Author’s response to reviews

Title: Validation of a portable nitric oxide analyser for screening in primary ciliary dyskinesia

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Version: 6
Date: 3 November 2013

Author’s response to reviews: see over
Dear Editors,
We would like to thank the reviewers for their careful consideration of our manuscript. We have addressed all issues that the reviewers raised, and believe that their suggestions have substantially improved the publication. The point-by-point responses are highlighted in red. We have tracked the changes in the document and have also provided a ‘clean’ revised copy.
With best wishes
Jane Lucas

Reviewer's report
Title: Validation of a portable nitric oxide analyser for screening in primary ciliary dyskinesia
Version: 1 Date: 4 March 2013
Reviewer: Mauro Maniscalco

Reviewer's report:
GENERAL COMMENTS
In their study Harris and coworkers have compared the reliability and usability of a hand-held analyser with a static nasal NO analyser in patients affected by primary ciliary dyskinesia and other respiratory diseases using different methods and different flow rates. They found that the hand-held device present a good sensitivity and specificity as a screening test for PCD, equating to the static analyser although they used a technique different by the manufacturer’s guidelines, because of the difficulties of patients to keep the breath-hold requirement. The study is well done and the question posed is well defined.

We thank the reviewer for these positive comments.

The main problem is the novelty of data. There is another study comparing nasal nitric oxide by hand–held and stationary devices in PCD and other respiratory diseases, showing that the hand-held device is as effective as the stationary analyzer for assessing nasal NO in PCD (Eur J Clin Invest. 2011 Oct;41(10):1063-70). This study must be quoted and discussed. Furthermore, a very recent study has showed that tidal breathing nNO using an hand-held NO devices discriminates significantly between PCD, CF and HS (PLoS One. 2013;8(2):e57262). This study must be quoted and discussed. Several studies have compared nasal NO using hand–held and stationary devices. These should be quoted (Rhinology. 2008 Mar;46(1):23-7. Eur J Clin Invest. 2008 Mar;38(3):197-200

We thank the reviewer for suggesting these manuscripts and have now cited them in the introduction. (In version with tracked changes: lines 70-79)

Methods
As the Authors decided not to use breath hold, have the patients breathed against a resistance to close the velum, as suggested by ATS guidelines? Tidal breathing nNO sampling is a non-velum closure procedure applying normal relaxed tidal breathing during sampling, as described in the method section. Non-velum closure techniques have been shown to be repeatable and robust (Mateos-Corral J Ped 2011). (line 121-160)

Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests: I declare that I have no competing interests

Reviewer's report
Title: Validation of a portable nitric oxide analyser for screening in primary ciliary dyskinesia
Version: 1 Date: 19 September 2013
Reviewer: Marieann Högman

Reviewer's report:
The study by Harris et al. has tested two NO analyzers from the same manufacturer (Aerocrine AB, Sweden). The aim was to investigate if NIOX MINO could differentiate patients with PCD from other respiratory diseases. The authors found that this hand-held NO analyzer is a promising screening tool for PCD.

Major comments
There are many papers during the last 15 years that has reported decreased values of nasal NO in PCD. The focus of this paper seems confusing. If set out to investigate whether NIOX MINO could discriminate PCD from healthy individuals or other respiratory disease the obvious method should be different. We have clarified the aim of the study (see comments to reviewer S Dell). Lines 81-8.

1. With NIOX Flex and NIOX MINO at 5 mL/s sampling rate there was a decrease about 41 – 56 %, except for CSLD patients were the difference was minimal in NO production. The NO production was quite similar between the two methods with NOIX MINO. This needs explanation since you use the NIOX Flex as “golden standard”. The difference between Niox Flex and Mino with sampling at 5ml/s was that we used a breath hold technique with Flex but a tidal breathing method with Mino. It is likely that the tidal breathing method is complicated by greater contamination from the lower airway. This has been added to the discussion as a limitation of this method. (Line 284-6)

2. From the ATS/ERS recommendations 2005 it is stated that the flow rate should be measured. How did you ensure that the flow rates were 5 resp. 2 mL/s for both analyzers? The Niox Mino was provided by the manufacturers for research use with a choice of two sampling rates: either 2 ml/sec or 5ml/sec. The Niox Flex in nasal research mode has a sampling rate of 5 ml/sec. This information has been clarified in the manuscript. (Line 122-8)

It is also stated that the velum should be closed and any method to monitor this is acceptable. What was your method? We have added more detail concerning the sampling techniques in the method section. (line 136-147)

3. Put your cut off value in comparison with Leigh et al. Standardizing Nasal Nitric Oxide Measurement as a Test for Primary Ciliary Dyskinesia. Ann Am Thorac Soc. 2013. Our cut off using each method was somewhat lower than the Leigh et al cut off values. Harris et al cut offs: NioxFlex 38 nL/min, NioxMINO5ml
30nL/min, NioxMINO2ml 42nL/min. Leigh et al cut off: 77nL/min. This has been added to the discussion. (lines 292-6)

4. Was the patient also participating in your article in Respir Med. 2013 Mar;107(3):380-6? This study was separate from the Respir Med study, and participants were recruited separately for each. Most participants were only included in one of the studies, although some were included in both.

5. Please relate your results to the ones by Montella et al. Measurement of nasal nitric oxide by hand-held and stationary devices. Eur J Clin Invest. 2011 Oct;41(10):1063-70. Why is your data that different? Montella et al compared the measurement of nasal nitric oxide by Niox MINO and by Niox Flex, during silent and humming exhalations. They therefore used different manoeuvres to those that were employed in our study which may explain their reported nNO levels which are different to those in our population, or those reported by Leigh et al 2013. However their findings are in line with our paper: PCD patients have lower nNO than healthy controls or CF patients, and the hand-held device is as effective as the stationary analyser for assessing nNO. This has been added to the discussion. (lines 298-303)

6. What scientific method did you use for the focus group interviews? If scientifically correct you should have themes and statements instead of table 2. One could think that you were commercially founded by Aerocrine? The meeting did not follow a formal focus group structure ie, the discussion was not transcribed and analysed using qualitative research analysis. The information included in table 2 was unanimously agreed by all PCD health care professionals in the meeting, none of whom had a conflict of interest. We believe that these expert opinions are valid. We have reworded to emphasise that these are opinions of the multidisciplinary PCD team rather than a formal consensus agreement. (262-270)

7. A group comparison should be used instead of T-test. Were the statistics done on loge-transformed values? The manuscript needs major statistical improvement. How was the cut off value determined (by GEE)? Thank you for detecting the error in our description of the statistical methods used to compare groups that has been corrected. The statistical analyses to compare groups were conducted correctly using unpaired t-tests groups and remain unchanged. The cut off values were determined from the ROC curves. (172-186)

8. Results: Why were there only 38 of 50 participants that could perform measurement at 2 mL/s compared to 5 mL/s? Uninterrupted sampling time at 2ml/s is 90secs in comparison to 45secs for the 5ml/s sampling rate. This has now been emphasised in the manuscript.(152-6)

9. The figures and tables do not clearly give answers to the scientific question. We have had a major revision of the tables and figures (see responses to S Dell).

Minor Comments
1. Introduction; line 8: What do you mean by scientists are required? The scientists are required to perform the high speed video analysis, electron microscopy and ALI-culture etc. The sentence has been expanded.(lines 59-62)

2. Participants: The participants should be more logical explained. The big groups first (healthy volunteers and PCD) then other respiratory disorders such as…. We have restructured the section describing participants as suggested. (line 96)
3. Is it really stated in the manufacturer guidelines that the patient should have long breath holds as up to 90 s? Was it a misunderstanding? \textit{Sadly not! 90 secs of uninterrupted sampling is required to obtain a result. This was also noted by Marthin J PLOS ONE (2013), which has recently been published. (lines 152-6)}

4. NO measurements: why was the highest NO recording taken and not mean value of three, as you did in your earlier publication? \textit{Our clinical protocol has changed in the past year so that we record the highest value. We have mirrored this in our research protocols.}

5. Usability and reliability of analyzers: Last sentence should be moved into section for NO measurements. \textit{This has been moved.}

6. Fraction of exhaled NO should be abbreviated FENO and a suffix of flow rate ex FENO0.005. \textit{As FENO was only mentioned once we have now removed the abbreviation.(line 71)}

7. All references by Eur Resp J are missing information. \textit{Thank you for noticing this. The reference list has been amended.}

8. Log transformed should be loge transformed \textit{The transformations were Log10 transformed. This has been clarified in the text.(line 174)}

9. The manuscript needs language correction.

\textbf{Level of interest:} An article of limited interest

\textbf{Quality of written English:} Needs some language corrections before being published

\textbf{Statistical review:} Yes, and I have assessed the statistics in my report.

\textbf{Declaration of competing interests:}

I declare

\textbf{Reviewer's report}

\textbf{Title:} Validation of a portable nitric oxide analyser for screening in primary ciliary dyskinesia

\textbf{Version: 1} \textbf{Date:} 3 October 2013

\textbf{Reviewer:} Sharon Dell

\textbf{Reviewer's report:}

\textbf{Introduction:}

1. NIOX mino uses an electrochemical sensing technique as opposed to the gold standard chemiluminescence. This difference should be described somewhere in the intro or methods. \textit{This information has been added to the methods section. (lines 122-7)}

2. The aim could be reworded for better clarity: you are evaluating ability of nNO measurement by the NIOX MINO to discriminate between PCD, other respiratory disease and healthy controls \textit{We have reworded the aims as suggested.(lines81-8)}

3. Throughout the manuscript you describe the older desktop NIOX chemiluminescent analyzer as “static” which is a misnomer, since of course it is measuring and outputting readings of NNO concentrations in a dynamic way. The word “stationary” would be a better descriptor. \textit{Thank you for this suggestion. We agree that ‘stationary’ is a better descriptor and have changed the wording throughout the manuscript.}
Methods:
1. It would be useful to describe the nasal NO measurement in terms of NO production in L/min (NO production nl/min = concentration in ppb x aspiration flow rate in L/min). This adjusts for the effect of flow rate and makes the measurements more comparable within studies (when using different flow rates) and across studies where different flow rates may be used (see 2005 ERS/ATS recommendations on this). It would also explain the differences seen between the NIOX mino 2ml/sec and 5 ml/sec protocol. Thank you for this suggestion. We have calculated NO output and added this data to the manuscript. (lines 144-7)

2. Were patients selected to be stable and free of upper respiratory tract infection for at least two weeks before the study? Patients were symptomatically free from infection. (135)

3. What diagnoses did the CSLD participants have? Did they have bronchiectasis? Was immunodeficiency ruled out? The patients labelled CSLD were all patients who had been referred to the PCD diagnostic service and had the diagnosis of PCD excluded. All had previously had CF excluded. They were all chronically productive of purulent sputum but had not necessarily had a HRCT; bronchiectasis had therefore not been radiologically excluded nor confirmed. This information has been added to the text. (lines 114-118)

4. Did you measure and account for the possible affect of ambient NO? The Niox Flex measures ambient NO which was consistently between 5 and 10 ppb. The MINO does not provide an ambient NO measure, but both machines were used in the same room. Since background levels were always very low we made no adjustment to participant measurements. (line 135)

5. Chemiluminescent technique: How often did you calibrate the machine and how did you make sure that it was calibrated with each test? The analyser is calibrated and maintained as per manufacturer’s guidelines. In accordance with these guidelines we calibrate with standard gases (for nasal mode) every 14 days and more frequently if the machine ‘requests’ a re-calibration. The machine automatically runs a self-check before each participant. We have added that the machine was maintained and calibrated according to manufacturer’s guidelines. (Line 131-4)

How long of a measurement plateau did you require? Did you require the 3 maneuvers to be reproducible within 10% to make sure that you had a proper “gold standard” technique for comparison? Why did you choose the maximum of the three values rather than the average? We have added the information to the manuscript (3 readings within 10%, 4 sec minimum plateau. We used the maximum of three values as this aligns with our clinical practice. (Line 141-144)

6. NIOX Mino electrochemical sensor technique: Please clarify timing for the 2ml/sec and 5ml/sec tidal breathing maneuvers. Was it 45 seconds and 90 seconds as mentioned in the previous sentence for the breath hold technique? Yes this is an unbelievably (and impossible) long sampling time, but is correct. If the sampling time is interrupted the analyser will not provide a result.

As a quality control criteria, did you require reproducibility between the 3 measurements in each patient in order to report it? Why did you choose the highest measurement instead of the average of the three? See comments above.
7. Statistical analyses: Paired t-tests should only be used for the comparisons between analysers (same patients) but not between groups (different patients). Please clarify this. Thank you for detecting the error in our description of the statistical methods which has been corrected. The statistical analyses were conducted correctly using independent t-tests for comparisons between groups and remain unchanged. (176)

Results:
1. Fig 1 ROC curves are not helpful. Suggest replacing these with a table that suggests cut-off values with sensitivity and specificity for PCD using the various measurement devices. We have removed the ROC curves and have replaced with a table of sensitivity/specificity as suggested.
2. Bar graphs or scatter plot to show the distribution of the data would be more descriptive of nNO values than table one. We have replaced the table with box plots to show the distribution of data. We agree that this is more descriptive than table 1 which has now been removed.
3. When comparing nNO values of NIOX mino at 5ml/sec versus 2 ml/sec, assuming the measurements are done correctly, one would expect the 2ml/sec procedure to have systematically higher values due to the lower flow rate (see ATS/ERS statement). This would be the reason to use NO output instead of concentration. If the measurements are similar using NO output, one would have more confidence in the device. Thank you for this suggestion. We have now presented the data as NO output, which takes account of the different flow rates. This shows similar output/production levels using the Mino at the two different flows.

Discussion:
1. The discussion should include obvious reasons for differences between the various measurements that are accounted for by factors other than the device such as flow rate and velum closure (non velum closure techniques result in systematically lower NNO values due to contamination with lower airway NO). We have added this to the discussion. (286, 302)
2. Did the two patients with CSLD and low NO have alternate diagnoses to PCD? Did they have in vivo studies of mucociliary clearance (nuclear medicine clearance scans)? The cause of CSLD in two patients with low NO is uncertain but using European guidelines we excluded PCD on the basis of normal ciliary beat frequency and pattern before and following culture at air liquid interface. They also had normal TEM. Radioaerosol MCC is not part of our diagnostic portfolio and was not undertaken in this patient group.
3. Please compare your results to the recently published article by June Marthin and Nielson in PLOS ONE Feb 2013, vol8, e57262 This has been added (79, Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests.