Letter to the Editor on "Feasibility of nasal NO screening in healthy newborns"

Nicole Beydon, Jane Lucas

To the Editor,

We read with great interest the article of Buechel and colleagues "Feasibility of nasal NO screening in healthy newborns" published online in September 2021 [1]. This useful study adds to the knowledge about nasal NO production in very young subjects in a context where reducing the age of diagnosis of Primary Ciliary Dyskinesia (PCD) diagnosis is an important issue. With this respect, Buechel and colleagues nicely showed that neonatal nasal NO measurement could not efficiently screen for PCD in the neonatal period because of low nasal NO production in both healthy and PCD subjects in this age group.

The authors reported two other aims such as determining the feasibility of the nasal NO measurement and comparing results of two different techniques (ie. chemiluminescence and electrochemical).

The feasibility was perfect using the chemiluminescence analyser (100% in the 62 newborns) while it was lower using the electrochemical device (85.5%). This was mainly due to the interruption of the measurement when an extra airflow (child's nose breathing) was detected and also to the mandatory lengthy duration of the sampling (45 s) using the electrochemical device. In contrast, the chemiluminescence device tolerated variable airflows and allowed the real time analysis of the NO trace (always < 20s). Therefore, it is not the technique in itself that limited the feasibility of the electrochemical technique, but the functioning of NO analysers. Electrochemical devices could be improved to tolerate higher flows and to be set for a shorter sampling time. Nasal breathing is physiological in newborns and the reason for which some of the study subjects achieved NO measurement without blocking the electrochemical device could be discussed. The possibility for healthy newborns to perform oro-nasal breathing during spontaneous sleep has been measured during 5 s up to 60 min [2]. During these periods, the nasal airflow was decreased about 70% (±12%) which could be sufficient to allow the continuation of an electrochemical measure of nasal NO. The decrease of the sampling duration depends on the size of the analyser chamber and on the sampling

flow. The former cannot be easily reduced while increasing the sampling flow to reduce the measurement duration might not be tolerated in newborns.

The comparison between nasal NO measured using chemiluminescence and electrochemical analysers in the same subjects required the similarity of the sampling flow, the respiratory manoeuver and the method to analyse the sample. Sampling flows were similar between the two devices, although we feel that the CLD-88sp was sampling at 0.33 $L.min^{-1}$ and the NIOX MINO at 0.30 $L.min^{-1}$ (and not the opposite), a difference which has been demonstrated as irrelevant when comparing NO outputs (in $nL.min^{-1}$) [3]. Regarding the method used to compute NO value it appears some discrepancy between that used with each device. While there is a lack of consensus, different ways of evaluating nasal NO from tidal breath sampling have been published. Using the electrochemical device NIOX MINO, there is no other choice than analysing the mean content of the last 30s of the sampling (the first 15 s are discarded). The use of the last version of this machine (NIOX VERO) could probably decrease the duration of the sampling, but this has not be validated. In contrast, chemiluminescence analysers offer to analyse either the mean of a period or the peaks present on the trace. In the present study, the NO trace must show a virtual plateau (≥ 5s) to validate the measure, and maximum stable peaks over a minimum of 5 s were averaged and recorded as NO result. While the recording of a plateau would have mimicked the electrochemical technique, especially if extended to 30s, the use of maximal peaks should always score higher than a plateau. To demonstrate this, we previously compared, from the same tidal breathing NO traces, the mean of 5 maximal peaks to the mean NO value of the last 10 s and 30 s in 138 and 110 measurements, respectively [4]. As expected, the mean biases [95% limits of agreement] were not negligible (42 [-52; 143] ppb and 49 [-56; 153] ppb, respectively) in favour of means of 5 NO peaks being higher than means over a 10 or 30 s duration. Buechel and colleagues reported 6/53 subjects with highest NO values recorded using the electrochemical technique which questions the chemiluminescence technique result (as electrochemical result does not involve any human intervention). We note than the shortest sampling duration was 5 s, meaning that, at least in that case, peaks were retrieved from

the virtual plateau. The authors should clarify the definition of the plateau and peaks. Also, in 5 s how many peaks could be present as no minimal number of peaks are stated and no definition of stable peaks is provided. It has been shown that maximal peak(s) during very rapid tidal breathing nasal NO sampling (2 – 4 – 6 s) could discriminate older PCD children and adults from unaffected subjects, but NO values increased with the duration of the sampling and were still significantly lower than that of the maximal 3 peaks obtained during a 30 s sampling [5]. Of course, these results obtained in older subjects with high nasal NO levels cannot be extrapolated to newborns with physiological low NO output. However, a strict definition of peaks, their minimal number and reproducibility is necessary to compare studies.

Lastly, the authors attributed the difference between chemiluminescence and electrochemical NO results to the different sampling method and analysis, but in Marthin and colleagues study, while subjects were similarly sampled (during breath hold or tidal breathing at 0.3 $L.min^{-1}$ flow), chemiluminescence analysers measured systematically higher NO values than electrochemical, in favour of a predominant if not single role of technology in the difference recorded [6].

We wish to draw attention on the ongoing ERS Task Force currently developing a technical standard which will include the measurement, reporting and interpretation of tidal breathing nasal NO. With this respect, the data from Buechel and colleagues will be helpful for illustrating the usefulness of this method.

Nicole Beydon
AP-HP.Sorbonne Université, Unité d'Exploration Fonctionnelle Respiratoire, Hôpital Armand-Trousseau, and Sorbonne Université, INSERM U938, Paris, France

Jane S Lucas

Primary Ciliary Dyskinesia Centre, NIHR Biomedical Research Centre, University of Southampton, and Primary Ciliary Dyskinesia Centre, University Hospital Southampton NHS Foundation Trust, Southampton, UK

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