

Supplement 1

Spirometry

Forced vital capacity (FVC) was measured in the seated position with a noseclip in all children over 5 years. A bacterial filter (SureGard; BirdHealthcare, Australia) attached to the Carefusion Vyntus PNEUMO system (Hoechberg, Germany) was used with SentrySuite software. Recordings were accepted if fulfilling criteria of the American Thoracic Society/European Respiratory Society guidelines (with a minimum of 3 satisfactory recordings)[1]. Predicted values and z-scores were calculated using Global Lung Initiative references (GLI)[2]. Height was predicted using ulna length for children who were unable to stand independently or spinal deformity was present[3].

Oscillometry

Oscillometry was attempted in all children over 5 years. Respiratory impedance measurement has been previously described[4]. A bacterial filter (SureGard; BirdHealthcare, Australia) attached to a commercially available device (i2M Chess medical, Gent, Belgium, marketed by Cosmed, Rome Italy) was used in accordance with the American Thoracic Society/European Respiratory Society guidelines (minimum of 3 and maximum of 12 acceptable measures obtained). A pseudo-random noise forcing function containing integer-multiple frequencies between 4 and 48 Hertz was applied at the airway opening. Reference data were used to calculate Z-scores from height[5].

Lung Clearance Index

Lung clearance index (LCI) was attempted in all children over 5 years. Measurements were obtained with the Multiple Breath Washout using endogenous nitrogen [6] with Eco Medics exhalizer D system (Durnten, Switzerland) attached to a bacterial filter and Spiroware software. Children were in the seated position and instructed to breathe normally with a nose clip. If the child was not able to maintain lip seal a flanged mouthpiece (Eco medics, Switzerland) or facemask (Quadralite mas; Intersurgical, UK) were used. A minimum of 3 tests with at least 2 acceptable tests within 1 LCI unit of each other were obtained. Post-hoc analysis for inadequate measurement quality was done and measurements were excluded if leak or excessive variation in breathing pattern of tidal volume was noticed.

Sniff Nasal Inspiratory Pressure

Sniff Nasal Inspiratory Pressures (SNIP) were attempted in all children over 5 years of age taken in the seated position. A nasal probe (careFusion, UK) was sized and placed in their nostril and measures were taken with a portable respiratory pressure meter (CareFusion MicroRPM) in each nostril. Maximal short, sharp sniffs were measured in each nostril (minimum 3 and maximum of 5). Values were recorded in cmH₂O and the highest repeatable value was reported as a z-score[7].

Peak Cough Flow

Peak Cough Flow (PCF) was attempted in all children over 5 years in the seated position with a noseclip and bacterial filter (SureGard, BirdHealthcare, Australia). CareFusion Vyntus PNEUMO system (Hoechberg, Germany) and SentrySuite software were used for the measurements. Children were instructed to perform a maximal inspiration followed by a cough into the spirometer (minimum 3 coughs and maximum 5 coughs). The highest value was recorded as L/min.

Maximum Inspiratory Pressures and Maximum Expiratory Pressures

In children who were able to perform spirometry, Maximum Inspiratory Pressures (MIPS) and Maximum Expiratory Pressures (MEPS) were attempted and recorded using a respiratory pressure meter (CareFusion microRPM). To obtain MIPS the children were instructed to exhale to residual volume, place a flanged mouthpiece (Echo Medics, Switzerland) attached to a bacterial filter (CareFusion, UK) in their mouth and perform maximal inspiration sustained for five seconds. For the MEPS manoeuvre, children were instructed to inspire to total lung capacity then place a flanged mouthpiece attached to bacterial filter in their mouth and perform a maximal exhalation sustained for 5 seconds. A minimum of 3 and maximum of 5 static pressure manoeuvres were performed and recorded in cmH₂O with the highest repeatable value being reported as a z-score[8].

Polysomnography

The following channels were used: electroencephalogram (EEG), electrooculography (EOG), chin and diaphragm electromyography (EMG), thoracic and abdominal effort Respiratory inductive plethysmography (RIP) bands, nasal airflow, thermistor, body position, electrocardiogram (ECG), Oximetry oxygen saturations (SpO₂), transcutaneous CO₂ (tCO₂) and a full audio and video recording [9]. The PSG were evaluated for sleep disordered breathing (SDB), obstructive (OSA), central (CSA) and mixed sleep apnoea and hypoventilation. The specific PSG data extracted for analysis were: Apnoea-hypopnea index (AHI) (total study and in Rapid Eye Movement (REM) and non-REM (NREM) sleep), central AHI (total study and in REM/NREM sleep), obstructive AHI (total study and in REM/NREM), tCO₂ (mean, maximum and time >50mmHg), and SpO₂ (mean and nadir). Use of NIV was noted. Studies were assessed for hypoventilation based on the AASM 2020 paediatric criteria for hypoventilation (tCO₂ greater than 50 mm Hg for greater than 25% of total sleep time) and the definition suggested by Birnkrant *et al* (2018) (TcCO₂ greater than 50mmHg for more than 2% of total sleep time) [10]. There were no differences in PSG scoring between the two individual pediatric sleep physicians (total AHI p=0.91; total central index p=0.90)

Muscle function testing scores

Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) is a 16-item motor assessment for infants with Spinal Muscular Atrophy

(SMA), scoring for each item ranges from 0 (no response) to 4 (complete response) with total scores range from 0-64 with higher scores indicating better motor function[11]. Hammersmith Functional Motor Scale—Expanded (HMFSE) is a 33-item measure of motor function that assesses activities related to daily living. Each of the 33 activities is scored on a scale ranging from 0 to 2 (no to full response) and total scores range from 0 to 66 with an increased in total score indicating an improvement in muscle function[12]. Revised Upper Limb Module (RULM) assesses upper limb functional performance items that are reflective of reachable space and activities of daily living. This test has 19 items that are scored ranging from 0 (unable) to 2 (full achievement). Total score range from 0 to 37 with higher scores indicating better function [13].

Table 1: Rate of decline for Oscillometry and respiratory muscle function variables during first year of nusinersen treatment.

| Rate of Decline in Rsr8 z-score (95%CI) per annum | | |
|--|------------------------|--------------|
| Total group (n=19) | -0.13 (-0.64, 0.37) | 0.60 |
| SMA type 2 (n=11) | -0.85 (-1.55, -0.15) | 0.02 |
| SMA type 3 (n=8) | 0.64 (0.02, 1.263) | 0.04 |
| Rate of Decline in Xrs8zscore (95%CI) per annum | | |
| Total group (n=19) | 0.06 (-0.35, 0.48) | 0.77 |
| SMA type 2 (n=11) | 0.06 (-0.48, 0.60) | 0.82 |
| SMA type 3 (n=8) | 0.05 (-0.59, 0.69) | 0.87 |
| Rate of Decline in MIP z-score (95%CI) per annum | | |
| Total group (n=14) | 0.17 (-0.55 to 0.22) | 0.40 |
| SMA type 2 (n=9) | -0.19 (-0.73 to 0.36) | 0.50 |
| SMA type 3 (n=5) | -0.13 (-0.61 to 0.35) | 0.59 |
| Rate of Decline in MEP z-score (95%CI) per annum | | |
| Total group (n=14) | -0.35 (-0.68 to -0.01) | 0.04 |
| SMA type 2 (n=9) | 0.70 (-1.18 to -0.21) | 0.005 |

| | | |
|--|----------------------|------|
| SMA type 3 (n=5) | 0.22 (-0.03 to 0.48) | 0.09 |
| Rate of Decline in SNIPcmH₂O (95%CI) per annum | | |
| Total group (n=14) | -0.23 (-7.10, 6.64) | 0.95 |
| SMA type 2 (n=9) | -1.66 (-11.21, 7.88) | 0.73 |
| SMA type 3 (n=5) | 2.15 (-6.91, 11.21) | 0.64 |

Respiratory Resistance at 8 hertz (Rrs8); Respiratory Reactance at 8 hertz (Xrs8); Mean Inspiratory Pressure (MIP); Mean Expiratory Pressure (MEP); Sniff Nasal Inspiratory Pressure (SNIP).

Table 2: Summary statistics of FVC z-score and AHI total by muscle responder & non-responder and by baseline and 12 months

| | Non-responder (n=5) | | | Responder (n=21) | | |
|--------------------------|----------------------|----------------------|---------|----------------------|----------------------|-------------|
| | Baseline | 12-months | P-value | Baseline | 12-months | P-value |
| FVC z-score median (IQR) | -2.15 (-5.86, -2.09) | -2.61 (-5.60, -2.26) | 0.84 | -2.41 (-6.62, -0.40) | -2.12 (-5.55, -0.81) | 0.94 |
| AHI total median (IQR) | 3.50 (2.20, 5.20) | 3.00 (1.25, 6.80) | 0.73 | 5.90 (2.10, 13.30) | 2.70 (0.50, 5.30) | 0.02 |

Forced Vital Capacity (FVC), Apnoea-Hypopnoea index (AHI)

Table 3: Polysomnography variables at baseline compared to 1 year: Statistically significant difference analysed by Mann-Whitney U test when comparing baseline to 1 year highlighted.

| PSG Variable | SMA type 1 N=6 | | | SMA type 2 N=12 | | | SMA type 3 N=8 | | | All SMA types N=26 | | |
|--------------------------------|---------------------|---------------------|-------------|---------------------|---------------------|---------|---------------------|---------------------|---------|-----------------------|---------------------|--------------|
| | Baseline | 1 year | p-value | Baseline | 1 year | p-value | Baseline | 1 year | p-value | Baseline | 1 year | p-value |
| AHI REM Median (IQR) | 42.10 (24.60-46.50) | 7.80 (0-15.00) | 0.02 | 6.95 (3.60-18.70) | 7.20 (1.60-18.90) | 0.92 | 5.05 (2.70-12.95) | 2.55 (0-6.90) | 0.17 | 9.85 (4.70-34.70) | 6.80 (0-13.20) | 0.06 |
| AHI NREM Median (IQR) | 3.30 (1.30-4.50) | 1.15 (0-3.20) | 0.20 | 2.70 (1.65-5.10) | 0.80 (0-6.10) | 0.18 | 2.30 (1.20-4.85) | 1.35 (0.55-1.70) | 0.14 | 2.75 (1.30-4.80) | 1.10 (0-3.20) | 0.02 |
| Total AHI Median (IQR) | 11.65 (5.90-18.30) | 3.65 (0.50-5.80) | 0.05 | 4.60 (2.15-8.30) | 3.10 (0.70-8.90) | 0.29 | 3.75 (1.80-6.30) | 2.0(0.65-3.80) | 0.11 | 5.50 (2.10-10.10) | 2.70 (0.70-5.30) | 0.02 |
| CAI REM Median (IQR) | 27.20 (21.20-46.50) | 0.60 (0-14.30) | 0.01 | 8.05 (1.20-21.55) | 7.20 (1.60-18.90) | 0.95 | 4.80 (2.70-12.00) | 2.55 (0-6.90) | 0.17 | 10.70 (3.60-22.40) | 6.50 (0-9.80) | 0.03 |
| CAI NREM Median (IQR) | 2.85(1.30-3.90) | 1.30 (0-3.20) | 0.30 | 2.50(1.0-3.95) | 0.80 (0-3.70) | 0.16 | 2.30 (1.15-4.85) | 1.30 (0.55-1.70) | 0.13 | 2.50 (1.20-4.30) | 1.10 (0.20-2.50) | 0.02 |
| Total CAI Median (IQR) | 10.10 (8.50-11.30) | 2.30(0.50-5.20) | 0.01 | 4.10 (1.95-6.35) | 2.90 (0.70-8.90) | 0.42 | 3.75 (1.60-5.95) | 2.00 (0.65-3.60) | 0.12 | 5.50 (2.10-9.20) | 2.50 (0.70-4.50) | 0.012 |
| OAI REM Median (IQR) | 1.10 (0-8.00) | 0.60 (0-4.00) | 1.00 | 0 (0-1.90) | 0 | 0.43 | 0 (0-0.50) | 0 | 0.06 | 0 (0-1.50) | 0 | 0.29 |
| OAI NREM Median (IQR) | 0 (0-0.90) | 0(0-0.30) | 0.70 | 0 (0-1.20) | 0 | 0.30 | 0 | 0 (0-0.20) | 1.0 | 0 (0-0.50) | 0 | 0.34 |
| Total OAI Median (IQR) | 0.45 (0-4.90) | 0.25 (0-1.00) | 0.51 | 0.05 (0-1.50) | 0.0 (0-0.20) | 0.29 | 0.05 (0-0.45) | 0 (0-0.05) | 0.36 | 0.01 (0-0.80) | 0 (0-0.20) | 0.15 |
| Max tCO2 REM Median (IQR) | 43.55 (39.60-46.40) | 42.00 (38.70-43.40) | 0.36 | 45.00 (42.10-52.10) | 47.20 (44.60-49.80) | 0.87 | 45.25 (44.15-47.60) | 44.85 (43.40-46.05) | 0.60 | 45.15 (42.05-47.40) | 44.6 (42.00-47.90) | 0.57 |
| Max tCO2 NREM Median (IQR) | 42.95 (38.90-47.00) | 42.70 (38.40-44.00) | 0.63 | 45.60 (42.80-50.70) | 48.40 (40.10-51.20) | 0.74 | 45.55 (44.8-48.10) | 46.00 (44.80-51.85) | 0.75 | 45.60 (44.20-48.40) | 46.00 (42.00-49.8) | 0.87 |
| Total Max tCO2 Median (IQR) | 41.00 (39.60-47.00) | 42.70 (39.60-44.20) | 0.93 | 47.50 (43.75-51.65) | 49.80 (45.40-51.20) | 0.60 | 45.80 (44.85-48.10) | 46.15 (45.25-51.85) | 0.75 | 46.50 (44.20-48.80) | 46.20 (43.40-50.20) | 0.72 |
| Min Sat REM Median (IQR) | 79.00 (76.00-87.00) | 93.00 (90.00-93.00) | 0.01 | 91.00 (89.00-92.00) | 91.50 (90.00-95.00) | 0.38 | 92.00 (90.00-94.00) | 92.0 (91.00-95.00) | 0.71 | 90.00 (87.50-925.0) | 92.00 (90.00-95.00) | 0.02 |
| Min Sat NREM Median (IQR) | 92.00 (91.00-92.00) | 94.00 (93.00-95.00) | 0.07 | 92.00 (87.00-93.00) | 93.00 (91.00-94.00) | 0.14 | 92.50 (90.50-93.50) | 92.00 (87.00-94.00) | 0.87 | 92.00 (90.00-93.00) | 93.00 (91.00-94.00) | 0.08 |
| Total Min Sat Median (IQR) | 79.00 (76.00-87.00) | 93.00 (90.00-94.00) | 0.01 | 90.50 (86.50-92.00) | 92.00 (89.00-94.00) | 0.20 | 90.50 (89.50-92.50) | 90.00 (87.00-93.50) | 0.79 | 89.50 (86.00-92.00) | 92.00 (89.00-94.00) | 0.02 |
| Mean Sat Median (IQR) | 96.55 (95.20-98.20) | 96.70 (96.70-96.90) | 0.62 | 95.55 (95.35-96.60) | 96.50 (95.90-97.40) | 0.11 | 97.60 (97.00-97.80) | 96.90 (96.35-97.40) | 0.13 | 96.60 (95.40-97.60) | 96.70 (96.20-97.40) | 0.64 |

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