

SUPPLEMENTARY FILE

Sheers *et al*: **Rapidly and slowly-progressive neuromuscular disease: Differences in pulmonary function, respiratory tract infections and response to lung volume recruitment therapy (LVR)**

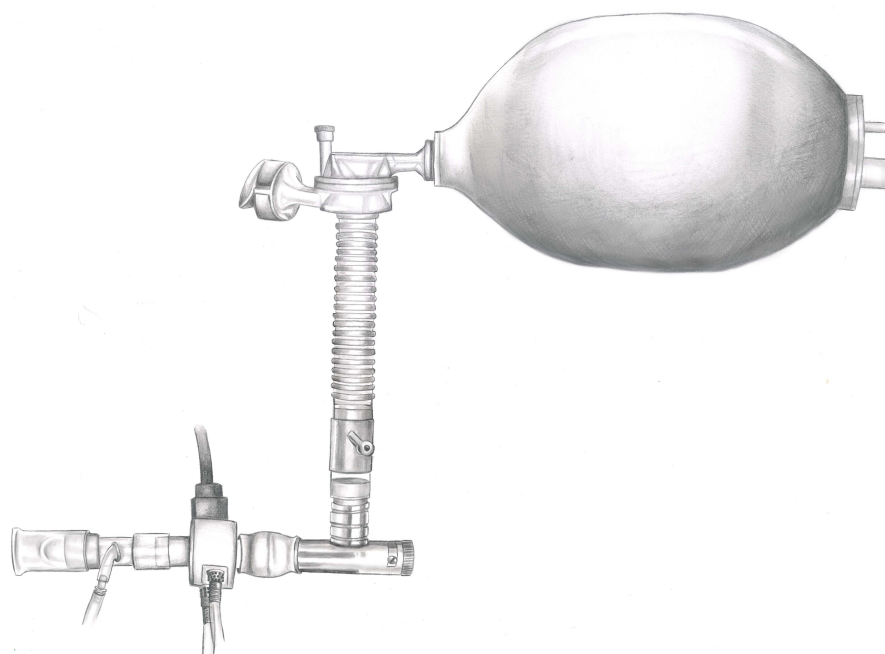
METHODS

Procedure

Vital capacity (VC), unassisted peak cough flow (PCF), respiratory system compliance (C_{rs}), lung insufflation capacity (LIC) and PCF from LIC (PCF_{LIC}), were collected using a calibrated heated pneumotachometer connected to a multi-channel data acquisition and analysis system (Hans Rudolph™ heated pneumotachometer Model 3700A, Pneumotachometer Heater Control, Pneumotach Amplifier 1, Series 1110; Hans Rudolph, inc., Kansas, USA. CED Micro 1401 hardware and Spike2 (version 7) software; Cambridge Electronic Design Limited, Cambridge, England). Raw flow, volume and pressure traces were analysed manually to derive LIC, VC, PCF, PCF_{LIC} and C_{rs} values.

A three-port tap connector was used in the circuit to obtain LIC, PCF_{LIC} and C_{rs} (Figures S1 and S2). The participant interface (mouthpiece or oro-nasal mask depending on test), pressure line connector and pneumotachometer were connected in series to the first port, which was always open (participant limb). The second port of the tap connector, distal to the pneumotachometer and participant, opened to the atmosphere (spontaneous limb). The third port (inflation limb) connected to either i) the LVR kit (for LIC and PCF_{LIC}), or ii) to a cylinder of compressed medical air with an adjustable flow regulator via a length of narrow bore tubing (for C_{rs}). A tap controlled the second and third ports, such that only one of these could be open and connect with the participant limb at any given time. Opening the tap connected the participant to the atmosphere and allowed spontaneous inhalation and exhalation whilst occluding flow from the inflation limb. When the tap was closed, the second port became occluded and the inflation limb opened, allowing i) LVR or ii) constant flow of medical air for C_{rs} measurements.

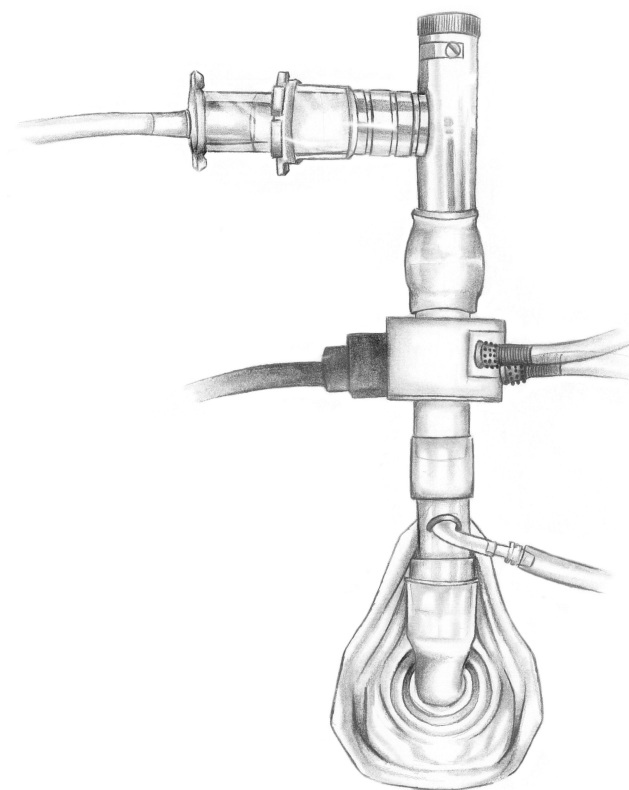
A commercially-available pulmonary function test system (Hyp'Air Compact Plus® Portable PFT System; Medisoft, Sorinnes, Belgium) was used to measure static lung volumes using the multiple-breath nitrogen washout technique.[1, 2] This same equipment also evaluated markers of respiratory muscle strength (maximal inspiratory and expiratory pressures sustained at the mouth for 1 second (MIP from RV, MEP from TLC), and sniff nasal inspiratory pressure (SNIP)).



Supplement Figure S1: Customised circuit, as used for measuring LIC

LIC = lung insufflation capacity

Components from left to right: mouthpiece, pressure line connector (*approximating mouth pressure, P_{mo}*), pneumotachometer and heater shell (*determining flow and volume*), three-port tap connector and lung volume recruitment (LVR) kit. The right-angled tap connector opened to the participant (via pneumotachometer) and LVR kit (via inflation limb). An additional port (outlet not visible) opened to the atmosphere and was controlled by twisting the tap's end. *Original artwork illustrated by Krisha Saravanan.*



Supplement Figure S2: Customised circuit, as used for measuring C_{rs}

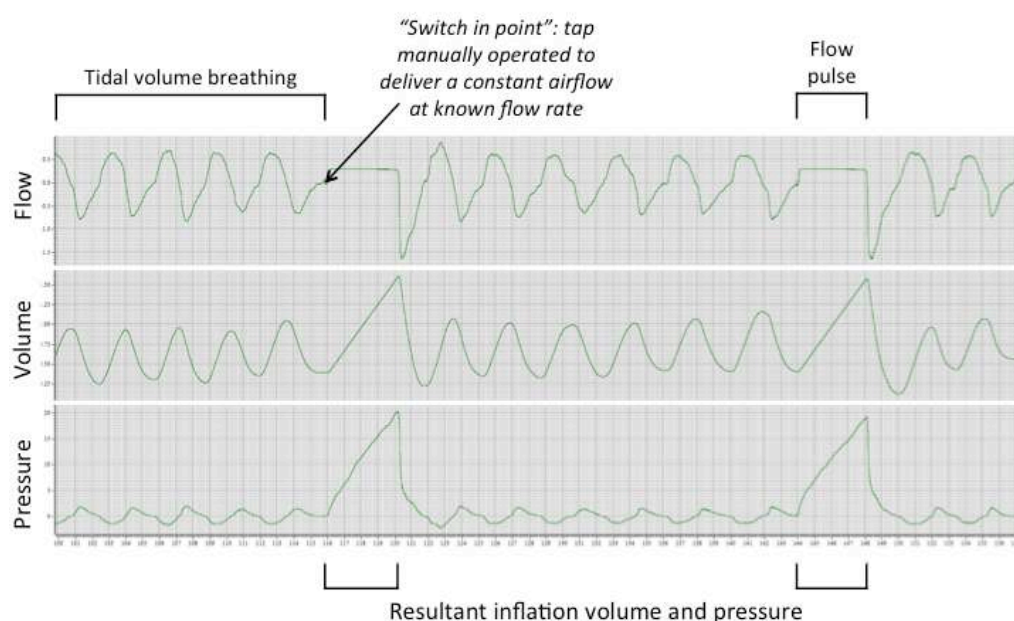
C_{rs} = respiratory system compliance

Components from top to bottom: three-port tap connector, narrow bore tubing and connector attaching to inflation limb (from cylinder of compressed medical air, not shown), pneumotachometer and heater shell (*determining flow and volume*), pressure line connector (*approximating mouth pressure, P_{mo}*), and non-vented oro-nasal mask. *Original artwork illustrated by Krisha Saravanan.*

Measurement of C_{rs} : Pulse inflation method

We employed a non-invasive measure of C_{rs} based on the pulse inflation method of Suratt *et al.*[3] During this technique, respiratory muscle relaxation occurs resulting in a quasi-static pressure-volume curve from which C_{rs} is calculated.[4] Advantages are that: it produces values comparable to other methods of C_{rs} , is reproducible within a single sitting, has been evaluated across a wide variety of diseases and can be performed by participants with severe respiratory muscle weakness.[5]

An assistant sealed the oro-nasal mask to the participant's face whilst the assessor controlled the tap. Participants were instructed to breathe gently (i.e., spontaneous tidal volume breaths) for three to five breaths, and then relax. After exhaling to approximate FRC, the assessor manually operated the tap to deliver a constant inflation airflow at a known flow rate. Participants were coached to cease any active respiratory efforts and allow the pulse of air to passively inflate their lungs. Flow was commenced at 0.3 L/s and modified if necessary to match the participant's flow rate, minimise visible respiratory efforts, and produce a linear pressure-time signal. The duration of the pulse inflation was controlled by the assessor; inflation continued until a volume greater than tidal breathing but less than a maximal inflation was delivered, confirmed by examining the real-time pressure and volume signals. Prolonged pulse durations were avoided as these could potentially recruit areas of atelectatic lung and alter compliance. Cessation of inflation and passive exhalation occurred by returning the tap to the starting position (Figure S3). A minimum of ten inflation manoeuvres were obtained during each C_{rs} test. Respiratory system compliance was calculated for each manoeuvre by generating a pressure-volume curve, with the line of best fit (least squares regression) representing the slope and C_{rs} value. The C_{rs} summary value (mean of all acceptable pulse inflation manoeuvres, from a minimum of three satisfactory manoeuvres) was used in analysis.



Supplement Figure S3: Measurement of C_{rs}

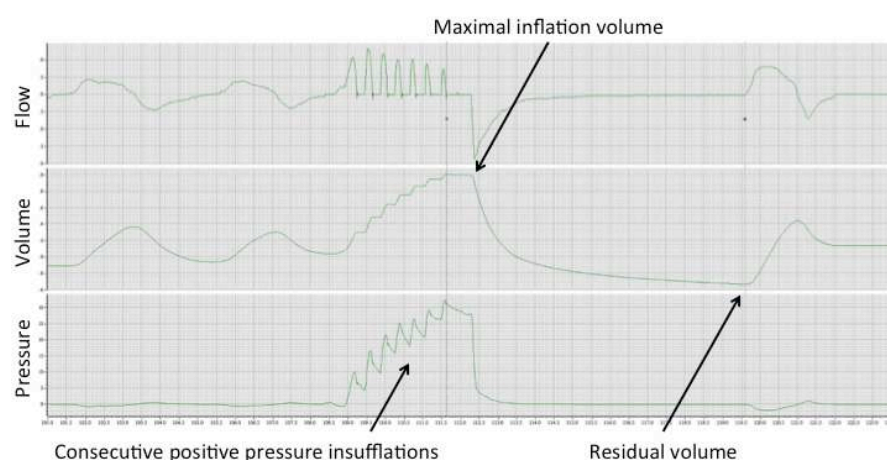
Respiratory system compliance (C_{rs}) was measured using the pulse inflation method. Each test generated flow (L/sec), volume (L) and mouth pressure (cmH₂O) traces as illustrated above. Traces were scored as per developed Scoring rules to determine C_{rs} ; a pressure-volume curve was generated (X-Y plot) for each inflation manoeuvre, with the line of best fit (least squares regression) representing the slope and hence C_{rs} value.

Measurement of lung insufflation capacity (LIC)

Following a short period of tidal volume breathing (inhalation and exhalation through open spontaneous limb), the three-way tap was manually operated to switch the participant to receive inflations via LVR bag compressions. Participants were inflated to their maximum tolerable volume, at which time they signalled the assessor who operated the tap to allow passive exhalation. Lung insufflation capacity (the maximum, tolerable insufflation capacity achieved with external assistance that does not involve the person controlling their glottis[6, 7]) was calculated for each manoeuvre from the volume signal. Cursors were positioned manually i) at the uppermost point achieved after delivery of consecutive positive pressure insufflations (maximum tolerable

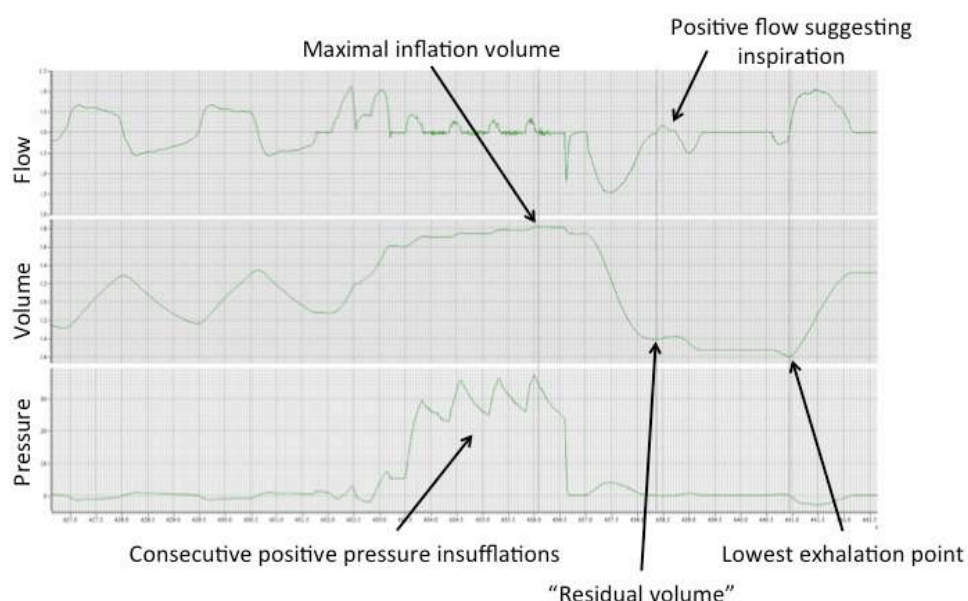
insufflation capacity / maximal inflation volume), and ii) at the lowermost point recorded on the subsequent exhalation (Figure S4). Lung insufflation capacity was taken as the volume between the two cursors, and the largest, technically acceptable LIC value was used in analysis.

The corresponding flow and pressure signals were cross-referenced to ensure that both the assisted inflation and expiration were technically acceptable. An assisted inflation trace was considered unacceptable if there were irregularities in pressure and flow traces, as seen during reflexive glottic closure or leak. Exhalation to RV was inferred if expiratory volume was more negative than tidal breathing, and was determined at the point when expiratory flow returned to zero. The expiratory component was assessed to ensure small spontaneous inspiratory efforts were not made, especially approaching the end of expiration, which may have artificially increased exhaled volume. If pressure or flow signals suggested an inspiratory effort was made, the exhaled volume immediately prior to this was taken as the end point (RV) (Figure S5).



Supplement Figure S4: Measurement of LIC

Lung insufflation capacity (LIC) was taken as the exhaled volume (in litres) following consecutive insufflation to the participant's maximum tolerable insufflation capacity (or maximal inflation volume to residual volume).



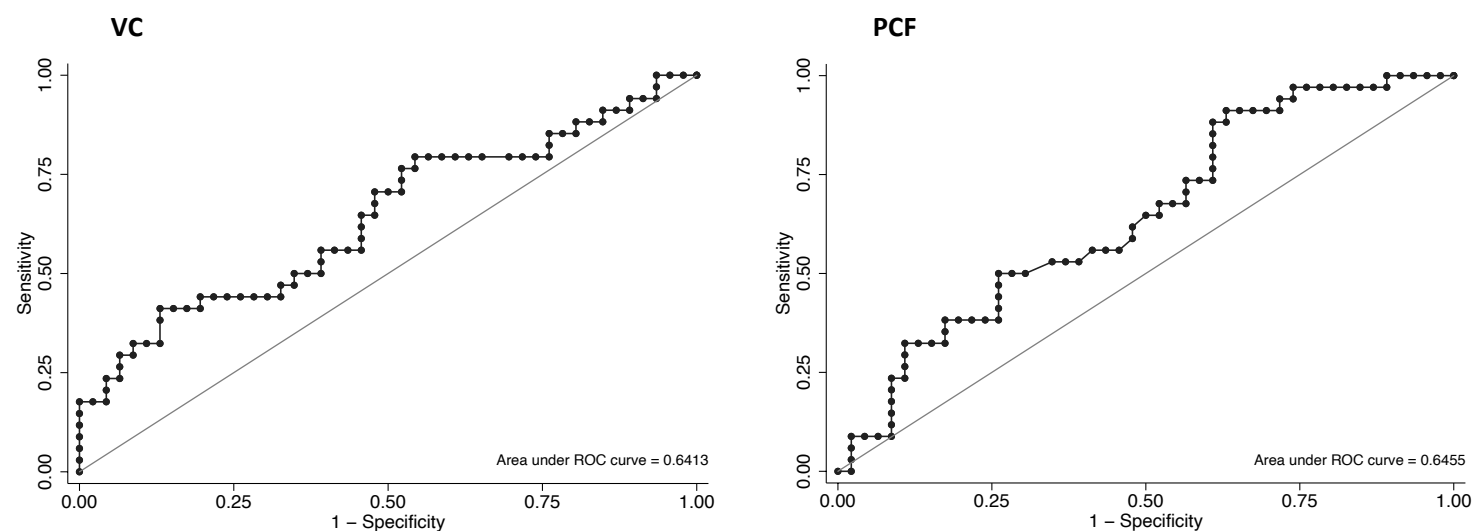
Supplement Figure S5: Measurement of LIC, in participant with inspiratory effort

Lung insufflation capacity (LIC) was taken as the exhaled volume (in litres) following consecutive insufflation to the participant's maximum tolerable assisted inflation capacity. Note small inspiratory efforts towards end-expiration; residual volume taken prior to these (maximal inflation volume to residual volume).

RESULTS

Respiratory tract infections

Receiver operating characteristic curves evaluating the sensitivity and specificity of VC (L) and PCF (L/min) suggest both parameters were poor at distinguishing past history of RTI in this cohort (AUC [95% CI] for VC = 0.64 [0.52, 0.77], PCF = 0.65 [0.52, 0.77]; Figure S6).



Supplement Figure S6: Receiver operator characteristic curves for past history of respiratory tract infection, for vital capacity (VC) and peak cough flow (PCF)

Data represents all participants. Vital capacity (L), peak cough flow (L/min).

Immediate effect of LVR

| Variable | Model | | | Time | | Disease | | Interaction | |
|--------------------|---------------------------|-------------|-------------------|----------|--------------|----------|-------------------|-------------|--------------|
| | log restricted likelihood | χ^2 | p-value | χ^2 | p-value | χ^2 | p-value | χ^2 | p-value |
| C _{rs} | 350.4 | 22.3 | 0.0001 | 9.38 | 0.002 | 10.04 | 0.002 | 7.69 | 0.006 |
| LIC | -139.9 | 31.5 | <0.0001 | 10.30 | 0.001 | 20.87 | <0.0001 | 0.20 | 0.654 |
| VC | -68.8 | 23.7 | <0.0001 | 2.90 | 0.088 | 20.60 | <0.0001 | 0.81 | 0.367 |
| FRC | -73.5 | 25.1 | <0.0001 | 3.97 | 0.046 | 18.00 | <0.0001 | 4.93 | 0.026 |
| TLC | -96.1 | 28.9 | <0.0001 | 6.11 | 0.013 | 18.85 | <0.0001 | 7.59 | 0.006 |
| RV | -53.1 | 20.0 | 0.0002 | 2.47 | 0.116 | 13.74 | 0.0002 | 4.71 | 0.030 |
| ERV | 11.6 | 17.2 | 0.0007 | 0.44 | 0.507 | 16.21 | 0.0001 | 0.02 | 0.885 |
| IC | -37.0 | 9.1 | 0.028 | 0.09 | 0.767 | 8.04 | 0.005 | 0.75 | 0.388 |
| PCF | -827.5 | 7.1 | 0.070 | 0.41 | 0.520 | 2.82 | 0.093 | 2.70 | 0.100 |
| PCF _{LIC} | -801.6 | 12.3 | 0.006 | 6.02 | 0.014 | 2.77 | 0.096 | 0.93 | 0.335 |

Supplement Table S1: Linear mixed models of the effect of Time and Disease on respiratory function in participants with neuromuscular disease, naïve to lung volume recruitment

Time represents Baseline and Immediately post a single-session of LVR; Disease signifies MND (amyotrophic lateral sclerosis / motor neurone disease) or Other NMD (slowly-progressive neuromuscular diseases); where Time and Disease are fixed effects and participant a random effect. *P*-values in **bold** indicate statistically significant values (*p*<0.05).

C_{rs} = Total respiratory system compliance, LIC = lung insufflation capacity, VC = vital capacity, FRC = Functional residual capacity, TLC = Total lung capacity, RV = Residual volume, ERV = Expiratory reserve volume, IC = Inspiratory capacity, PCF = Peak cough flow, PCF_{LIC} = PCF from LIC.

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7. Naughton PE, Sheers N, Berlowitz DJ, *et al.* Objective measurement of lung volume recruitment therapy: Laboratory and clinical validation. *BMJ Open Resp Res* 2021;8:e000918.