



# Nitrogen multiple breath washout test for infants with cystic fibrosis

*To the Editor:*

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* sulfur hexafluoride washout (SF<sub>6</sub>-MBW), the nitrogen variant (N<sub>2</sub>-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 cystic fibrosis patients because N<sub>2</sub>- and SF<sub>6</sub>-MBW cannot be used interchangeably. In infants, the N<sub>2</sub>-MBW has not been fully investigated to date.

We report our data regarding safety, feasibility and repeatability of N<sub>2</sub>-MBW in cystic fibrosis 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 cystic fibrosis. 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<sup>-1</sup> 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<sup>-1</sup>) was tightly sealed around the mouth and nose. At first, N<sub>2</sub>-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 s of medical air breathing followed (normalisation of breath pattern, nitrogen back wash-in). Afterwards, SF<sub>6</sub>-MBW was performed using the same device and software within a subgroup of 10 patients, who were tested after March 2016 (SF<sub>6</sub>-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<sub>2</sub>-MBW [5] modified for infants and SF<sub>6</sub>-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. A pair-wise t-test was used to compare results from SF<sub>6</sub>- and N<sub>2</sub>-MBW in individual patients with both measurements available (n=8). Patient's characteristics between the N<sub>2</sub>-MBW and SF<sub>6</sub>-MBW group were compared by the t-test. The reliability of N<sub>2</sub>-MBW was assessed using estimates of intraclass correlation (ICC). Coefficient of repeatability was calculated as follows:  $1.96 \times \sqrt{2} \times \text{within-subject standard deviation (sd)}$ . Calculations of repeatability and reliability data were based on appropriate one-way ANOVA models and performed on N<sub>2</sub>-MBW data only (n=23).

The feasibility of both tests in infants was high. Sedation with chloral hydrate failed in five patients (14.3%). The success rate of N<sub>2</sub>-MBW reached 76.7% (23 successful measurements in 30 sedated infants), while, in SF<sub>6</sub>-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<sub>2</sub>-MBW trial was 3:57 min; SF<sub>6</sub>-MBW trial took 2:44 min on average. N<sub>2</sub>-MBW yielded higher values of FRC (functional residual capacity), LCI<sub>2.5</sub> and LCI<sub>5</sub> (defined as the 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



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**Nitrogen multiple breath washout test is a safe, feasible and repeatable method for infants with cystic fibrosis. It may outperform sulfur hexafluoride variant with respect to its better suitability for long time follow up and lower technical demands.** <http://ow.ly/hqnB30k9REW>

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volume ( $V_T$ ). 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  $V_T$  during nitrogen washout).

The lower  $V_T$  during nitrogen washout seems to be a consequence of hyperoxic conditions during washout phase ("peripheral chemodenervation") [8, 9]. Although the  $V_T$  recovers or even exceeds the normoxic values after approximately 60 s, in the end effect, average  $V_T$  is lower compared to normoxaemia. 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 3–18 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 coefficient of variation of LCI in infants, while coefficient of repeatability was slightly higher.

We conclude that  $N_2$ -MBW is an attractive alternative to  $SF_6$  washout even in cystic fibrosis 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.

TABLE 1 Nitrogen and sulfur 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
LCI <sub>2.5</sub>	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
LCI <sub>5</sub>	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 breaths·min <sup>-1</sup>	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
$V_T$ 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±SD or estimated value [95% confidence interval], unless otherwise stated. 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: sulfur hexafluoride multiple breath washout test; CV: coefficient of variation (calculated as  $100 \times SD$  of the repeated measurements in one patient divided by their mean); ICC: intraclass correlation (calculated as  $\frac{\sigma_{\text{between}}^2}{\sigma_{\text{between}}^2 + \sigma_{\text{within}}^2}$ , where  $\sigma_{\text{between}}^2$  is the variance of a measured quantity in the study population (variability between subjects) and  $\sigma_{\text{within}}^2$  is the variance of repeated observations made on one subject (within subject variability)); CR: coefficient of repeatability (calculated as  $1.96 \times \sqrt{2} \times \text{within subject SD}$ ); FRC: functional residual capacity; LCI<sub>2.5</sub> and LCI<sub>5</sub>: lung clearance index at 2.5% or 5% of starting concentration; RR: respiratory rate;  $V_T$ : tidal volume; tPTEF/tE: time to peak expiratory flow to expiratory time ratio.

Further data (e.g. regarding the normative values) are needed before widespread use of N<sub>2</sub>-MBW may be recommended.

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