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Early View

Research letter

Nitrogen multiple breath washout test for infants with cystic fibrosis

Václav Koucký, Veronika Skalická, Petr Pohunek

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Title: Nitrogen multiple breath washout test for infants with cystic fibrosis

Authors: Václav Koucký[1,2], Veronika Skalická[1,2], Petr Pohunek[1,2]

Affiliation:
[1] Department of Paediatrics, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic
[2] Department of Paediatrics, University Hospital Motol, Prague, Czech Republic

Corresponding author:
Václav Koucký
Department of Paediatrics, 2nd Faculty of Medicine, Charles University and University Hospital Motol
V Úvalu 84
Praha 5
150 06
Czech Republic
E-mail: vaclav.koucky@seznam.cz

Take-home-message: Nitrogen multiple breath washout test is a safe, feasible and repeatable method for infants with cystic fibrosis. It may outperform sulphur hexafluoride variant with respect to its better suitability for long time follow up and lower technical demands.

Multiple breath washout test (MBW) with lung clearance index (LCI) as a main outcome parameter has proven to be a valuable research tool in patients with cystic fibrosis (CF) [1,2]. Moreover, there is growing evidence of its relevance in routine clinical practice [3]. Nevertheless, some technical limitations remain that hamper its widespread use. While most of the MBW data in infants have been acquired via sulphur hexafluoride washout (SF_6-MBW), the nitrogen variant (N_2-MBW) is recommended by the European Cystic Fibrosis Society Clinical Trial Network (ECFS-CTN) for older children. However, this methodological discrepancy may limit long-term follow-up of CF patients because N_2- and SF_6-MBW cannot be used interchangeably. In infants, the N_2-MBW has not been fully investigated to date.

We report our data regarding safety, feasibility and repeatability of N_2-MBW in CF infants. Between January 2015 and January 2017, we performed lung function testing in 35 infants aged 6.7–25.2 months (median 14.7) with classical form of CF. Testing was performed in patients without any signs of acute respiratory infection for at least 14 days. Quiet sleep was induced by administering chloral hydrate 80–100 mg/kg per rectum. The patients were placed in a supine position with their head and neck in a neutral position, and a Rendell-Baker face mask Nr. 1 or 2 (selected to keep the instrument dead space under 2 ml/kg) was tightly sealed around the mouth and nose. At first, N_2-MBW was performed using the Exhalyzer D device (Ecomedics Duernten, Switzerland) with the Spiroware 3.2.0 software, following all the relevant recommendations [4,5]. When finished, at least 120 seconds of medical air breathing followed (normalization of breath pattern, nitrogen back wash-in). Afterwards, SF_6-MBW was performed using the same device and software within a subgroup of 10 patients, who were tested after March 2016 (SF_6-MBW became available in our centre); their anthropometric parameters and age were not significantly different from those of the whole study group. Both measurements were performed in fixed order within the same sedation period and without changing the body and face mask position. The technical acceptability of trials was assessed by a physician with ECFS-CTN certification in this method and adhered to the standard operating procedure for N_2-MBW [5] modified for infants and SF_6-MBW. Both tests were repeated at least three times to obtain a coefficient of variation (CV) lower than 10%. Pulse rate and haemoglobin oxygen saturation were continuously monitored during MBW measurements and until full consciousness was regained. Pair-wise t-test was used to compare results from SF_6- and N_2-MBW in individual patients with both measurements available (n=8). Patient's characteristics between the N_2-MBW and SF_6-MBW group were compared by the t-test. The reliability of N_2-MBW was assessed using estimates of intraclass correlation (ICC). Coefficient of repeatability was calculated as follows: 1.96 * √2 * within subject standard deviation (SD). Calculations of repeatability and reliability data were based on appropriate one-way ANOVA models and performed on N_2-MBW data only (n=23).

The feasibility of both tests in infants was high. Sedation with chloral hydrate failed in 5 patients (14.3 %). The success rate of N_2-MBW reached 76.7 % (23 successful measurements in 30 sedated infants), while in SF_6-MBW it reached 80.0%. Irregular breathing pattern and face mask leaks were the most common reasons for data rejection. Mean number of trials performed per patient was 3.6 (range 3-6) resulting in 2.5 successful trials (range 2-4). Mean estimated duration of one N_2-MBW trial was 3:57 minutes; SF_6-MBW trial took 2:44 min on average. N_2-MBW yielded higher values of FRC (functional residual capacity), LCI2.5 and LCI5 (defined as a number of lung volume turnovers required to decrease the inert gas concentration to 2.5% and 5% of its starting concentration, respectively) and lower values of tidal volume (Vt). Respiratory rate (RR) and ratio of time to peak expiratory flow to expiratory time (tPTEF/tE) did not differ between the two methods. Both reliability and repeatability of N_2-MBW measurements was high with ICC exceeding 0.9 for most of the parameters; for detailed results see Table 1. We did not observe any adverse reactions such as haemoglobin desaturation < 94%, apnoea, tachycardia, aspiration, or prolonged sedation.

Our work is one of the two reports offering direct comparison of N_2- and SF_6-MBW in infants. It is also the largest study to date on N_2-MBW in CF infants. The presented data clearly indicate that nitrogen washout is a safe method for infants. Although inhalation of pure oxygen during nitrogen washout lowers tidal volume and minute ventilation (as shown by Singer et al [6]), we did not notice any adverse reaction. Unfortunately the aforementioned work [6] does not comment on the outcome parameters (LCI, FRC). The first direct comparison of both modifications of MBW was performed by Gustafsson et al [7] in 10 healthy infants. They reported similar differences in outcome parameters (higher FRC and LCI, lower Vt during nitrogen washout).

The lower Vt during nitrogen washout seems to be a consequence of hyperoxic conditions during washout phase ("peripheral chemodenervation") [8,9]. Although the Vt recovers or even exceed the normoxic values after approximately 60 seconds, in the end effect, average Vt is lower compared to normoxemia. Consequently, assessment of tidal breath parameters during washout phase is inaccurate. Moreover, the increased dead space ventilation makes the washout less efficient and contributes to LCI overestimation. The solubility of nitrogen in blood also interferes with methodology of N_2-MBW. Its back-diffusion may increase washout time and LCI as suggested by Sullivan et al [10]. Based on these physiological aspects, nitrogen washout cannot estimate ventilation inhomogeneity and end expiratory lung volume (i.e. FRC) as precisely as SF_6 washout does. However, in our opinion, N_2-MBW may still be helpful in clinical setting. As previously shown by our group, N_2-MBW is capable to reveal poorer lung function in *Pseudomonas aeruginosa* ever-infected infants [11]. Moreover, compared to SF_6-MBW it is technically less demanding (no need of this expensive and poorly available gas, its severe greenhouse effect) and consequently more convenient for long term follow up.

Our results regarding the feasibility of N_2-MBW are fully comparable to the SF_6-MBW data published by Stahl et al. They reported feasibility of 67.5% in children aged 0-3 years [12] and 78.9-100% in older children from a multicentric study [13]. Jensen et al [14] compared the N_2-MBW and SF_6-MBW data in children aged 3 to 18 years and nitrogen washout proved as a feasible alternative to SF_6 washout with similar discriminative power and repeatability. Our data on repeatability of N_2-MBW in infants are comparable to the data of Singer et al [15] from older children. We obtained lower CV of LCI in infants, while CR was slightly higher.

We conclude that N_2-MBW is an attractive alternative to sulphur hexafluoride washout even in CF infants. It is safe and feasible. Moreover, in some aspects it may outperform the SF_6 method because of its lower cost and lower technical demands of the measurement. The suitability of this variant for longitudinal follow-up is also higher. On the other hand, it must be acknowledged, that SF_6 washout offers more precise measurement with respect to a few physiological mechanisms interfering with the methodology of MBW. Further data (e.g., regarding the normative values) are needed before widespread use of N_2-MBW may be recommended.

Table 1.Nitrogen and sulphur washout data summary

Parameter	N_2-MBW (n = 23)	CV [%]	ICC (95% CI)	CR	SF_6-MBW (n = 8)	CV [%]	Comparison for n = 8 (p-value)
FRC [ml]	315.6 ± 84.6	3.7 ± 2.7	0.97 (0.93; 0.99)	48.3 (39.6; 62.0)	306.8 ± 93.6	2.9 ± 1.8	0.003
LCI2.5	10.47 ± 1.84	3.7 ± 1.8	0.92 (0.84; 0.96)	1.58 (1.30; 2.03)	8.08 ± 0.90	2.5 ±1.1	0.001
LCI5	6.4 ± 0.63	2.8 ± 2.2	0.85 (0.71; 0.93)	0.77 (0.63; 0.99)	6.08 ±0.43	1.9 ± 0.9	0.008
RR [min^{-1}]	29.7 ± 4.6	3.4 ± 2.4	0.96 (0.92; 0.98)	4.16 (3.40; 5.34)	28.3 ± 3.2	3.8 ±1.7	0.305
Vt [ml]	89.9 ± 28.9	3.6 ± 4.1	0.98 (0.96; 0.99)	11.3 (9.3; 14.5)	106.4 ± 26.2	2.5 ± 2.4	0.015
tPTEF/tE [%]	31.7 ± 10.8	7.5 ± 4.9	0.86 (0.74; 0.94)	12.2 (10.0; 15.7)	32.5 ± 16.5	6.7 ± 4.1	0.973

Data stated as mean ± standard deviation (SD), in case of ICC and CR as estimated value (95% confidence interval). The comparison was performed in a subgroup of patients with both SF_6- and N_2-measurements available (n=8), pair-wise t-test used for calculation. N_2-MBW – nitrogen multiple breath washout test; SF_6-MBW – sulphur hexafluoride multiple breath washout test; CV – coefficient of variation (calculated as 100 * SD of the repeated measurements in one patient divided by their mean); ICC – intraclass correlation (calculated as $(\sigma^2_{between}) / (\sigma^2_{between} + \sigma^2_{within})$, where $\sigma^2_{between}$ is the variance of a measured quantity in the study population (variability between subjects) and σ^2_{within} is the variance of repeated observations made on one subject (within subject variability); CR - coefficient of repeatability (calculated as 1.96 * √2 * within subject SD);FRC – functional residual capacity; LCI2.5 and LCI5 – lung clearance index at 2.5% or 5% of starting concentration; RR – respiratory rate; Vt – tidal volume; tPTEF/tE – time to peak expiratory flow to expiratory time ratio.

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Conflict of interest disclosure

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References:

1. Lum S, Gustafsson P, Ljungberg H, Hülskamp G, Bush A, Carr SB, Castle R, Hoo AF, Price J, Ranganathan S, Stroobant J, Wade A, Wallis C, Wyatt H, Stocks J; London Cystic Fibrosis Collaboration. Early detection of cystic fibrosis lung disease: multiple-breath washout versus raised volume tests. Thorax 2007; 62: 341-347.

2. Hall GL, Logie KM, Parsons F, Schulzke SM, Nolan G, Murray C, Ranganathan S, Robinson P, Sly PD, Stick SM; AREST CF, Berry L, Garratt L, Massie J, Mott L, Poreddy S, Simpson S. Air trapping on chest CT is associated with worse ventilation distribution in infants with cystic fibrosis diagnosed following newborn screening. PLoS One 2011; 6: e23932. doi: 10.1371/journal.pone.0023932.

3. Davies G, Aurora P. The use of multiple breath washout for assessing cystic fibrosis in infants. Expert Rev Respir Med. 2017 Jan;11(1):21-28. doi: 10.1080/17476348.2017.1269604.

4. Robinson P D, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, Thamrin C, Arets HGM, Aurora P, Fuchs S I, King GG, Lum S, Macleod K, Paiva M, Pillow JJ, Ranganathan S, Ratjen F, Singer F, Sonnappa S, Stocks J, Subbarao P, Thompson BR, Gustafsson PM. Consensus statement for inert gas washout measurement using multiple- and single breath tests. Eur Respir J 2013; 41: 507-522.

5. Renee Jensen on behalf of working group: Green K, Gustafsson P, Latzin P, Pittman J, Ratjen F, Robinson P, Singer F, Stanojevic S, Yammine S: Standard Operating Procedure: Multiple Breath Nitrogen Washout, EXHALYZER® D, ECO MEDICS AG, November 2013.

6. Singer F, Yammine S, Schmidt A, Proietti E, Kieninger E, Barben J, Casaulta C, Regamey N, Gustafsson P, Frey U, Latzin P. Ventilatory response to nitrogen multiple-breath washout in infants. Pediatr Pulmonol 2014; 49: 342-347. doi: 10.1002/ppul.22841.

7. Gustafsson PM, Bengtsson L, Lindblad A, Robinson PD.The effect of inert gas choice on multiple breath washout in healthy infants: differences in lung function outcomes and breathing pattern. J Appl Physiol (1985). 2017 Dec 1;123(6):1545-1554. doi:10.1152/japplphysiol.00524.2017.

8. Rigatto H, Desai U, Leahy F, Kalapesi Z, Cates D. The effect of 2% CO2, 100% O2, theophylline and 15% O2 on "inspiratory drive" and "effective" timing in preterm infants. Early Hum Dev. 1981 Feb;5(1):63-70.

9. Foong RE, Harper AJ, Hall GL, Ramsey KA; AREST CF. The effect of 100% oxygen on tidal breathing parameters in preschool children. EurRespir J. 2017 Jun 1;49(6). pii: 1601959. doi: 10.1183/13993003.01959-2016.
10. Sullivan L, Forno E, Pedersen K, Nielsen JG, Weiner DJ. Nitrogen back-diffusion during multiple-breath washout with 100% oxygen. Eur Respir J. 2017 Sep 9;50(3). pii: 1700679. doi: 10.1183/13993003.00679-2017.

11. Koucky V, Skalicka V, Martinu V, Pohunek P. Do bacterial infection and number of exacerbations have an impact on lung function and nutritional status in the youngest patiens with cystic fibrosis? J Cyst Fibros. 2017 Jun 16; S1: 25.

12. Stahl M, Joachim C, Blessing K, Hämmerling S, Sommerburg O, Latzin P, Mall MA. Multiple breath washout is feasible in the clinical setting and detects abnormal lung function in infants and young children with cystic fibrosis. Respiration 2014; 87: 357-363.

13. Stahl M, Graeber SY, Joachim C, Barth S, Ricklefs I, Diekmann G, Kopp MV, Naehrlich L, Mall MA. Three-center feasibility of lung clearance index in infants and preschool children with cystic fibrosis and other lung diseases. J Cyst Fibros. 2017 Aug 12. pii: S1569-1993(17)30836-6. doi: 10.1016/j.jcf.2017.08.001.

14. Jensen R, Stanojevic S, Gibney K, Salazar JG, Gustafsson P, Subbarao P, Ratjen F. Multiple breath nitrogen washout: a feasible alternative to mass spectrometry. PLoS One 2013;8: e56868. doi:10.1371/journal.pone.0056868.

15. Singer F, Kieninger E, Abbas C, Yammine S, Fuchs O, Proietti E, Regamey N, Casaulta C, Frey U, Latzin P. Practicability of nitrogen multiple-breath washout measurements in a pediatric cystic fibrosis outpatient setting. Pediatr Pulmonol 2013; 48: 739-746. doi: 10.1002/ppul.22651.