An international survey on nasal nitric oxide measurement practices for the diagnosis of primary ciliary dyskinesia

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Shareable abstract (@ERSpublications)
There is considerable variability in routine practices for measuring nasal nitric oxide in patients suspected of having primary ciliary dyskinesia. Guidance is needed for professionals using different techniques, equipment and methodology in children. https://bit.ly/3JJndRh

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Abstract

Nasal nitric oxide (nNO) measurements are used in the assessment of patients suspected of having primary ciliary dyskinesia (PCD), but recommendations for performing such measurements have not focused on children and do not include all current practices. To guide the development of a European Respiratory Society-supported technical standard for nNO measurement in children, an international online survey was conducted to better understand current measurement practices among providers involved in PCD diagnostics.

78 professionals responded, representing 65 centres across 18 countries, mainly in Europe and North America. Nearly all centres measured nNO in children and more than half performed measurements before 5 years of age. The test was often postponed in children with signs of acute airway infection. In Europe, the electrochemical technique was more frequently used than chemiluminescence. A similar proportion of centres performed measurements during exhalation against a resistance (49 out of 65) or during tidal breathing (50 out of 65); 15 centres used only exhalation against a resistance and 15 used only tidal breathing. The cut-off values used to discriminate PCD were consistent across centres using chemiluminescence analysers; these centres reported results as an output ($nL \cdot min^{-1}$). Cut-off values were highly variable across centres using electrochemical devices, and nNO concentrations were typically reported as ppb.

This survey is the first to determine real-world use of nNO measurements globally and revealed remarkable variability in methodology, equipment and interpretation. These findings will help standardise methods and training.

Introduction

Primary ciliary dyskinesia (PCD) is a rare inherited disorder affecting motile cilia. It is characterised by impaired mucociliary clearance leading to chronic upper and lower respiratory tract infections from infancy; laterality defects occur in 50% of individuals and subfertility is common. The diagnosis is often delayed or missed because the presentation can be variable and individual symptoms nonspecific [1]. The diagnosis currently relies on the identification of typical ultrastructural cilia defects by transmission electron microscopy or biallelic pathogenic variants in a known PCD-associated gene. Ciliary beat analysis using high-speed video microscopy and immunofluorescent labelling of ciliary proteins are also used as diagnostic tools. However, these methods require highly specialised resources that are not widely available [2, 3].

Nitric oxide (NO) production is reduced in the nasopharynx and paranasal sinuses of most people with PCD [4]. Thus, measurement of nasal nitric oxide (nNO) production has become a useful adjuvant test in those presenting with compatible symptoms, once cystic fibrosis or immune defects have been excluded [5, 6]. There are established, validated methods using chemiluminescence analysers, in which nNO transnasal flow is measured in series during a respiratory manoeuvre with velum closure [7]. The North American PCD Foundation Clinical Center Network and the North American Research Network (Genetic Disorders of Mucociliary Clearance Consortium) has recently updated these recommendations. Their technical standard focuses on chemiluminescence devices for nNO measurements while exhaling against a resistor in children 5 years of age or older, and tidal breathing methods in preschool children or others who are unable to exhale into a resistor [8]. Alternative approaches, such as breath-holding, pursed-lip breathing through the mouth, voluntary elevation of the soft palate by a trained subject or nasal exhalation during humming, have also been suggested but are used less often [7]. In addition, these standards highlighted other important factors, such as the flow at which nasal air was sampled or the impact of ambient NO levels [7].

Recently, more affordable portable devices have emerged that measure NO using electrochemical technology. For these procedures, the test is predefined by industrial specifications without the benefit of a graphical (online) display of the NO level, hence hindering the investigator's ability to select the optimal NO sample [9–11]. While such devices are CE marked and approved for use in Europe, they have not been fully validated against established PCD diagnostic tests in large multicentre trials. As a consequence, there is still a need to standardise and validate nNO measurements using these analysers [8, 10].

Perhaps more important, infants and younger children cannot correctly perform respiratory manoeuvres to close the velum as recommended in existing guidelines, leaving no choice but to sample the nNO during tidal breathing [12]. Similarly, there is no standardised approach to measure or interpret nNO measured during tidal breathing.

A European Respiratory Society (ERS)-supported international task force (TF) was recently created and charged to produce a technical standard for nNO measurements in children suspected to have PCD, reviewing various equipment and methodologies. Little is known regarding current practices for measuring nNO worldwide, so to better understand current practice and needs, we surveyed professionals who routinely use nNO as an adjuvant tool for PCD workup. We assessed equipment and routine procedures used to measure and interpret nNO in various settings, focusing on children, and assessing how clinicians resolve technical and environmental factors which might influence the result in younger populations. False positive tests mainly due to technical issues would likely expose children to unnecessary and costly invasive tests. Alternatively, false negative tests would delay the diagnosis of PCD.

Material and methods

Questionnaire

In December 2020, three members of the TF (N.B., A.L.H., J.S.L.) constructed a brief survey which was circulated between TF members for comments, revisions and amendments. The final version was created as an electronic survey using Microsoft Forms software in January 2021.

After questions concerning the respondents and their service, the survey included 37 stem questions and seven sub-questions, each depending on the answer to a stem question. The last item of the questionnaire was a free text box for any relevant comments (survey is in supplementary annex 1).

Briefly, the questionnaire asked about the number of clinical tests undertaken annually at the centre, the designation and training of the professionals performing the measurements, age categories of the population routinely tested, equipment available for measuring nNO at the centre and response to environmental considerations (respiratory infections, ambient NO). Additional information included the testing protocol and number of measurements performed on each patient, the respiratory manoeuvres performed to measure nNO and repeatability criteria. Lastly, the survey queried standardisation of the nNO measurement interpretation and reporting.

Participants

We sent an explanatory e-mail with a link to Chairs to circulate their international clinical members and research collaboratives; these included the ERS-funded BEAT-PCD Clinical Research Collaboration (CRC), ERS group 7.1 (Paediatric respiratory physiology & sleep) and group 9.1 (Respiratory function technologists/Scientists), the National Institutes of Health-funded Genetic Disorders of Mucociliary Clearance Consortium and the PCD Foundation's Clinical Center Network. In addition, paediatric pulmonologists from countries represented in the American Thoracic Society (ATS) Virtual International Pediatric Pulmonology Network (VIPPN) roster contacted their national respiratory societies and other national working groups to distribute the invitation to participate in the survey.

The e-mail asked individuals to complete the survey if they were responsible for measuring nNO at their centre; if measured by somebody else, they were asked to forward the link to that colleague.

Analyses

Responses collected between January and March 2021 were extracted into an Excel file. The number and proportion of centres are reported as a percentage with the 95% confidence interval. Comparisons between groups were performed using Chi-square test or Fisher exact test, as appropriate, using GraphPad Prism (version 6.01). The p-value confirmed a statistical relationship when $\leqslant 0.05$ (two-sided).

Results

Questionnaire respondents

There were 147 individuals who followed the link to the survey. 78 (53%) were eligible and completed the survey. These respondents were from 65 different centres and 18 countries; we used only one respondent per centre for analysis when there was duplication to avoid bias. Respondents were mostly from centres in Europe and North America. All participating centres and the main results are available in tables 1 and 2.

Sixty-seven (46%) individuals did not perform nNO measurements, representing 56 different centres located in 11 different countries (Algeria, Australia, Bolivia, Bulgaria, France, Ireland, Morocco, the Netherlands, UK, Spain, Thailand, and Turkey), and they were therefore not eligible to complete the survey. Two individuals started the survey but did not complete the questionnaire; their surveys were not included in the final analysis.

Centre activity and personnel training

More than 25 annual tests were regularly performed in most of the responding centres, some exceeding 100 tests per year (main results per centre are shown in the table S1). As many as four physicians or staff members performed nNO measurements in two-thirds of the centres, but a larger number of professionals could be routinely involved (between 12 to 25 providers) without relationship to the number of tests performed annually. Notably, 11/27 centres performing fewer than 25 tests per year had between 6 and 15 professionals/staff obtaining the nNO measurements.

The professional backgrounds of staff who perform nNO measurements are listed in table 1. Physiologists and respiratory scientists most often obtained the measurements and, along with paediatric pulmonologists, accounted for the main professionals answering the survey. A training programme to teach personnel how to perform nNO measurements existed in more than half of the centres. It was usually delivered in a standardised format by a specific member of the team or consortium. However, at seven sites, all using electrochemical devices, the only source of training was provided by an industry representative. Comparisons between centres using chemiluminescence or electrochemical devices are included in the supplementary material.

Population tested and equipment used

Both children and adults were tested in two-thirds of the responding centres, and roughly a quarter of centres tested only children. Children younger than 5 years of age could be tested in approximately half

TABLE 1 Main results for routine performance of nasal nitric oxide (nNO) measurements in 65 centres responding completely in the survey

	Centres n (%)	95% CI of percentages
Countries		
UK	14 (22)	12–33
USA	13 (20)	11–32
France	9 (14)	7–25
The Netherlands	7 (11)	4–21
Belgium	4 (6)	2–15
Australia	3 (5)	1–13
Others with one or two respondents[#]	14 (22)	12–33
Number of tests performed per year		
<25 tests	27 (42)	29–54
>25 tests	38 (58)	46–70
Of which >100 tests	15 (23)	14–35
Qualification of professionals performing measurement		
Physiologists/respiratory therapists	21 (32)	21–45
Pulmonary function technicians	9 (13)	6–24
Paediatric pulmonologists	5 (8)	3–17
Nurses	4 (6)	2–15
Others or association of professionals	26 (40)	30–53
Training programme for measuring nNO		
Yes, standardised	26 (40)	30–53
Yes, informal	9 (13)	6–24
No	30 (46)	34–59
Population tested		
Children and adults	44 (68)	55–79
Only children	17 (26)	16–39
Only adults	4 (6)	2–15
Younger age of children tested in 61 centres		
⩽2 years	25 (41)	29–54
3–4 years	9 (15)	7–26
⩾5 years	27 (44)	31–58
Test postponed if recent respiratory infection		
Total	64	
No	20 (31)	20–44
Yes	44 (69)	56–80
Delay for 2 weeks	22/44 (50)	35–65
Delay for 4 weeks	15/44 (34)	20–50
Technique used to measure NO		
Total	64	
Electrochemical	31 (48)	36–61
Chemiluminescence	23 (36)	24–49
Both	10 (15)	8–27
Sampling flow of the device		
Unknown	22 (34)	23–47
0.25–0.40 L·min^{-1}	30 (46)	34–59
>0.40 L·min^{-1}	13 (20)	11–32

[#]: Canada, Chile, Czech Republic, Switzerland, Brazil, Denmark, Germany, Ireland, Mexico, Palestine, Portugal and Tunisia.

of the centres (34 out of 65). The majority of centres would postpone measurements if concerned about confounders, especially recent respiratory tract infections, which typically delayed testing for 2 to 4 weeks.

More responding centres used only an electrochemical device compared to sites that used only chemiluminescence devices. The NiOX VERO (Circassia®) was used at most centres (35 out of 41) as an electrochemical device, while CLD 88 (Ecomedics®) was the predominant chemiluminescence analyser (30 out of 33). Higher annual number of tests performed and younger age of children tested were more likely in centres using a chemiluminescence device (supplementary material).

TABLE 2 Methods used to measure and report nitric oxide (NO) results in 65 study centres

	Centres n (%)	95% CI of percentages
Measurement of ambient NO		
Daily or before each test	41 (63)	50–75
Not systematically	6 (9)	3–19
Never	18 (28)	17–40
Number of nostrils sampled during one test		
Always in both nostrils	41 (63)	50–75
Not systematically in both nostrils	15 (23)	13–35
Never in both nostrils	9 (14)	6–25
Number of measurements per nostril		
One	14 (22)	12–33
Two	32 (49)	37–62
More than two	19 (29)	19–42
Methods used in centres to sample nasal NO[#]		
Exhalation against a resistor	49 (75)	63–85
Tidal breathing	50 (77)	65–86
Breath-hold	10 (15)	8–26
Humming	2 (3)	0–11
Between-measurement repeatability within individuals		
Not reported	17 (26)	16–39
Reported, expressed as ppb	3 (5)	10–13
Reported, expressed as %	35 (54)	41–66
Reported, expressed as ppb and %	10 (15)	8–26
Report of nasal NO results includes		
Practical issues during measurements[¶]	55 (85)	74–92
Sampling flow of the device	31 (48)	35–60
Ambient NO	27 (42)	29–54
Nasal NO results in $nL \cdot min^{-1}$	16 (25)	15–37
Nasal NO results in ppb	33 (50)	38–63
Nasal NO results in $nL \cdot min^{-1}$ and ppb	16 (25)	15–37

[#]: more than one method per centre; [¶]: practical issues such as poor cooperation, difficulty with the respiratory manoeuvre, obstructed nostrils, crying, *etc.*

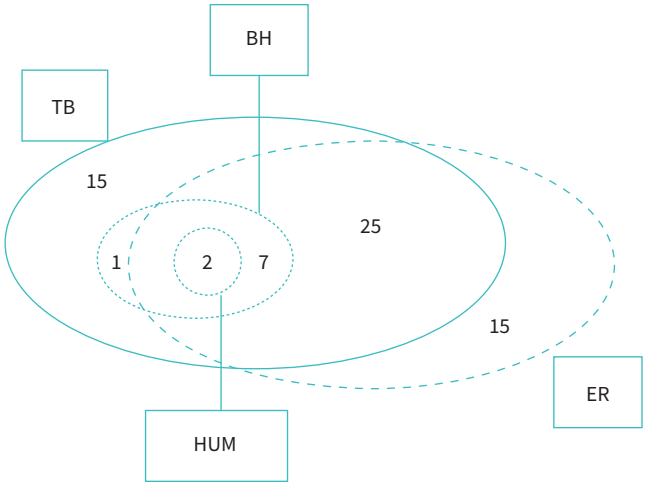

FIGURE 1 Methods used to measure nasal nitric oxide across 65 centres. ER: exhalation against a resistance; BH: breath-hold; TB: tidal breath; HUM: humming.

The sampling flow of the device was usually between 0.25 and 0.40 L·min^{-1} among the two-thirds of centres that disclosed this information (table 1). The survey did not determine whether the sampling flow was regularly checked before tests, but most North American sites measured this as part of their standard operating procedure. Ambient NO was measured daily or before each nNO measurement in nearly two-thirds of the centres, particularly in centres having a chemiluminescence device (table 2 and supplementary material).

Nasal nitric oxide measurements

The sampling of both nares with at least two measurements per naris was performed in most centres (table 2), but sites that had only an electrochemical device on site performed fewer repeated measurements (supplementary material). Only four centres performed a single measurement in one nostril (supplementary table S1). All centres reported using either exhaling against a resistance or tidal breathing methods to measure nNO (figure 1). Tidal breathing with an open mouth was used in 44% of centres that performed this method. The maximum acceptable intra-measurement repeatability of nNO was set at 10% by most sites (32 out of 45).

Nasal nitric oxide results and interpretation

A standardised report for nNO results was used by approximately two-thirds of the centres. Nasal NO was more frequently reported as a concentration (ppb) than as an output (nL·min^{-1}) (table 2), particularly by those sites that used electrochemical devices (supplementary material).

The cut-off value for nNO output was set at 77 nL·min^{-1} for exhalation against a resistance or for tidal breathing methods at most centres. Nevertheless, cut-offs for "normal" nNO values varied among sites, especially among centres using electrochemical analysers, which more often expressed the nNO cut-off as a concentration. The cut-off values, respiratory manoeuvres and units used to report nNO results are shown in figure 2.

Discussion

Repeatedly low nNO values in people with key clinical features are highly suggestive of PCD. nNO measurements have become a useful first-line diagnostic tool, provided the measurement is performed accurately. Accurate measurements are imperative to avoid false positive and false negative results.

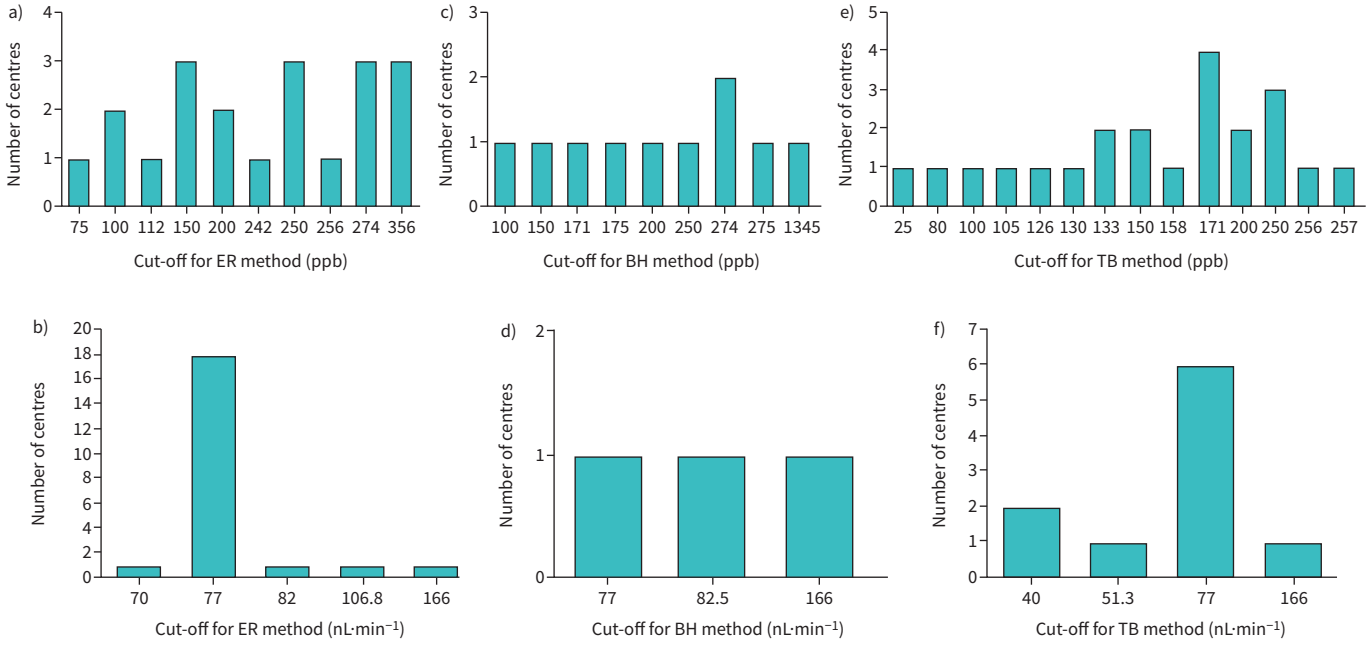

FIGURE 2 Cut-offs of nasal nitric oxide values used by primary ciliary dyskinesia centres to screen patients according to the respiratory manoeuvre and expression of the result. a), c) and e) show the number of centres using cut-offs expressed as ppb during a) an exhalation against a resistance (ER) (n=20), c) breath-hold (BH) (n=10) or e) tidal breathing (TB) (n=22). b), d) and f) show the number of centres using cut-offs expressed as nL·min^{-1} during b) ER (n=22), d) BH (n=3) or f) TB (n=10).

Results of an international survey, mostly answered by European and North American centres, were collected and analysed to determine the variability in nNO measurement practices (table 1 and supplementary table S1). In North America, a standard operating procedures and technical standards document that focuses on use of the chemiluminescence technique is likely to account for the more uniform responses across these centres compared to the rest of the world [7, 8]. Indeed, the individual practices highlighted, such as age of children tested, recording of ambient NO, number of measurements performed in a subject and reporting standards, may be due to the use of specific devices or techniques (chemiluminescence or electrochemical). More worrisome was that a large proportion of centres did not have standardised protocols or provide formal training for staff performing nNO measurements. These findings confirm the need for standardised and well-validated methods for nNO measurements, and these are particularly lacking for the electrochemical technique.

Most responding centres routinely tested children. Interestingly, more than half of the centres measure nNO in children under 5 years of age despite the absence of guidelines or established cut-off values for infants and preschool children [2, 3].

At nearly two-thirds of the centres, nNO measurements were postponed if the child had a recent or intercurrent respiratory infection. Recent respiratory infections have been shown to reduce nNO values in infants, but the time required for the injured sinonasal epithelium to recover and return to the usual NO production level is unknown [13].

Ambient NO level was assessed in roughly two-thirds of the centres. Ambient NO can increase measured nNO, but there is no consensual cut-off for high ambient NO or an effective way to "correct" the measurement [14–17]. Nevertheless, the majority of centres measuring ambient NO levels (34 out of 47 centres) took into account its value when interpreting nNO results [7, 18]. Not all sites reported ambient NO levels (table 2).

Repeatability was assessed in the vast majority of centres by sampling both nares and performing more than one measurement per naris. All published guidelines recommend repeating nNO measurements to confirm reliability and accuracy of the measured value. Better between-naris repeatability has been reported in methods involving velum closure compared to the tidal breathing method [14], which may reflect the variable airflow in nares during tidal breathing. Regardless of the method used, nNO can significantly vary between nares due to mechanical or anatomical factors, demonstrating the necessity to sample both nares, and report the highest value.

Because the recommended method to measure nNO when exhaling against a resistance requires the subject to be cooperative, it was expected that centres testing young children would use alternative approaches, such as tidal breathing. This was reflected in survey results which showed that these two methods (tidal breathing and exhaling against a resistance) were equally used across the responding centres (figure 1). The tidal breathing method has been extensively used, and results have been shown to correlate with measurements obtained using the exhalation against a resistance method, though the tidal breathing method may be less accurate in distinguishing PCD children from unaffected children [12, 14]. 15 centres exclusively used the tidal breathing method, even though there are no published guidelines describing its accuracy or interpretation of results in children of all ages [9, 12–14, 19–24].

Finally, cut-off values varied between centres, depending on the technique and equipment used, and the method of measurement (figure 2). The cut-off measures were much more uniform across centres that expressed nNO levels as an output ($nL \cdot min^{-1}$) when compared to those reporting NO concentration (ppb), which reflect the differences between chemiluminescence *versus* electrochemical techniques, respectively (see supplementary material). The unique cut-off in $nL \cdot min^{-1}$ was determined in a large multicentre study that included results from different chemiluminescence analysers sampling gas at 0.50, 0.33 or $0.30\ L \cdot min^{-1}$ [25]. The sampling flow of the device has an effect on NO output and measured concentration, but it has been demonstrated that in this narrow range of sampling flows, typical for measurements in children (table 1), nNO output can confidently be compared [25]. However, significantly larger sampling flows would hamper the comparison between cut-off values [14, 26]. It is to be noted that in the survey, one-third of the responding centres did not know about the sampling flow of their device (table 1), and others did not record it (table 2), thus hampering inter-centre comparisons of results. Discrepant cut-off values for PCD have been published depending on the study population (number of subjects, genetic background, diagnostic criteria and health status) included in the receiver operating characteristic analyses and the sensitivity and specificity chosen by the investigators [9, 10, 14, 20, 21, 27, 28].

The cut-off values used for nNO output were similar, regardless of the method used, exhalation against a resistance or tidal breathing, even though cut-off values measured during tidal breathing are often lower than cut-off values measured during exhalation against a resistance in which the velum is closed [12, 14, 19–21]. However, accurately measuring nasal output flow during tidal breathing is problematic, because the tidal breath superimposes local flow to the device's sampling flow, resulting in variability that can increase when subjects close or open their mouths [19]. We do not know whether these limitations would strongly impact the difference between nNO cut-off values using tidal breathing or exhalation against a resistance method, but similarity between the two methods when values are expressed using $nL \cdot min^{-1}$ is doubtful.

The strength of this study is the large number of international professionals who responded. There were few missing responses, which ensures the reliability of the data presented. However, the questionnaire was not distributed to paediatric otolaryngologists or out-of-hospital practices; therefore, our results may not fully reflect the true variability in practices if they occur in the community. It is likely that these settings would more frequently use electrochemical devices and different protocols, possibly with fewer measurements per subject, and variable cut-off values, thus adding to the practice variability that was noted.

In conclusion, this survey clearly shows the need to standardise and validate nNO testing in children worldwide for both chemiluminescence and electrochemical analysers. Standardisation will improve test consistency, reliability and accuracy. Education and training are also needed. The current international efforts to produce technical standards for nNO testing in children for PCD will be critical towards achieving this goal.

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