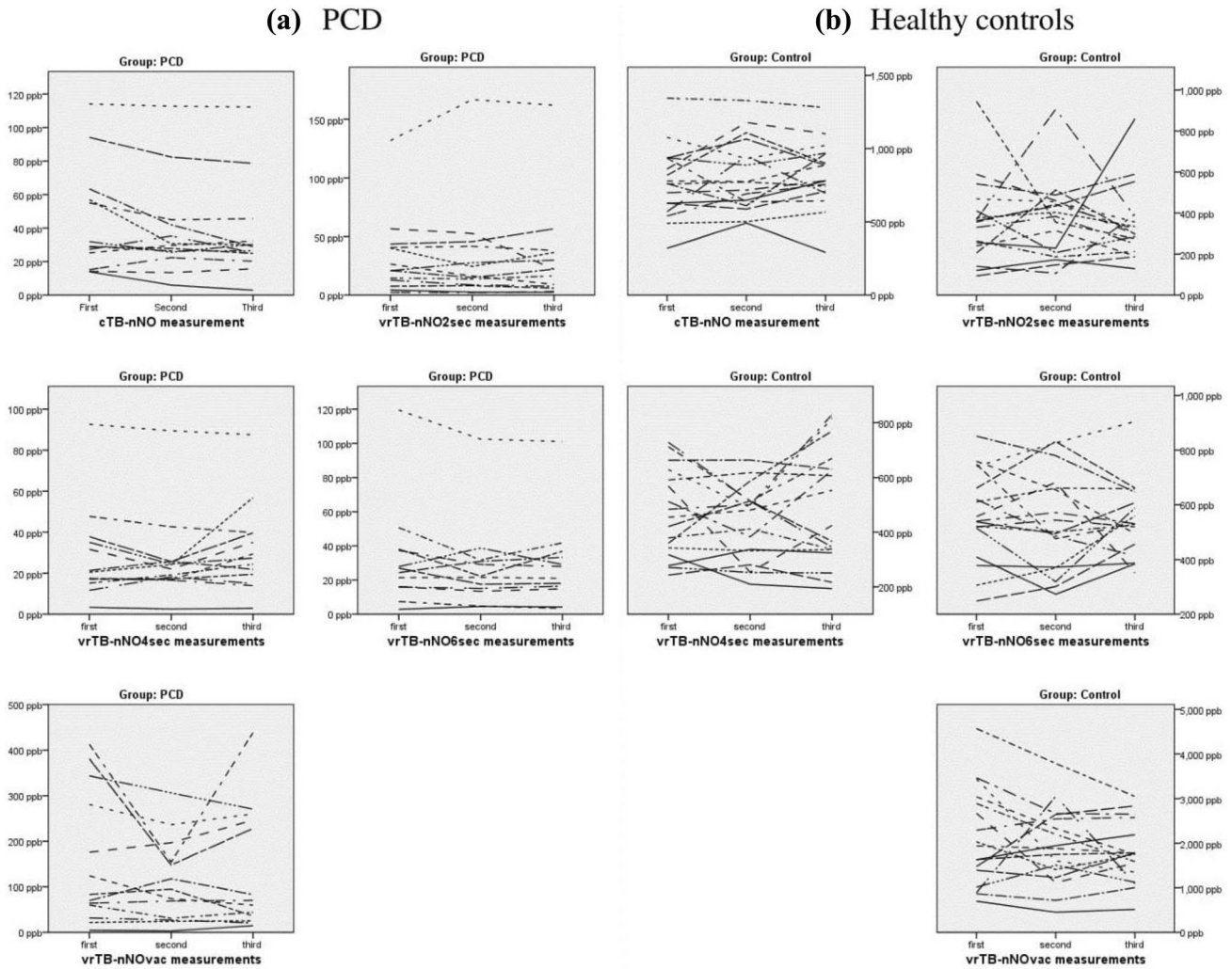

Fig. 2 Overview of intra-measurement differences

information by preserving great separation of PCD from HC, supporting the potential of vrTB-nNO in this patient subgroup.

nNO can be suppressed temporarily during respiratory tract infections in subjects without PCD, potentially overlapping with PCD values. This emphasises the importance of measuring nNO during infection-free intervals to avoid false-positive results [15]. Furthermore, nNO values within normal range have been reported in PCD patients with RSPH mutations [17, 18]; therefore, nNO should be used to supplement the PCD workup rather than as a standalone procedure.

There is currently no consensus on how to standardize TB-nNO measurements. Moreover, cutoff values to separate PCD from healthy infants and young children are still lacking, although a cutoff value of 77 nL/min can probably be used from approximately 2 years of age [15]. Although no definitive cut off has been agreed on, a feasible measurement modality for young children is desirable to serve as a triage test for further diagnostic workup.

In this study, vrTB-nNO$_{vac}$ resulted in very high nNO values even in PCD patients, likely due to increased flow rate from pinching the sampling tube; however, exact flow rates were unreadable because the analyser was not built for this, so the nNO values can only be reported as ppb and not calculated as nL/min. The exact explanation of the mechanism behind this modality needs further elaboration.

If care is not taken to avoid probe adhesion to nasal mucosa during nNO sampling, probe flow can stop and the related nNO reading will be low, risking a false-positive result in patients without PCD. Conversely, stopped flow causes negative pressure to build in the sampling tube and its abrupt release may result in a sudden rise in nNO (thereby risking a false-negative reading in patients with PCD). Awareness of probe adhesion during sampling is important, as are triple measurements.

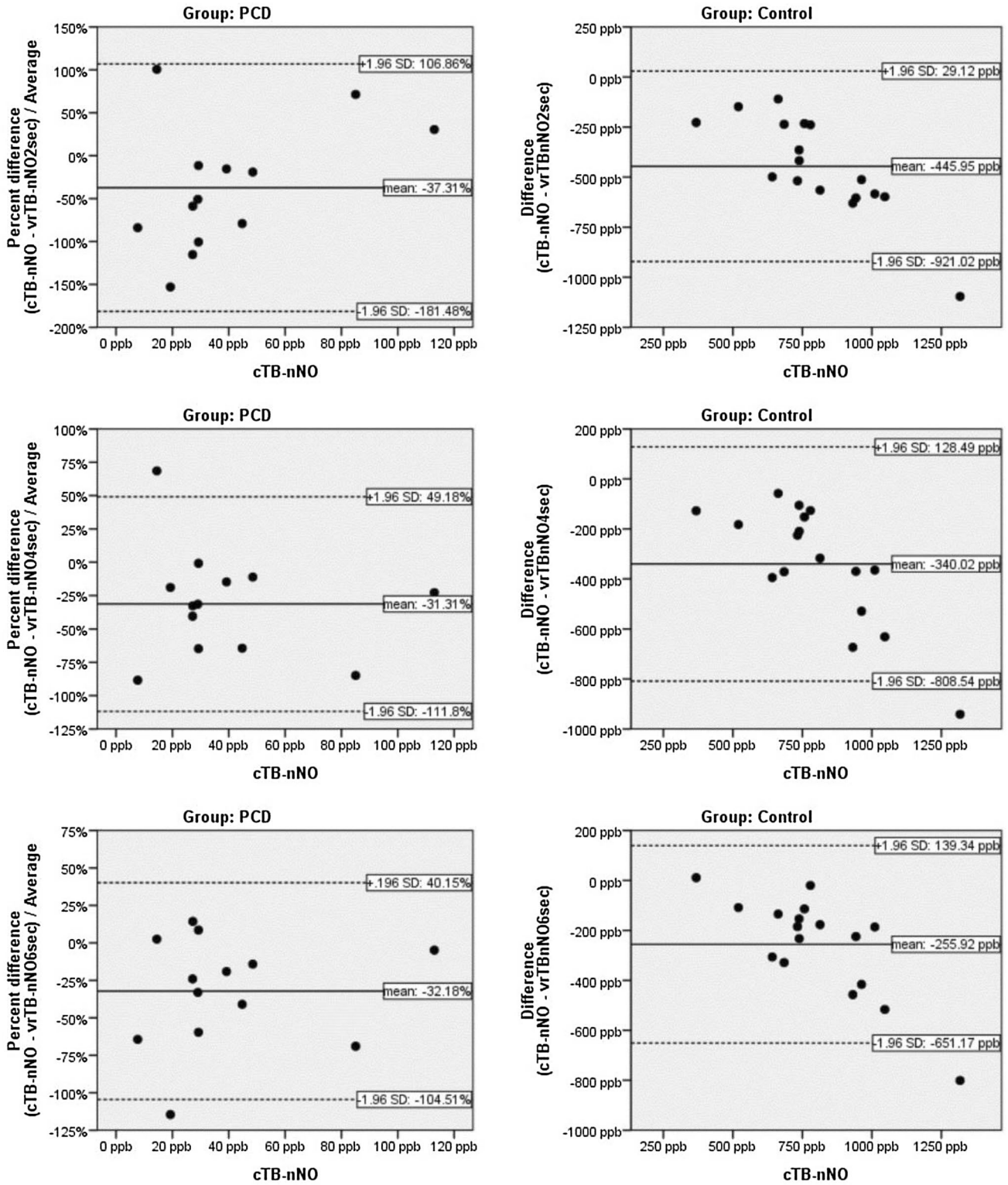

Fig. 3 Bland–Altman plots (difference plots with 2 s limits of the difference)

Conclusion

This method comparison and proof of concept study introduced novel, very rapid TB-nNO sampling modalities that discriminated strongly between PCD and HC with as little as 4 s of sampling. We included a completely new vacuum TB-nNO sampling modality in which nasal air is obtained nasally via the probe in < 2 s and found it equally discriminating, providing great potential for use in uncooperative patients due to the very short probe-in-nose time requirement. Our results indicate very rapid TB-nNO sampling methods can be used during the PCD workup as an alternative to VC nNO and cTB-nNO. Very rapid TB-nNO needs standardisation, and further investigation in patients referred for PCD workup and in infants and young children, where tolerance of nasal probing is crucial to successful measurement.

Compliance with Ethical Standards

Conflict of interest KGN and JKM are members of BEAT-PCD (COST Action BM1407). KGN is partly financially supported by The Children's Lung Foundation. MGH declares that he has no conflict of interest.

Ethical Approval The study was approved by the Committee on Health Research Ethics in the Capital Region of Denmark in March 2017 [Journal No. H-17002138].

Informed Consent Informed consent was obtained from all individual participants, and their parents and/or guardians when necessary, included in the study prior to enrollment.

Research Involving Human Participants All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. Knowles M, Zariwala M, Leigh M (2016) Primary ciliary dyskinesia. Clin Chest Med 37(3):449–461
2. Brown D, Pittman J, Leigh M, Fordham L, Davis S (2008) Early lung disease in young children with primary ciliary dyskinesia. Pediatr Pulmonol 43(5):514–516
3. Marthin J, Petersen N, Skovgaard L, Nielsen K (2010) Lung function in patients with primary ciliary dyskinesia: a cross-sectional and 3-decade longitudinal study. Am J Respir Crit Care Med 181(11):1262–1268
4. Goutaki M, Meier A, Halbeisen F, Lucas J, Dell S, Maurer E et al (2016) Clinical manifestations in primary ciliary dyskinesia: systematic review and meta-analysis. Eur Respir J 48(4):1081–1095
5. Behan L, Dimitrov BD, Kuehni CE, Hogg C, Carroll M, Evans HJ et al (2016) PICADAR: a diagnostic predictive tool for primary ciliary dyskinesia. Eur Respir J 47(4):1103–1112
6. Kuehni C, Frischer T, Strippoli M, Maurer E, Bush A, Nielsen K et al (2010) Factors influencing age at diagnosis of primary ciliary dyskinesia in European children. Eur Respir J 36(6):1248–1258
7. Behan L, Dunn Galvin A, Rubbo B, Masefield S, Copeland F, Manion M et al (2016) Diagnosing primary ciliary dyskinesia: an international patient perspective. Eur Respir J 48(4):1096–1107
8. Shapiro A, Davis S, Polineni D, Manion M, Rosenfeld M, Dell S et al (2018) Diagnosis of primary ciliary dyskinesia. An official American Thoracic Society Clinical Practice guideline. Am J Respir Crit Care Med 197(12):1535–4970
9. Lucas J, Barbato A, Collins S, Goutaki M, Behan L, Caudri D et al (2017) European Respiratory Society guidelines for the diagnosis of primary ciliary dyskinesia. Eur Respir J. https://doi.org/10.1183/13993003.01090-2016
10. Beydon N, Chambellan A, Alberti C, de Blic J, Clement A, Escudier E et al (2015) Technical and practical issues for tidal breathing measurements of nasal nitric oxide in children. Pediatr Pulmonol 50(12):1374–1382
11. Marthin J, Nielsen K (2011) Choice of nasal nitric oxide technique as first-line test for primary ciliary dyskinesia. Eur Respir J 37(3):559–565
12. American Thoracic Society, European Respiratory Society (2005) ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 171(8):912–930
13. Leigh M, Hazucha M, Chawla K, Baker B, Shapiro A, Brown D et al (2013) Standardizing nasal nitric oxide measurement as a test for primary ciliary dyskinesia. Ann Am Thorac Soc 10(6):574–581
14. Shapiro A, Josephson M, Rosenfeld M, Yilmaz O, Davis S, Polineni D et al (2017) Accuracy of nasal nitric oxide measurement as a diagnostic test for primary ciliary dyskinesia. A systematic review and meta-analysis. Ann Am Thorac Soc 14(7):1184–1196
15. Marthin J, Philipsen M, Rosthoj S, Nielsen K (2018) Infant nasal nitric oxide over time: natural evolution and impact of respiratory tract infection. Eur Respir J. https://doi.org/10.1183/13993003.02503-2017
16. Mateos-Corral D, Coombs R, Grasemann H, Ratjen F, Dell SD (2011) Diagnostic value of nasal nitric oxide measured with non-velum closure techniques for children with primary ciliary dyskinesia. J Pediatr 159(3):420–424
17. Knowles M, Ostrowski L, Leigh M, Sears PR, Davis S, Wolf W et al (2014) Mutations in RSPH1 cause primary ciliary dyskinesia with a unique clinical and ciliary phenotype. Am J Respir Crit Care Med 189(6):707–717
18. Frommer A, Hjeij R, Loges N, Edelbusch C, Jahnke C, Raidt J et al (2015) Immunofluorescence analysis and diagnosis of primary ciliary dyskinesia with radial spoke defects. Am J Respir Cell Mol Biol 53(4):563–573
19. Lowry R. Clinical calculator 1 2001. http://vassarstats.net/clin1.html. Accessed 1 Jun 2018
20. Krouwer J (2008) Why Bland-Altman plots should use X, not (Y + X)/2 when X is a reference method. Stat Med 27(5):778–780
21. Marthin J, Nielsen K (2013) Hand-held tidal breathing nasal nitric oxide measurement a promising targeted case-finding tool for the diagnosis of primary ciliary dyskinesia. PLoS ONE 8(2):e57262