Multiple breath washout lung function reveals ventilation inhomogeneity unresponsive to mechanical assisted cough in patients with neuromuscular disease

Mathis Steindor1*, Anna Pichler1, Laura Heitschmidt1, Eva Pitsikoulis1, Alexandra Kavvalou1, Eser Orhan2, Margerete Olivier1 and Florian Stehling1

Abstract

Background: Respiratory involvement defines the clinical outcome of neuromuscular diseases (NMD). The lung clearance index (LCI) is a marker of lung ventilation inhomogeneity and indicates small airway disease. It is determined by multiple breath washout lung function (MBW). The merit of LCI is undisputed for primary lung diseases like cystic fibrosis, but its role in NMD is unclear.

Methods: We investigated the role of MBW in patients with NMD and the effect of two different tracer gases and cough assist devices on the LCI. Patients and controls performed MBW with nitrogen (N2) and sulfur hexafluoride (SF6), whereas the latter analysis was repeated after the use of a cough assist device in the NMD group. LCI was compared to forced vital capacity (FVC) and peak cough flow (PCF).

Results: 24 NMD patients (12 Duchenne Muscular Dystrophy, 8 Spinal Muscular Atrophy, 4 other NMDs) and 15 healthy controls were enrolled. In the NMD group, overall LCI N2 was higher than LCI SF6 (9.67 ± 1.56 vs. 8.71 ± 1.47; mean ± SD; p < 0.033). In controls, LCI N2 did not differ significantly from LCI SF6 (7.03 ± 0.37 vs. 7.05 ± 0.67; p = 0.882). Both LCI N2 and LCI SF6 were significantly higher in NMD patients as in controls (9.67 ± 1.56 vs. 7.03 ± 0.37, p < 0.001, and 8.71 ± 1.47 vs. 7.05 ± 0.67, p < 0.001). In the NMD group, both LCI N2 and LCI SF6 showed a negative correlation to FVC (r = −0.525; p = 0.008 and r = −0.526; p = 0.008, respectively) and PCF (r = −0.590; p = 0.002 and r = −0.641; p = 0.001, respectively). LCI N2 and LCI SF6 correlated well in the NMD group. LCI SF6 did not change significantly after the use of the cough assist in NMD patients (n = 22; 8.65 ± 1.52 pre vs. 8.79 ± 2.03 post, p = 0.667).

Conclusion: Lung involvement of patients with neuromuscular diseases goes beyond weakness of respiratory muscles. MBW with both N2 and SF6 is suitable to detect ventilation inhomogeneity in NMD patients with respiratory impairment. Cough assist devices with low to moderate pressure levels do not immediately improve the LCI.

Keywords: Chronic respiratory insufficiency, Pediatric pulmonology, Airway clearance, Lung function

Introduction

After being widely neglected over decades following its introduction in 1952 [1], multiple-breath-washout (MBW) lung function techniques have been rediscovered in recent years. The lung washout of tracer gases (usually nitrogen, N2, or Sulphur hexafluoride, SF6) to fractions...
capacity (FVC) and PCF manoeuvres values were noted. The best of three consecutive expiratory forced vital (PCF; Pocket Peak, nSpire Health Ltd., UK) and MBW. Performed spirometry (Easy One, NDD Medical Technologies, Switzerland) on Exhalyzer D (Ecomedics, Switzerland) using the Spiroware Version 3.2.1 software for all study subjects and controls and LCI was calculated for the 2.5% stopping point (LCI 2.5) for both tracer gases. For the NMD group, MBW and LCI SF₆ calculation was performed again after the use of the subject’s personal cough assist device with the personal settings or, if the patient was not equipped with a personal device, with a default device ad hoc titrated to the patients’ requirements. Cough assist use in the study comprised three consecutive cycles of three cough manoeuvres (3 × 3) over a period of 5 min. Patients unfamiliar with the use of cough assist devices received initial instructions and training before the 3 × 3 manoeuvres. All cough assist manoeuvres and cough assist titrations were performed and supervised by a respiratory nurse. LCI N₂ was compared to LCI SF₆ within each group and both analyses were compared between NMD patients and controls, LCI SF₆ measurements before and after the use of the cough assist were compared for the NMD study group. Correlation analyses of LCI, FVC and PCF were performed. All MBW measurements and interpretations were performed according to ERS/ATS quality standards under the supervision of trained staff [16]. For each subject and analysis, two technically acceptable sequential MBW measurements with a coefficient of variation (CV) < 5% or three technically acceptable sequential MBW measurements with a CV < 10% were considered eligible for inclusion.

Statistical analyses were performed with SPSS Version 27 (IBM, USA) and Graphpad Prism Version 9.2.0 (GraphPad, USA). Kolmogorov–Smirnov-Lilliefors- and Shapiro–Wilk-test were used to determine normal distribution of non-descriptive study datasets. Unpaired t-test were used for comparison between two groups and pre-/post-analyses, respectively. The relation between study parameters was determined using Pearson’s correlation and linear regression. Bland–Altman-plots were used to compare LCI results with different tracer gases to estimate the risk of bias.

Methods
Inclusion criteria for NMD patients were established diagnosis of NMD with respiratory muscle involvement (diagnosed clinically and/or by pulmonary function testing). NMD patients and age matched healthy controls performed spirometry (Easy One, NDD Medical Technologies, Switzerland), peak cough flow measurement (PCF; Pocket Peak, nSpire Health Ltd., UK) and MBW. The best of three consecutive expiratory forced vital capacity (FVC) and PCF manoeuvres values were noted. MBW was performed sequentially with the tracer gases N₂ and SF₆ on an Exhalyzer D (Ecomedics, Switzerland) using the Spiroware Version 3.2.1 software for all study subjects and controls and LCI was calculated for the 2.5% stopping point (LCI 2.5) for both tracer gases. For the NMD group, MBW and LCI SF₆ calculation was performed again after the use of the subject’s personal cough assist device with the personal settings or, if the patient was not equipped with a personal device, with a default device ad hoc titrated to the patients’ requirements. Cough assist use in the study comprised three consecutive cycles of three cough manoeuvres (3 × 3) over a period of 5 min. Patients unfamiliar with the use of cough assist devices received initial instructions and training before the 3 × 3 manoeuvres. All cough assist manoeuvres and cough assist titrations were performed and supervised by a respiratory nurse. LCI N₂ was compared to LCI SF₆ within each group and both analyses were compared between NMD patients and controls, LCI SF₆ measurements before and after the use of the cough assist were compared for the NMD study group. Correlation analyses of LCI, FVC and PCF were performed. All MBW measurements and interpretations were performed according to ERS/ATS quality standards under the supervision of trained staff [16]. For each subject and analysis, two technically acceptable sequential MBW measurements with a coefficient of variation (CV) < 5% or three technically acceptable sequential MBW measurements with a CV < 10% were considered eligible for inclusion.

Statistical analyses were performed with SPSS Version 27 (IBM, USA) and Graphpad Prism Version 9.2.0 (GraphPad, USA). Kolmogorov–Smirnov-Lilliefors- and Shapiro–Wilk-test were used to determine normal distribution of non-descriptive study datasets. Unpaired t-test were used for comparison between two groups and pre-/post-analyses, respectively. The relation between study parameters was determined using Pearson’s correlation and linear regression. Bland–Altman-plots were used to compare LCI results with different tracer gases to estimate the risk of bias.

Results
We enrolled 27 patients and 15 age matched healthy controls in the study. Three extremely weak NMD patients failed to perform valid MBW measurements due to persistent mouth leaks (two female SMA patients age 13 and 17 years and one female patient with unspecified NMD aged 5 years) and were excluded. Of the 24 NMD patients finally enrolled, 12 had DMD, 8 had SMA and 4 had other NMDs. Most patients showed severe restrictive patterns on spirometry and had measurable cough insufficiency (patient characteristics and study datasets
are given in Table 1). All patients but three out of 24 (87.5%) were supplied with a cough assist device before enrolment in the study. The same proportion of patients had established nocturnal non-invasive ventilation (Table 1). Cough assist inspiratory pressure levels were 24.8 ± 4.0 cmH2O (mean ± SD), expiratory pressure levels were −23.3 ± 5 cmH2O. After uniform technical synchronization of MBW analyses, three SMA patients showed CV values slightly above the 5%-threshold for their two consecutive LCI N2 measurements (CV 6.7, 7.2 and 8.7%, respectively), one of those showed an elevated CV (8.7%) compared to healthy controls, indicating ventilation inhomogeneity in NMD patients with profound thoracic restriction. We demonstrate that lung ventilation inhomogeneity in NMD patients with muscular or profound thoracic restriction. The agreement of LCI N2 and LCI SF6 before the use of the cough assist as well as after the use of the cough assist (9.54 ± 0.667 vs. 7.03 ± 0.590, respectively) (Fig. 1, Panel B/C). LCI SF6 did not change significantly after the use of the cough assist in NMD patients (n = 22; 8.71 ± 1.52 pre vs. 8.45 ± 1.32 post, p = 0.214) (Fig. 3). One patient with DMD had a marked increase of LCI SF6 after the use of the cough assist (9.54 vs. 15.82), widely exceeding the changes seen in all other patients. Notably, exclusion of this patient from pre-/post-analyses did not change the statistical outcome (n = 21; 8.61 ± 1.54 pre vs. 8.45 ± 1.32 post, p = 0.067) (Fig. 3, Panel A). One patient with DMD had a marked increase of LCI SF6 after the use of the cough assist (9.54 vs. 15.82), widely exceeding the changes seen in all other patients. Notably, exclusion of this patient from pre-/post-analyses did not change the statistical outcome (n = 21; 8.61 ± 1.54 pre vs. 8.45 ± 1.32 post, p = 0.214) (Fig. 3).

**Discussion**

In the present study both LCI N2 and LCI SF6 were significantly higher in NMD patients (applying as well to every subgroup of NMD) as compared to healthy controls (Additional file 1: Figure S1). LCI N2 and the LCI SF6 correlated very well in the NMD group but not in the control group with bias being ± 0.96 for NMD patients and -0.03 for controls according to Bland–Altman analysis (Fig. 1, Panel B/C). LCI SF6 did not change significantly after the use of the cough assist in NMD patients (n = 22; 8.71 ± 1.52 pre vs. 8.79 ± 2.03 post, p = 0.667) (Fig. 3, Panel A). One patient with DMD had a marked increase of LCI SF6 after the use of the cough assist (9.54 vs. 15.82), widely exceeding the changes seen in all other patients. Notably, exclusion of this patient from pre-/post-analyses did not change the statistical outcome (n = 21; 8.61 ± 1.54 pre vs. 8.45 ± 1.32 post, p = 0.214) (Fig. 3).
before for DMD patients with profound thoracic restriction [5]. Our study now corroborates these findings for other groups of NMD as well. We show that the underlying ventilation inhomogeneity can be detected and monitored by both SF₆ and N₂ MBW. However, though both elevated, LCI N₂ values were significantly higher in NMD patients than LCI SF₆ values, which is in accordance to earlier observations in other disease cohorts but was not observed in our control group [15]. The reason for the relatively high difference between N₂- and SF₆-LCI values in our study cohort remains speculative. The different physical properties of both tracer gases might lead to distinct lung clearance especially in the context of altered ventilation mechanics in complex restrictive patterns of NMD patients. Speculatively, as hinted by higher LCI values for N₂ in comparison to SF₆, N₂ MBW might be more sensitive towards detection of ventilation inhomogeneity in NMD patients. This might represent another reason to use N₂ MBW in clinical practice in addition to environmental aspects regarding the greenhouse effects of SF₆ [17]. We speculated that the use of cough assist devices might have an immediate influence on the observed ventilation inhomogeneity of NMD patients, for example by recruitment of (micro)atelectatic lung areas and/or
mucus clearance. A moderate short-lasting increase of vital capacity could be demonstrated by the use of cough assists in NMD patients earlier [12]. However, despite a tendency towards lower values, LCI did not change significantly after the use of the cough assist. Though disappointing from a therapeutic perspective, this finding renders MBW lung function timing in NMD patients independent from cough assist use in future studies or in a clinical setting. Notably, the pressure levels of the cough assist devices used in this study can be regarded as low to moderate. Some centres use and recommend higher pressure levels (up to 60cmH₂O), which might alter the influence on LCI [18]. Additionally, all but three out of 24 NMD patients used positive-pressure nocturnal non-invasive ventilation, which might decrease the effect of additional positive pressure on lung ventilation homogeneity delivered by cough assist devices. Future studies should investigate the effect of higher pressure levels in cough assist devices on the LCI, desirable in larger cohorts of NMD patients to allow comparison of NMD patient subgroups. Notably, the potential risk of pneumothorax through cough assist devices should be kept in mind, especially when increasing pressure levels [19].

In conclusion, NMD patients with respiratory muscle involvement develop lung ventilation inhomogeneity as a marker of secondary structural lung disease, which can be detected and monitored by N₂ and SF₆ MBW. This lung disease worsens with increasing muscular weakness. Mechanical assisted cough with low to moderate pressure levels does not have an immediate influence on lung ventilation inhomogeneity.

**Abbreviations**

MBW: Multiple breath washout; LCI: Lung clearance index; NMD: Neuromuscular disease; DMD: Duchenne muscular dystrophy; SMA: Spinal muscular atrophy; NIV: Non-invasive ventilation; VC: Vital capacity; PCF: Peak cough flow.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12890-022-02012-z.

**Figure S1**

LCI N₂ and SF₆ plotted for the different NMDs of the study group and controls. LCI of all NMD subgroups differ significantly from the respective control group with the same tracer gas (p < 0.001). Mean and standard deviation are indicated. DMD Duchenne Muscular Dystrophy SMA Spinal Muscular Atrophy uNMD uncategorized neuromuscular disease.

**Acknowledgements**

We thank Ina Fuge, Claudia Bock, Nadine Kordt and Manuela Groch-Seidler for their excellent technical assistance.

**Author contributions**

FS and MS conceived and designed the study. AP performed all measurements. AP, EO, FS and MS analysed the data. AP, FS and MS wrote the paper with significant intellectual input from all authors. All authors read and approved the final manuscript.

**Funding**

Open Access funding enabled and organized by Projekt DEAL. No funding was obtained for the study.

**Availability of data and materials**

The datasets generated and/or analysed during the current study are available in the Figshare repository, https://figshare.com/s/942c1de31e1b9a26d63 (Private link. Public link will be generated after publishing of the paper.)

**Competing interests**

The authors declare that they have no competing interests.

**Ethics approval and consent to participate**

The study was reviewed and approved by the ethics committee of the University of Duisburg Essen (reference 18–8165-BO). The participants or their legal guardians provided their written informed consent to participate in this study. All research was performed in accordance with the Declaration of Helsinki.

**Consent for publication**

Not applicable.

**Author details**

1Pediatric Pulmonology and Sleep Medicine, Department of Paediatrics III, University Children’s Hospital Essen, University of Duisburg-Essen, Hufelandstr. 55, 45122 Essen, Germany. 2Pediatric Research Network Essen, Essen, Germany.
References
1. Beckläcke MR. A new index of the intrapulmonary mixture of inspired air. Thorax. 1952;7(1):111–6.
2. Gustafsson PM, Aurora P, Lindblad A. Evaluation of ventilation maldistribution as an early indicator of lung disease in children with cystic fibrosis. Eur Respir J. 2003;22(6):972–9.
3. Gustafsson PM, De Jong PA, Tiddens HA, Lindblad A. Multiple-breath inert gas washout and spirometry versus structural lung disease in cystic fibrosis. Thorax. 2008;63(2):129–34.
4. Clay RD, Iyer VN, Reddy DR, Sionts B, Scanlon PD. The 'complex restrictive' pulmonary function pattern: clinical and radiologic analysis of a common but previously undescribed restrictive pattern. Chest. 2017;152(6):1258–65.
5. Stehling F, Dohna-Schwake C, Mellies U, Grosse-Onnebrink J. Decline in lung volume with duchenne muscular dystrophy is associated with ventilation inhomogeneity. Respir Care. 2015;60(9):1257–63.
6. Estenne M, Gevenois PA, Kinneer W, Soudon P, Heilporn A, De Troyer A. Lung volume restriction in patients with chronic respiratory muscle weakness: the role of microatelectasis. Thorax. 1993;48(7):698–701.
7. Stehling F, Steinmann J, Mellies U. The microbiome and secondary lung disease in neuromuscular patients: Is it time to change our clinical practice? - Reply. Respirology. 2017;22(5):1036.
8. Chatwin M, Ross E, Hart N, Nickol AH, Polkey MI, Simonds AK. Cough augmentation with mechanical insufflation/exsufflation in patients with neuromuscular weakness. Eur Respir J. 2003;21(3):502–8.
9. Chatwin M, Toussaint M, Goncalves MR, Sheers N, Mellies U, Gonzales-Bermejo J, et al. Airway clearance techniques in neuromuscular disorders: a state of the art review. Respir Med. 2018;136:98–110.
10. Dohna-Schwake C, Ragette R, Teschler H, Voit T, Mellies U. IPPB-assisted coughing in neuromuscular disorders. Pediatr Pulmonol. 2006;41(6):551–7.
11. Merc H, Falaize L, Pradon D, Lacombe M, Petitjean M, Orlikowski D, et al. Short-term effect of volume recruitment-derecruitment manoeuvre on chest-wall motion in Duchenne muscular dystrophy. Chron Respir Dis. 2017;14(2):110–6.
12. Veldhoen ES, Vercoelen F, Ros L, Verweij-van den Ouderenijt LP, Wosten-van Asperen RM, Hulzebos EH, et al. Short-term effect of air stacking and mechanical insufflation-exsufflation on lung function in patients with neuromuscular diseases. Chron Respir Dis. 2022. https://doi.org/10.1177/14799731221094619.
13. Stahl M, Joachim C, Wielputz MO, Mall MA. Comparison of lung clearance index determined by washout of N2 and SF6 in infants and preschool children with cystic fibrosis. J Cyst Fibros. 2019;18(3):399–406.
14. Bayfield KJ, Horsley A, Alton E, Irving S, Bush A, Davies JC. Simultaneous sulfur hexafluoride and nitrogen multiple-breath washout (MBW) to examine inherent differences in MBW outcomes. ERJ Open Res. 2019. https://doi.org/10.1183/23120541.00234-2018.
15. Yammine S, Lenher N, Nyulas S, Singer F, Latzin P. Using the same cut-off for sulfur hexafluoride and nitrogen multiple-breath washout may not be appropriate. J Appl Physiol (1985). 2015;119(2):1510–2.
16. Robinson PD, Latzin P, Verbanck S, Hall GI, Horsley A, Gappa M, et al. Consensus statement for inert gas washout measurement using multiple- and single-breath tests. Eur Respir J. 2013;41(3):507–22.
17. Dervos CT, Vassiliou P. Sulfur hexafluoride (SF6): global environmental effects and toxic byproduct formation. J Air Waste Manag Assoc. 2000;50(1):137–41.
18. Boitano LJ. Management of airway clearance in neuromuscular disease. Respir Care. 2006;51(8):913–22.
19. Suri P, Burns SP, Bach JR. Pneumothorax associated with mechanical insufflation-exsufflation and related factors. Am J Phys Med Rehabil. 2008;87(11):951–5.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:
- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.
Learn more biomedcentral.com/submissions