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ABSTRACT PRESENTED AT NATIONAL AND INTERNATIONAL CONFERENCES

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Trucco F, Ridout D, Finkel R, Scoto M, Mercuri E, Muntoni F on behalf of the international SMA consortium (iSMAc). *Respiratory function in SMA type 2 and non-ambulant SMA type 3 longitudinal data from the international SMA consortium (iSMAc).* **World Muscle Society 2019 (Poster Presentation awarded)**

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Trucco F, Bush A, Tan HL. *Reducing the need for carbon dioxide monitoring in the investigation of paediatric sleep disordered breathing.* **International Conference European Respiratory Society 2018 (Oral Communication)**

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LIST OF ABBREVIATIONS

4SC: four stairs climb

6MWT: 6-minute walking test

CMD: Congenital muscular dystrophy

CMS: Congenital myasthenic syndromes

CMYO: Congenital myopathy

CO_2: Carbon dioxide

CPAP: Continuous positive airway pressure

CR Poly: Cardio-respiratory polygraphy

CRP: C-reactive protein

CS: Corticosteroids

DM1: Congenital myotonic dystrophy

DMD: Duchenne muscular dystrophy

EBC: Exhaled breath condensate

EEG: Electroencephalogram

EMG: Electromyography

EOG: Electro oculogram

et$PaCO_2$: End-tidal partial pressure of carbon dioxide

FOT: Forced Oscillation technique

FVC: Forced Vital Capacity (absolute, L)

FVC %: Forced Vital Capacity % predicted

GPB: Glosso-pharyngeal breath

ICAM-1: Intercellular adhesion molecule 1

IFN-γ: Interferon gamma

IL-1β: Interleukin 1 beta

IL-6: Interleukin 6

IL-10: Interleukin 10

IL-17: Interleukin 17

IL-23: Interleukin 23

IOPD: Infantile-onset Pompe Disease

IQR: Interquartile range

LoA: Loss of ambulation

LOPD: Late-onset Pompe Disease

MCP-1: Monocytes chemoattractant protein 1

MEP: Maximal Expiratory pressure

MIP: Maximal Inspiratory pressure

NF-κB: Nuclear Factor κB

NIV: Non-invasive ventilation

NMD: Neuromuscular disorders

NREM: Non REM sleep

O_2: Oxygen

O_2CO_2: Oxycapnography

OEP: Optoelectronic Plethysmography

Pab: Abdominal pressure

$PaCO_2$: Partial pressure of carbon dioxide

PCF: Peak Cough Flow

Pdi: Trans diaphragmatic pressure

PEF %: Peak Expiratory Flow % predicted

Pgas: Gastric pressure

Poes: Oesophageal pressure

PMN: Polymorphonucleates

Ppl: Pleural pressure

PSG: Full polysomnography

REM: Rapid eye movement

RIP: Respiratory inductive plethysmography

ROS: Reactive oxygen species

RSBi: Rapid shallow breathing index

RV: Residual Volume

SDB: Sleep disordered breathing

SLP: Structured Light Plethysmography

SMA: Spinal Muscular Atrophy

SNIP: Sniff Nasal Inspiratory Pressure

SpO_2: Oxygen saturation

$tcPaCO_2$: Transcutaneous partial pressure of carbon dioxide

Th: Lymphocytes T helper

TLC: Total lung capacity

TLR: Toll-like receptors

TNF-α: Tumour necrosis factor alpha

VC: Vital capacity

VCAM-1: Vascular cellular adhesion molecule 1

VOC: Volatile organic compounds

Vt: Tidal Volume

Chapter 1

INTRODUCTION

1. RESPIRATORY IMPAIRMENT IN PAEDIATRIC NEUROMUSCULAR DISORDERS

1. a. Pathophysiology

Neuromuscular disorders (NMD) are a heterogeneous group of conditions affecting muscles, nerves or the neuromuscular junctions. Children with neuromuscular weakness have a high risk of developing significant respiratory morbidity throughout life [1]. Respiratory failure is also the main cause of mortality. The likelihood of respiratory failure varies greatly among different conditions and, with some exceptions, it is more likely in children with more severe global weakness. Each NMD diagnostic group has a different extent of respiratory impairment and a different rate of progression. Muscular and other features of each disease have different effects on the respiratory system causing this differential involvement. Nevertheless, it is possible to generalise about the pathophysiological processes underlying respiratory decline in NMD [2].

In children with NMD, respiratory failure results from the combination of lung parenchymal involvement, reduction of the thoracic cavity volume, airway obstruction by an enlarged heart chamber and impairment of cough and of the respiratory muscle pump [3].

- Lung parenchymal involvement is caused by atelectasis due to chronic aspiration secondary to impaired swallowing or gastro-oesophageal reflux. In addition, recurrent lower respiratory tract infections secondary to weak cough/ expiratory muscle dysfunction may cause lung damage. Scoliosis, a common feature in NMD children,

can worsen atelectasis by reducing lung volume. In addition, it reduces the gas exchanging area and reduces the mechanical advantage of respiratory muscles. Lung parenchymal involvement is primarily manifest as hypoxemia, due to excessive ventilation-perfusion mismatch.

- Impaired cough. The volume inspired at the end of the inspiratory cough phase is the most important determinant of cough as it affects the mechanical advantage of the expiratory muscles [4]. It is impaired in NMD children due to the combination of inspiratory muscle weakness and decreased lung and chest wall compliance. The expiratory cough phase may be limited by expiratory muscle weakness, a poorly compliant chest wall, mechanical disadvantage of the expiratory muscles, limited passive lung elastic recoil during expiration, and a diminished cross-sectional diameter of the airway related to impaired lung inflation [5].
- The respiratory muscle pump consists of central nervous centres controlling voluntary breathing, brainstem pathways controlling automatic breathing, and spinal cord and motor neurons transmitting nerve impulses to the respiratory muscles that work as effectors. All the above steps are crucial and can be differentially affected in children with NMD, and thus to contribute to ventilatory failure.

Ventilatory failure is defined as the inability to remove carbon dioxide (CO_2) and clinically manifests by first intermittent nocturnal and then daytime hypercapnia [6]. This results from the combination of weak inspiratory muscles and a high inspiratory load caused by the combination of reduced lung and chest compliance, upper airway collapsibility and which may be further worsened by an impaired central respiratory drive [3, 7].

The respiratory muscles are classified as:

- Inspiratory. The diaphragm is the major muscle of inspiration and accounts for ~70% of the inhaled tidal volume in the normal individual. Its contraction creates a negative intrathoracic pressure causing air to flow into the lung. Diaphragm contraction also increases the intra-abdominal pressure, causes a transverse swing ('bucket-handle' motion) of the lower rib cage and an upward vertical force on the lower ribs. These latter two mechanisms of the diaphragm are important adjunct inspiratory actions.

  The intercostal muscle fibres run between the ribs in the costal spaces. The external intercostal are accessory inspiratory muscles, expanding the rib cage during inspiration. The abdominal muscles also may play a minor role in inspiration.
- Expiratory. The internal intercostal lie deeper and act to decrease the size of the thoracic cavity during expiration. Expiration is normally a passive process by controlled lung recoil. The abdominal muscles (rectus abdominis, internal oblique, external oblique and transversus abdominis) may augment expiration in pathological states.
- Accessory muscles of respiration. They are sternocleidomastoid, scalene, trapezii, pectoralis major and minor, which enhance inspiratory efforts when there is an increased respiratory load.
- Muscles of the upper airway maintain its patency.

All NMD patients experience different extents of respiratory muscle weakness according to the underlying diagnosis and progression. In these children, respiratory muscles are also prone to fatigue (as expressed by the Tension Time Index) [8].

Factors increasing inspiratory load are:

- Lung compliance. If lung compliance is low, the work of breathing is increased. Reduced compliance may be caused by micro-macro atelectasis, and fibrosis induced by recurrent aspiration and other parenchymal disease. The elastic properties of lungs

may also be affected by chronic and progressive impairment of respiratory muscles because of reduced tidal volumes [3, 8].

  - Chest wall compliance. In children with NMD a combination of factors contributes to the alteration of the elastic properties of chest wall: muscle atrophy, extra-articular contractures and kyphoscoliosis. The reduced ribcage movements, consequent on impaired inspiratory muscle function and decreased physical activity lead to ankyloses of costovertebral and costo-chondral joints and to stiffening of the ribcage.
  - Upper airway collapsibility particularly during inspiration due to any increased negative pleural pressure generated to overcome increased inspiratory work of breathing

- Respiratory drive can be impaired in children with NMD for several reasons. Disordered afferent signalling from affected muscles, upper motor neuron dysfunction and/or abnormal control of ventilation could all account for a reduced ventilatory response to abnormalities of blood gases. However, the depressed ventilatory responses to hypoxia or hypercapnia may result from the inability of the muscles to respond to a normal or even increased drive [8]. The assessment of respiratory control in NMD patients is challenging, and therefore it might be underestimated [9].

Figure 1 summarises the consequences of respiratory system failure.

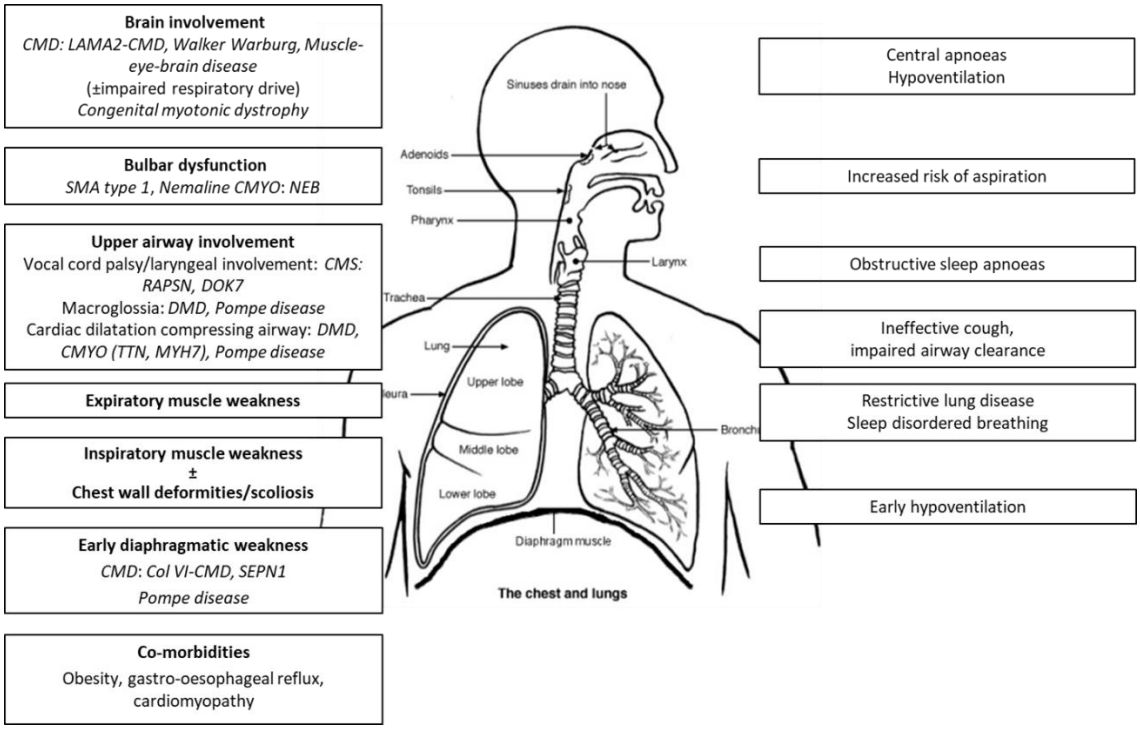


Figure 1. *Muscular and other factors contributing to respiratory impairment and sleep disordered breathing in neuromuscular disorders*

- Restrictive pulmonary function is characterised by a decreased total lung capacity (TLC), residual volume (RV) and forced vital capacity (FVC) when due to parenchymal disease. Extra pulmonary restriction due to scoliosis may result in raised RV. The extent of restriction is also linked to the degree of respiratory muscle impairment, such that decline in FVC will occur over time as the disease progresses. The development and progression of scoliosis will further decrease FVC in NMD children (Figure 2).

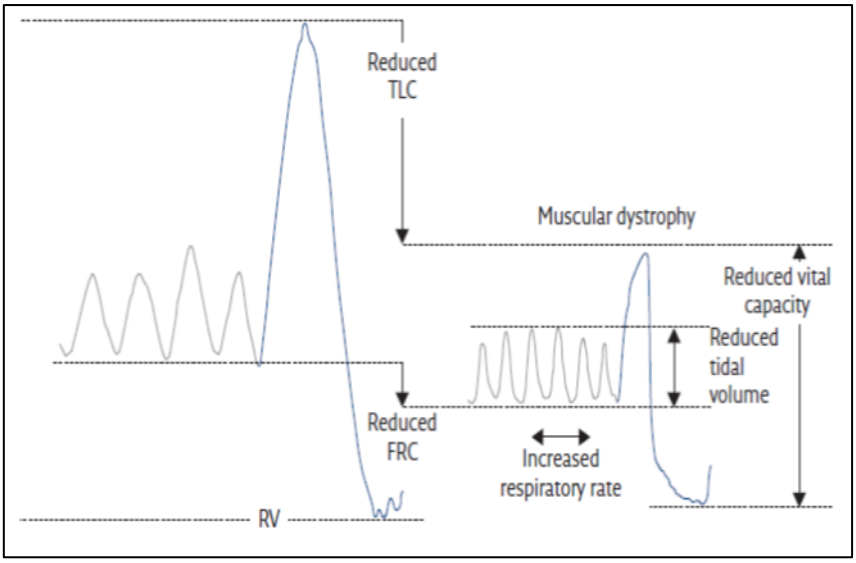


Figure 2. *Lung volume variations during quiet breathing (grey lines) and vital capacity (blue lines) in a healthy subject (left) and a patient with muscular dystrophy (right).*

Patients with muscular dystrophy breathe at a reduced vital capacity with rapid and shallow breathing (reduced tidal volume and increased respiratory rate). RV: residual volume; FRC: functional residual capacity. Adapted from Lo Mauro et al. Breathe 2016.

- Abnormal thoraco-abdominal pattern of breathing. With the progression of the disease and respiratory muscle involvement, NMD patients are more prone to rapid shallow breathing (RSB) and asynchronous movements of the ribcage and abdomen.
- Dyspnoea and orthopnoea. Diaphragm weakness causes orthopnoea, whereas breathlessness occurs in the upright position in the case of intercostal (expiratory) muscle weakness.
- Hypoventilation. Children with NMD are more prone to rapid shallow breathing (low tidal volumes (Vt)) as a strategy to maintain gas exchanges. When muscle weakness progresses, they develop hypercapnia.
- Hypercapnia. Chronic CO_2 retention is strongly related to both respiratory muscle weakness, and to the imbalance between respiratory muscle strength and the load they are working against.

1. b. Assessment of respiratory function

Clinical assessment of respiratory function is crucial in children with NMD and should be part of every medical consultation [10]. The identification of respiratory muscle weakness helps predict whether the patient will decompensate with respiratory infections and the likelihood of occurrence of sleep-disordered breathing. The evaluation of respiratory function relies on different tests of varying degrees of invasiveness [7, 11, 12].

Non-invasive tests

- Spirometry

Spirometry should be carried out at every clinic appointment in all patients who can perform the technique. The flow/volume loop may appear normal in shape despite significant loss of lung volume. See Figure 3. If there is disproportionate weakness of expiratory muscles, the flow/volume loop may display an abrupt decrease in flow at low lung volumes [8].

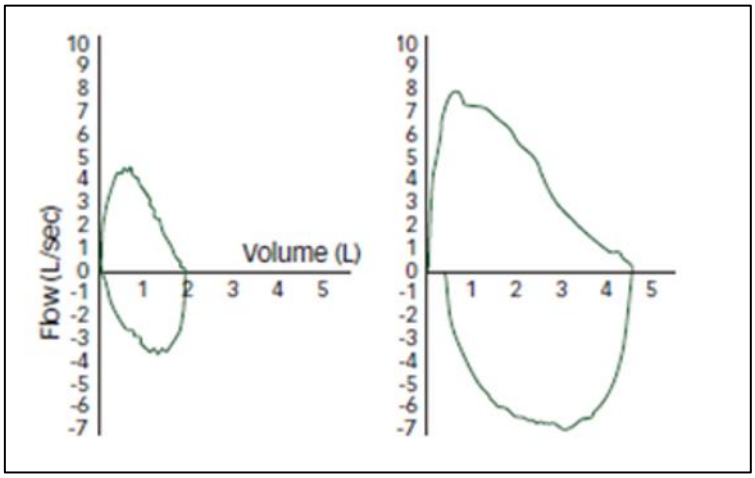


Figure 3. *Flow-volume loop a. restrictive respiratory pattern in an 18-year-old boy with Duchenne muscular dystrophy and b. normal spirometry in an age matched healthy subject. The restrictive spirometry pattern in children with neuromuscular disorders is characterised by reduced volumes and flows. Adapted from Buyse GM. et al. Europ Neurol Rev, 2015*

Measurement of FVC in the upright and supine positions is recommended if diaphragm weakness is suspected, as a >25% fall FVC% from upright to supine is associated with diaphragm weakness [13]. However, in advanced disease with lower lung volumes, there may be a much lesser percentage fall even with complete bilateral diaphragm paralysis [14].

FVC is also a predictor of increased risk of developing SDB [15]. FVC<60% is associated with SDB onset, FVC<40% SDB is associated with overnight hypoventilation, and FVC<25% is associated with SDB with associated daytime respiratory failure. It has been shown in Duchenne muscular dystrophy that an absolute FVC < 1 litre (L) is associated with a higher risk of mortality in the following 5 years [16, 17].

Technical quality standards for lung function tests have been published but they may be difficult to fulfil in young children and in patients with facial weakness. Spirometry that is acceptable and reproducible according to the American Thoracic Society/European Respiratory Society lung function criteria can generally be performed by healthy children 6 years of age and above [18]. The Global Lung Health Initiative has developed spirometry reference ranges for essentially all ages (range of 3–95 years) which takes into account age, height and ethnicity [19]. The major limitation of these tests is that they depend on the patient's motivation, cooperation and ability.

- Peak Cough Flow (PCF)

The strength of a cough manoeuvre is measured using an oro-nasal mask/mouthpiece, connected to a pneumotachograph or peak flow meter. The child should be in the upright position, inspiring to total lung capacity and then forcefully coughing into the device via a mouthpiece or (usually) a mask. PCF can be objectively assessed in cooperative children starting at around 6 years of age. An inefficient PCF is related primarily to the weakness of expiratory muscle and, to a lesser extent, to an impaired inspiration secondary to inspiratory

muscle weakness [20]. Normal PCF values for adults are greater than 400 L/min, and PCF values of 270 L/min or less have been shown to increase the risk of pneumonia in adults with NMD [21]. A PCF value below 160 L/min is generally considered a sign of impaired cough and an indication for starting cough augmentation techniques [22, 23]. There are currently normative data for cough peak flow in healthy children. The 90th centile PCF for females 4-18 years range from 202 l/min (4 years of age) to556 l/min (18 years of age) while in males it ranges from 226 l/min (4 years of age) to 898 l/min (18 years of age) [24]. See Figure 4.

TABLE 2 Percentile values of cough peak flows (liters/min) by gender and age

	Females						
Age, yrs	5th	10th	25th	50th	75th	90th	95th
4	110	112	124	147	179	202	209
5	125	132	171	185	219	245	273
6	161	161	191	230	242	284	317
7	179	200	228	247	265	302	330
8	200	219	270	299	321	340	351
9	270	270	290	311	347	369	369
10	270	284	299	330	361	380	399
11	296	299	347	380	399	441	478
12	305	340	361	399	412	450	459
13	311	330	361	395	441	508	545
14	361	372	399	428	478	518	561
15	344	384	424	469	508	550	596
16	358	412	428	469	508	550	626
17	369	416	433	469	513	550	633
18	399	420	441	488	513	556	639
	Males						
Age, yrs	5th	10th	25th	50th	75th	90th	95th
4	130	132	143	162	194	226	230
5	138	153	179	194	226	262	270
6	166	171	204	226	250	279	293
7	200	211	235	270	299	340	351
8	215	247	279	299	321	340	347
9	217	237	293	311	340	372	424
10	250	260	296	321	351	380	428
11	290	299	340	369	399	420	441
12	311	317	334	369	399	450	498
13	321	337	392	450	518	567	578
14	380	395	498	608	672	713	750
15	380	428	534	633	706	788	829
16	493	518	539	652	713	728	871
17	498	545	561	645	846	898	944
18	518	545	602	728	880	898	944

Figure 4. *Normative data of Peak Cough Flow for healthy children according to age and gender. Adapted from Bianchi et al. Am J Phys Med Rehabil 2008.*

- Tests of respiratory muscle strength

The measurement of maximum static inspiratory (MIP) or expiratory (MEP) pressures at the mouth allows the assessment of global respiratory muscle strength in clinical settings [25]. In the absence of distal airway obstruction, when the glottis is open, mouth pressure can be assumed to equal alveolar pressure. Mouth pressures are measured via a flanged mouthpiece connected to a pressure transducer. Patients are required to make maximum inspiratory (Mueller manoeuvre) and expiratory (Valsalva manoeuvre) efforts at or near RV and TLC, respectively. The inspiratory and expiratory pressure should be maintained ideally for at least 1.5 seconds, so that the maximum pressure sustained for one second can be recorded. Subjects are normally seated and nose clips are not required [25]. MIP is measured at RV and MEP at TLC. Guidelines suggest recording the maximum of three manoeuvres which should vary by less than 10% [26].

The main disadvantages are that these manoeuvres require active cooperation, and are difficult to perform if there is significant facial weakness. They have a positive correlation with VC in NMD [16, 27]. MIP is considered a useful tool for the early evaluation of inspiratory muscle strength, especially of the diaphragm, in patients with NMD and it is currently used as a respiratory endpoint in clinical trials for NMD [28]. There are normative data for MIP but not MEP in healthy children [29].

The measurement of maximal sniff nasal inspiratory pressure (SNIP) relies on inspiratory pressure recorded by a pressure transducer connected to a catheter placed in the nostril. SNIP has proven useful in monitoring the respiratory decline in Duchenne muscular dystrophy patients [30], and other adult NMD [31] and as a predictor of survival in ALS [32]. SNIP is feasible and reproducible in NMD children [33] and correlates with VC [34], for example in patients with Spinal muscular atrophy types 2 and 3 [35]. The most significant advantage of

SNIP is its feasibility in young children, in patients with at least developmental delay, and in patients with bulbar dysfunction and facial weakness. No studies have assessed whether the relationship between SNIP, lung volumes and respiratory muscle tests differ in different NMDs. There are reference values for SNIP for adults which vary between genders [36] and for Caucasian children aged 6-17 years. SNIP reference values for age range 6-12 years are 99 ± 22 cmH_2O in boys, 92 ± 22 cmH_2O in girls. SNIP reference values for age range 13-17 years are 117 ± 31 cmH_2O in boys and 97 ± 26 cmH_2O in girls [37]. See Figure 5.

RESPIRATORY PRESSURES IN BOYS AND GIRLS*

	n	Pn_{sn} ($cm\ H_2O$)	$P_{I_{maxFRC}}$ ($cm\ H_2O$)	$P_{I_{maxRV}}$ ($cm\ H_2O$)	$P_{E_{maxFRC}}$ ($cm\ H_2O$)	$P_{E_{maxTLC}}$ ($cm\ H_2O$)
Boys, 6 to 12 yr	67	99 ± 22	80 ± 20	88 ± 19	80 ± 24	96 ± 24
		55–155	38–134	48–141	37–147	55–159
Boys, 13 to 17 yr	26	117 ± 31	107 ± 22	110 ± 23	105 ± 23	123 ± 22
		70–186	66–153	60–152	56–145	91–162
Girls, 6 to 12 yr	63	92 ± 22	68 ± 17	77 ± 19	65 ± 22	80 ± 22
		51–151	32–109	38–124	28–119	37–137
Girls, 13 to 16 yr	24	97 ± 26	81 ± 20	86 ± 21	74 ± 23	91 ± 24
		55–155	54–131	53–137	42–142	53–155

* Values are means ± SD and range.

Figure 5. *Normative data of Sniff Nasal Inspiratory Pressure for healthy children according to age and gender. Adapted from Stefanutti et al. Am J Respir Crit Care Med 1999.*

- Breathing pattern

The monitoring of breathing pattern is based on the recording of respiratory frequency, tidal volume (Vt) and minute ventilation. It allows the calculation of the rapid shallow breathing index (rSBi), which is the response to an increased respiratory workload. Optoelectronic plethysmography (OEP) is a technique that consists of the analysis of the displacement of the thoracic and abdominal compartments by means of special cameras. The system, based on eight infrared video cameras working at a sampling rate of 60 Hz, computes the 3D coordinates of

52 retro-reflective markers placed, according to specific anatomical points, over the anterior chest wall surface of the subject lying supine [20]. See Figure 6.

The analysis of the thoraco-abdominal pattern of breathing by OEP has been performed in children with Duchenne muscular dystrophy and Spinal Muscular Atrophy. In DMD it showed that the average abdominal contribution of to the tidal volume, a surrogate for diaphragm function, significantly decreased with age and impairment was associated with increased risk of developing SDB[38, 39]. In SMA patients, OEP showed that the severity of bell-shaped chest and the extent of paradoxical breathing strongly correlated with the severity of disease [29, 36].

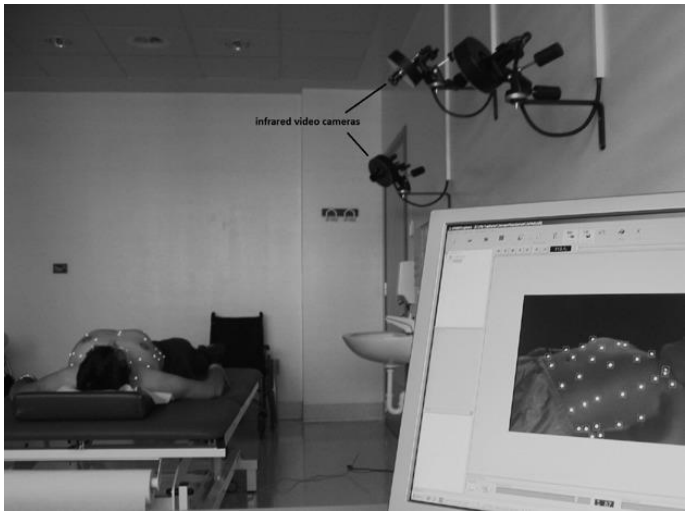


Figure 6. *Set-up of respiratory pattern recording via Optoelectronic plethysmography (adapted from Lo Mauro et al. Pediatric Pulmonology 2014)*

Recently, Structured Light Plethysmography (SLP), a novel non-contact method of assessing lung function has been described in NMD patients [40]. SLP projects a light in a grid pattern onto the patient's thoraco-abdominal wall. Changes in lung volume during respiration are assessed by measuring the displacement of the thoraco-abdominal wall, the right and left chest wall and the chest versus abdominal movements during breathing. See Figure 7.

FVC measured via SLP correlated with FVC measured via spirometry, but repeated measures of FVC via SLP had only a moderate repeatability. SLP was able to detect small changes in thoraco-abdominal wall motion during tidal breathing.

Figure 7. *Structured light plethysmography PneumaCare Thora3DI® System device and acquisition of respiratory pattern in a child with SMA type 1. The PneumaCare Thora3DI® projects a light grid on the chest of the patient lying in supine position. PneumaView™ bi-dimensionally reconstructs the displacement of the thoraco-abdominal light grid.*

- Imaging of respiratory muscles

Different imaging techniques have been used for the static and dynamic assessment of respiratory muscles. These include are chest fluoroscopy, computed tomography, two-dimensional B (brightness)-mode ultrasound to measure diaphragm thickness in static images, and M (motion)-mode ultrasound to measure direction of diaphragmatic motion. Magnetic resonance provides both static and dynamic images [41].

Invasive tests

- Oesophageal and gastric pressures

Measurement of transdiaphragmatic pressure (Pdi) is used to diagnose diaphragmatic dysfunction. Pdi, defined as the difference between pleural (Ppl) and abdominal pressure (Pab) is assumed equal to the measured difference between oesophageal (Poes) and gastric pressure (Pga) recorded via air-filled balloon catheters, i.e. Pdi = Pga-Poes [25, 26]. These pressures can be measured under different conditions [25].

- During tidal breathing. Contraction of the diaphragm and inspiratory muscles produces a positive change in Pgas and a negative change in Poes. As such, the ΔPgas/ΔPoes ratio reflects the relative contribution of the diaphragm and the other respiratory muscles to quiet breathing. A value ranging between −1 and 1 indicates an ever-increasing contribution of the rib cage and the expiratory muscles, as compared with the diaphragm, to tidal breathing. With total diaphragmatic paralysis, this ratio becomes equal to 1.This analysis of spontaneous breathing was shown to correlate with clinical severity in children with muscular dystrophy caused by collagen VI deficiency [42].
- During voluntary manoeuvres. In many children with NMD pressures produced during sniff and cough are less than normal [11, 42]. Assessments during sniff are particularly useful when SNIP yields suspiciously low values, e.g. in patients with upper airway obstruction (hypertrophy of the adenoids, rhinitis and polyps) or lower airway obstruction. In clinical practice, sniff Poes and sniff Pdi are the most accurate and reproducible volitional tests available to assess global inspiratory and diaphragmatic strength in cooperative children over 6–8 years of age [43], albeit they are invasive and time-consuming.

Glottal closure during the early part of expiratory efforts during coughing allows pressure to build up, increasing the expulsive force. In patients with ALS and bulbar involvement leading to of glottal dysfunction, MEP values were low but Cough Pga was normal [44].

- Diaphragm EMG

Diaphragm EMG is a surrogate of respiratory effort, and can be used to distinguish between central and obstructive sleep apnoea events and to assess exertional breathlessness during exercise. When combined with tidal volume recordings, it assesses upper airway resistance [26].

2. SLEEP DISORDERED BREATHING IN PAEDIATRIC NEUROMUSCULAR DISORDERS

2. a. Pathophysiology

SDB is one of the main co-morbidities associated with NMD, with a reported prevalence of > 40% in children [45]. Gas-exchange abnormalities may occur in >80% of patients with NMD [8]. Four main factors variably contribute to the development of SDB [46].

- Reduction of lung volumes

Even in healthy individuals, in the supine position VC falls by as much as 19% [47] and FRC by approximately 25% [48]. In NMD children, sleep compounds the reduction in lung volumes imposed by the supine position with the further reduction of functional residual capacity by approximately 6% to 15% [49].

The low lung volume is thought to represent a risk factor for the development of obstructive sleep apnoea by reducing traction on and stability of the upper airway. It has been shown in otherwise healthy patients that expiratory reserve volume was correlated with both nocturnal OSA and oxygen desaturation frequency. The activity of both the laryngeal muscles and the diaphragm increase when negative inspiratory pressure increases. In addition, otherwise healthy patients with OSA have impaired capacity to increase respiratory muscle drive during increased respiratory loading[50]. Furthermore, the drop in oxygen saturation for a given degree of SDB is more profound and accelerated at lower lung volumes [50] [46]. The nadir and mean nocturnal oxygen saturations are inversely correlated with the decline in vital capacity between sitting and supine positions [51].

- Loss of muscle tone

There is normally a loss of muscle tone during rapid eye movement (REM) sleep that mainly affects intercostal muscles causing a reduction in the rib cage contribution to the tidal volume from 44% in wakefulness to 19% in quiet sleep [52]. Respiratory motor neurons are differentially susceptible to REM reduction in tone, with most suppression in inspiratory laryngeal, expiratory pharyngeal and inspiratory and expiratory intercostal nerve activities compared to NREM sleep. As a consequence, ventilation in REM sleep relies predominantly on the function of the diaphragm [53].

- Reduced chemosensitivity

The control of breathing physiologically relies on four different respiratory sensors that provide afferent input into the central system: (1) peripheral arterial chemoreceptors; (2) central (brainstem) chemoreceptors; (3) intrapulmonary receptors; and (4) chest wall and muscle mechanoreceptors. In healthy humans, during sleep, the cognitive influences on ventilatory control are largely eliminated and respiratory responses to hypoxia and hypercapnia are generally reduced. Ventilatory compensation is also severely reduced during rapid eye movement (REM) sleep. The arterial $PaCO_2$ normally increases by 4 to 7 mmHg during slow-wave sleep due to the reduced tidal volume and respiratory rate. Additionally hypercapnic and hypoxic ventilatory responses and central drive are reduced to less than one third of the wakefulness values during REM sleep. Several mechanisms causing abnormal ventilatory control have been suggested in patients with NMD. However, even in presence of a normal ventilatory drive, some patients have hypoventilation because of abnormalities in muscle function and neuromuscular transmission [53] [54].

- Anatomical causes

Macroglossia contributes to obstructive sleep apnoea in DMD. In one study, approximately 64% of young male patients with DMD had obstructive sleep apnoea (OSA), due to the combination of macroglossia with obesity related to the combination of immobility and systemic steroids used as standard therapy in DMD. [55]. Macroglossia is also highly prevalent (29% to 62%) in children with the infantile form of acid maltase deficiency (Pompe disease) [46].

Craniofacial abnormalities and vocal cord palsy in some CMS (*RAPS*, *DOK7* mutation) or congenital myopathies can predispose to OSA [56]. Bulbar involvement in SMA type 1 and congenital myopathies such as nemaline rod myopathies can increase the risk of OSA during REM sleep due to reduced tone of upper airway dilator muscles. However, studies phenotyping SDB according to the underlying disease and its risk factors have not been performed.

2. b. Classification of sleep disordered breathing

The progression of sleep disordered breathing in neuromuscular children depends on the nature of respiratory and bulbar muscle involvement [6]. The spectrum ranges from obstructive sleep apnoea to diaphragmatic and pseudo-central sleep-disordered breathing to REM-related and overnight hypoventilation [46, 53, 57].

Obstructive sleep apnoea (OSA)

OSA is defined as partial or complete upper airway obstruction during sleep, associated with at least one of the following: (1) sleep disruption; (2) hypoxemia; (3) hypercapnia or (4) compatible daytime symptoms [58]. Risk factors for OSA in NMD children are similar to the general population. Furthermore, additional pathophysiologic features contribute to increased upper airway resistance in NMD. Patients with DMD have a predictable pattern of progression,

going from reduced upper airway muscle tone causing OSA, to chest wall muscle hypotonia and diaphragm weakness leading to nocturnal hypoventilation [59]. In one study, 31% of DMD children (median age 8 years) had OSA, whereas 32% young adults (median age 13 years), had hypoventilation [60]. In Pompe disease, the early diaphragmatic involvement leads to a combination of OSA and hypoventilation early in life particularly in the most severe cases. In infantile-onset Pompe disease children (age below 18 months), 41% had OSA and 38% hypoventilation whereas in late-onset disease (mean age 39 years) 11% had OSA and 44% hypoventilation [61, 62]. OSA has also been reported in 55% of adult subjects with myotonic dystrophy type 1 [63].

Pseudocentral or diaphragmatic sleep-disordered breathing

Pseudocentral or diaphragmatic sleep-disordered breathing are early warnings of respiratory muscle involvement. The events are due to impaired intercostal muscle activity and reduced contribution of the rib cage to the tidal volume and the consequent increased burden on an already weak diaphragm [64]. In NMD diagnostic groups with preserved diaphragm function, e.g. children with Spinal Muscular Atrophy (SMA) type 2, mild hypoxaemia with minimal hypercapnia or normocapnia may be seen overnight.

Nocturnal hypoventilation

Different thresholds in adults and children define nocturnal hypoventilation. In adults, the American Academy of Sleep Medicine adopts two definition, i.e. $PaCO_2 \geq 55$mmHg for ≥10 minutes or, alternatively, a ≥10mmHg increase in the arterial $PaCO_2$ relative to the awake supine value, to a value exceeding 50 mmHg for ≥ 10 minutes [65]. The prevalence of hypoventilation according to each of these criteria, in a population of 232 NMD adults affected by Steinert disease (DM1), SMA, DMD or Becker Muscular Dystrophy (BMD), mean age 43.1±15.4 years, varied from 4% to 9%. Overall, at least six other different criteria for

definition of hypoventilation have been proposed in adults, affecting the estimated prevalence in patients with neuromuscular disorders [66].

In children, sleep-related hypoventilation is defined according to the American Academy of Sleep Medicine as carbon dioxide levels above 50 mmHg for more than 25% of the total sleep time [65]. The prevalence of nocturnal hypoventilation in 46 children (mean age 12±5 years) with a combination of DMD, SMA and congenital myopathies (CMYO) was 24% by the above definition [67] but it become commoner with age.

Normally, relative hypoventilation occurs during sleep as a result of blunting of the hypoxic and hypercapnic drive. In NMD hypoventilation develops initially in REM sleep with decreased intercostal EMG activity [27], then in NREM sleep [68]. As diaphragm involvement progresses rapid eye movement (REM)-related hypoventilation is seen, which progresses to persistent hypoventilation during non-REM and REM sleep usually once vital capacity (VC) falls below 40% [68]. Hypoventilation is initially compensated for with an increased arousal response that prevents prolonged oxygen desaturation or hypercapnia but causes sleep fragmentation resulting in daytime fatigue and hyper somnolence. With the progression of the disease the arousal response becomes blunted, allowing longer periods of REM sleep, during which alveolar hypoventilation occurs. Eventually, respiratory drive becomes depressed, and severe hypoventilation then becomes present day and night [57].

Central sleep apnoea, periodic breathing, Cheyne-Stokes breathing

There is very little information about central apnoea in children with NMD. Predisposing factors are possible cardiac dysfunction, gastro-oesophageal reflux and central drive abnormalities that are present to different degrees in the various NMDs.

Cheyne-Stokes respiration (CSR) is a type of periodic breathing characterised by periods of hyperventilation (crescendo-decrescendo pattern) that alternates with central apnoea. Central sleep disturbances in NMD are manifest by Cheyne-Stokes breathing in association with cardiomyopathy, as can be seen in DMD and BMD, limb girdle muscular dystrophies (LGMD) as well as Pompe disease, or to an instability in control of breathing as in Congenital Myotonic Dystrophy (DM1). Cheyne Stokes respiration in patients with cardiomyopathy is associated with increased mortality. A lower awake and sleep CO_2 level, and circulatory delay are key pathological mechanisms [46, 53].

2. c. Assessments of sleep disordered breathing

The British Thoracic Society guidelines for respiratory management of children with neuromuscular weakness [10], and the most recent standard of care guidelines for DMD [69], SMA [70] congenital muscular dystrophies (CMD) and CMYO [71] strongly advocate a pro-active approach targeted to the regular assessment of daytime respiratory function and sleep physiology.

Daytime tests

Daytime symptoms of SDB, especially hypoventilation, are vague. They may include morning lethargy and headaches, anorexia, and poor growth. Many patients with slower disease progression develop symptoms insidiously. As a result, they are often only appreciated once they are corrected with treatment [57]. Physical examination may yield some clues to the presence of SDB, although findings are not specific for this condition. Questionnaires have been proposed as screening tools. The “Sleep Disordered Breathing in Neuromuscular Disease Questionnaire” has five items enquiring about symptoms of dyspnoea and sleep quality and a score ranging from 0 to 10. A score of ≥5 had a poor positive predictive value (69%) but a good negative predictive value (95%) for identifying SDB [72].

Oxycapnopgraphy (O_2CO_2 monitoring)

Overnight of O_2 and CO_2 should be regularly assessed. Oximetry can be performed in conjunction with measurement of end-tidal CO_2 or coupled with transcutaneous CO_2 monitoring [73]. According to the most recent British Thoracic Society guidelines, in asymptomatic NMD children, a nadir SpO2 ≥ 93% during episodes of desaturation is considered sufficient to exclude clinically significant hypoventilation in children with neuromuscular disease. However, there are no studies that specifically evaluate whether oxygen saturation monitoring alone is adequate as a method for screening for nocturnal hypoventilation in children or adult with NMD [10].

Pulse oximetry also enables calculation of the oxygen desaturation index (ODI), which is the mean number of desaturation episodes per hour of recording. Typically, ODI is reported as the number of 3% desaturations (3%ODI) and/or the number of 4% desaturations (4%ODI). They have the same ability to detect OSA in otherwise healthy children [74]. In otherwise healthy children, the normal 4%ODI 95centile is 2.2/h and 97.5th centile 2.4/h [75].

McGill score quantifies severity of oximetry based on the number and depth of desaturations/night. It has been proposed as screening tool to prioritize children requiring treatment for OSA. In otherwise healthy children, it has a high positive predictive value (PPV) and a low negative predictive value (NPV) in diagnosing OSA compared to full polysomnography (PSG) [76].

Clear-cut thresholds of nocturnal oxygen desaturation indicating hypoventilation have not been established. Additionally, the absence of significant nocturnal desaturations does not necessarily exclude hypoventilation [73].

Cardiorespiratory polygraphy (CR Poly)

CR Poly allows determination of whether there is a central or obstructive (or both) cause of SDB. CR Poly set-up includes a. Respiratory inductive plethysmography (RIP) which uses chest and abdominal bands providing both an assessment of the chest/abdominal asynchrony and a semi-quantitative measurement of airflow and tidal volume. b. Movement probe. c. Body position monitor. d. Snoring microphone (optional). e. Video. f. ECG. g. Nasal pressure. h. Oronasal airflow (thermister). i. End-tidal/transcutaneous $PaCO_2$. j. Arterial oximeter with pulse waveform [10, 68, 77].

Rules for the scoring of sleep and respiratory events in polysomnography or polygraphy recordings are provided by the American Academy of Sleep Medicine (AASM) manual [65].

Apnoea Hypopnoea Index, AHI, is the number of mixed, obstructive and central apnoeas and hypopnoeas per hour of total sleep time and is the most commonly used polysomnography parameter for the description of SDB severity.

AASM defines an obstructive apnoea as the drop in the peak signal excursion by ≥ 90% of the pre-event baseline for at least the duration of 2 breaths and is associated with the presence of respiratory effort throughout the entire period of absent airflow. A respiratory event is scored as a central apnoea if it meets apnoea criteria (drop in flow≥ 90%), associated with absent inspiratory effort throughout the entire duration of the event, and at least one of the following: 1. The event lasts 20 seconds or longer. 2. The event lasts at least the duration of two breaths during baseline breathing and is associated with an arousal or ≥ 3% oxygen desaturation. 3. For infants younger than 1 year of age, the event lasts at least the duration of two breaths during baseline breathing and is associated with a decrease in heart rate to less than 50 beats per minute for at least 5 seconds or less than 60 beats per minute for 15 seconds. Hypopnoea is defined as

a drop of the peak signal excursions by ≥ 30% of pre-event baseline for a duration of at least 2 breaths associated with a ≥ 3% desaturation from pre-event baseline or an arousal [65].

A recent European Respiratory Society taskforce proposed new definitions and criteria for the diagnosis of OSA in children 2-18 years. Definition 1. OSA is defined as an oAHI ≥2/h or an oAI (obstructive apnoeas per total sleep time) ≥1/h in the presence of SDB symptoms. OSA are mild when oAHI is 2–5/h while they are moderate-to-severe when oAHI >5/h. Definition 2. OSA is defined as an AHI ≥1/h in presence of SDB symptoms. OSA is considered moderate-to-severe when AHI is >5/h [78]. In children younger than 2 years of age, the ERS taskforce has issued separate, yet similar, cut-off for the definition of OSA and its severity. Mild OSA is defined as obstructive AHI 1–5/h, moderate OSA is defined as an obstructive AHI >5–10/h, severe OSA is defined as an obstructive AHI >10/h. In young children with underlying conditions at risk for OSA such as Pierre-Robin sequence and Down syndrome the McGill oximetry score can be used as surrogate for CR Poly results. When abnormal (>2) the score indicates moderate-to-severe OSA [79].

In children without SDB symptoms or morbidity, or abnormalities predisposing to SDB, the 90th percentile for the AHI is 3.2/h for the second year of life, up to 2.5/h for the ages > 2 and ≤6 years, and ≤ 2.1/h for the ages > 6 and <18 years.

Full polysomnography (PSG)

The gold standard for the assessment of SDB is full polysomnography (PSG) [10, 53, 57]. It provides information about sleep efficiency and sleep quality as well as detailed characterisation of respiratory patterns and abnormalities.

Besides the standard CR Poly set-up, PSG contains: a. six-channel Electroencephalogram (EEG), 2 frontal (F), 2 central (C) and 2 occipital (O) leads, b. Electrooculogram (EOG) (right and left) and c. Electromyogram (EMG): Submental and bilateral tibial. Sleep staging involves

the combined measurement of the EEG, EOG to record rapid eye movements, and EMG to record submental and tibial muscle activity [80].

Sleep alternates between REM and NREM. REM and NREM sleep have defining EEG patterns, and both neurological and physiological features. NREM sleep occurs with a period of relatively low brain activity. NREM sleep consists of three stages: stage 1 (transition from wakefulness to sleep), stage 2 (initiation of true sleep), and stage 3 (deep sleep; previously divided into stages 3 and 4). REM sleep is thought to have a role in consolidating memories and in the development of the central nervous system. REM sleep is characterized by a burst of rapid eye movements; there is a high brain metabolic rate, a variable heart rate and an active suppression of peripheral muscle tone. Well-defined sleep stages similar to those in adults are easily identifiable in children above 6 months of age, although differences in the characteristics of the voltage and waveforms of the EEG occur with maturation beyond this age. Special criteria have been used to define sleep stages in infants younger than 6 months of age [68].

The knowledge about sleep architecture in children with NMD is very limited as most of the studies published to date aimed to investigate the respiratory component of sleep, i.e. SDB [81-85]. Patients with SMA type 1 and type 2 assessed with PSG were shown to have reduced arousability during non-REM sleep compared to age matched healthy controls suggesting a potential central nervous system involvement [82, 83]. Adults with congenital myotonic dystrophy have been reported to suffer of narcoleptic-like sleep-onset REM period. It seems however to only partially explain their daytime sleepiness [85]. In DMD boys, PSG has confirmed the well-known pattern of REM-associated hypoventilation [84].

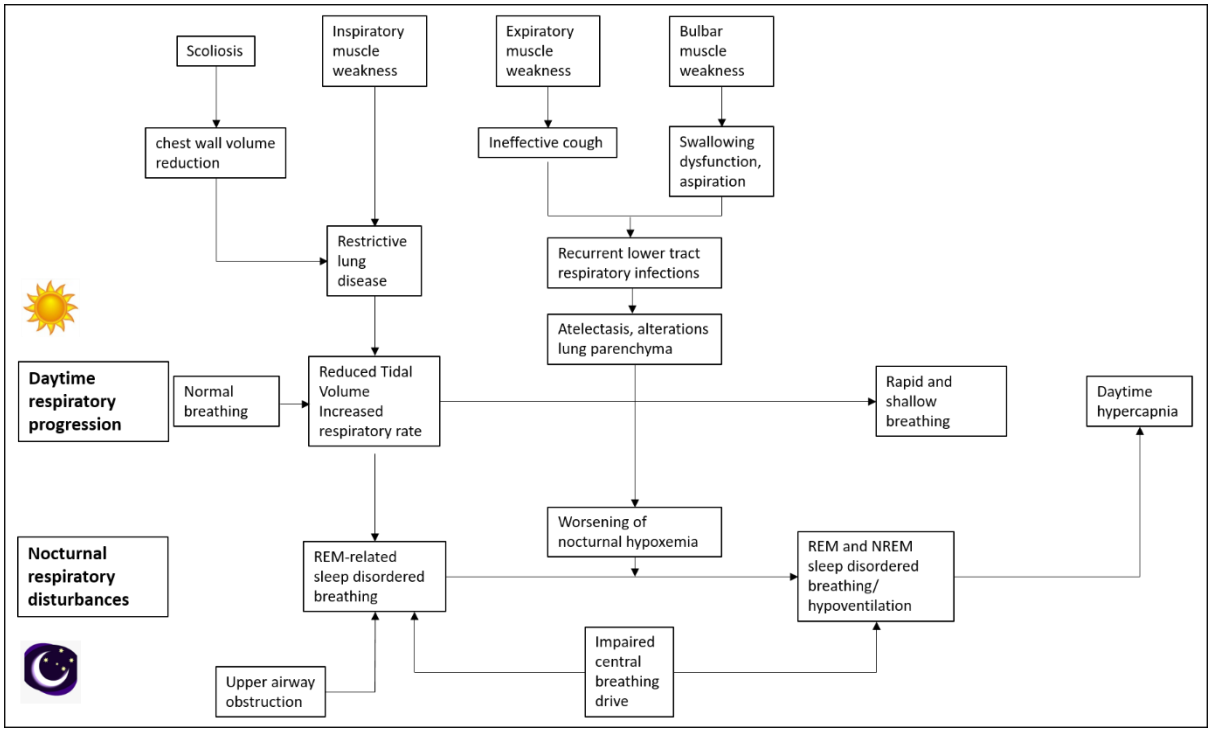


Figure 8. *Algorithm of the typical evolution of respiratory status in children with neuromuscular disorders. Daytime breathing pattern evolves usually towards a rapid shallow breathing due to various contributing factors such as weak inspiratory muscles, restriction of ribcage. The impaired expiratory muscle strength negatively affects cough and airway clearance leading to parenchymal lung damage.*

Overnight sleep disordered breathing are usually REM-related hypoventilation progressing towards overnight hypoventilation. However, depending on specific diagnostic groups, central and obstructive events are often detected.

3. CURRENT MANAGEMENT OF RESPIRATORY IMPAIRMENT IN NEUROMUSCULAR CHILDREN

3. a. Swallowing and management of secretions

Swallowing impairment, often associated with the severity of the underlying disorder as well as concomitant involvement of the central nervous system, plays a significant role in worsening the respiratory status of children with NMD. Anticholinergic drugs such as glycopyrrolate are used in the management of sialorrhea because they block the cholinergic input to the salivary glands and help in drying up the secretions. The main adverse effect of these medications is that they can thicken oral secretions, making difficult to mobilize them and increasing the risk of airway plugging [86].

Dysphagia may lead to complications, such as malnutrition, aspiration pneumonia, and other pulmonary sequelae. Initially rehabilitative interventions such as exercises to improve the strength, skill, or the biomechanical support needed for swallowing are put in place[87]. If patients become unable to adequately meet their hydration and nutrition needs, the placement of a gastrostomy tube may become necessary [88].

3. b. Respiratory physiotherapy and airway clearance

Any therapeutic effects of respiratory muscle training in children with NMD rely on the appropriate training and the being patient in relatively good clinical condition with preserved inspiratory muscle function. To date, the benefits are controversial. The main limitations of these studies are the small number of patients and the lack of standardized protocols [89].

Techniques aimed to actively recruit lung volumes are recognised as helpful in preventing the occurrence of atelectasis or secretion plugs. Lung volume recruitment is performed using positive pressure administered with a manual self-inflating resuscitation bag attached via

tubing containing a one-way valve to a mouthpiece. It consists of an inspiratory pressure applied to the airway, a breath-hold, and then a spontaneous or assisted forced expiratory manoeuvre. Regular use was associated with a reduction of long-term decline of pulmonary function in DMD patients [90].

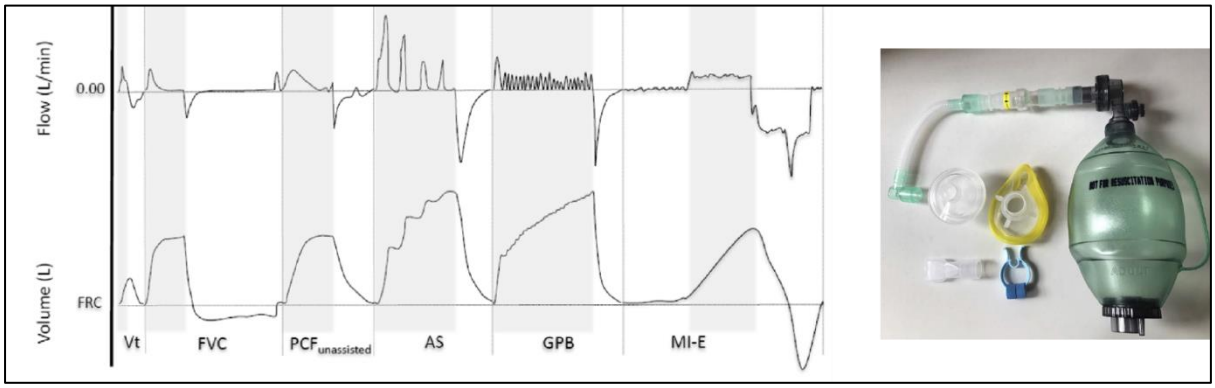


Figure 9. *Equipment for lung volumes recruitment. Self-inflating resuscitation bag, extension and mouthpiece.*

Volumes and flow augmentation during spirometry (Forced Vital Capacity), cough (Peak Cough Flow) and manoeuvres of lung recruitment, namely air stacking with self-inflating bag (AS), glossopharyngeal breathing (GPB), cough assist (MI-E). Adapted from Chatwin M et al. Respiratory Medicine 2018.

Glossopharyngeal breathing (GPB) is a lung-inflation manoeuvre obtained by inflating one gulp of air at a time .particularly in the late stages of the disease. The regular performance of GPB was shown to increase lung volumes and to delay the onset of daytime ventilator use. It can be performed in the event of ventilator failure and during the inspiratory cough phase, as cough flow correlates with pre-cough volume [91].

The cough assist technique by mechanical in-exsufflation produces a manoeuvre that mimics a cough. This device provides both an inspiratory phase, generating limited positive pressure and an expiratory phase generating a rapid negative pressure. Both manual and automatic modes can be used [92].

Figure 10. *Devices for Cough assistance*

A recent Cochrane Review concluded that there is not enough evidence for or against the usefulness of mechanical in-exsufflation to help airway clearance in NMD patients [93]. Conversely, a recent state of the art review written by international experts in the field provided details about effectiveness and indications also taking into account the severity of patients. Experts suggest that using cough assist techniques in very weak patients is a priority. Short-term and bench studies as well as case reports have suggested that mechanical in-exsufflation improves peak cough flow sufficiently to aid mucus clearance [22, 23].

3. c. Non-Invasive Ventilation

Non-invasive Ventilation (NIV) has been associated with dramatically improved life expectancy for NMD patients [22, 94]. It requires two main pieces of equipment: a pressure/ volume generator that to provide ventilation and an interface that connects the ventilator to the patient. In most cases, the pressure/volume generators are pressure-support bi-level type devices that are adjusted for a set expiratory positive airway pressure that functions to prevent airway obstruction and a set inspiratory positive airway pressure that serves to ventilate the individual. In almost all cases, because patients are often too weak to trigger breaths from the ventilator and because of the presence of central sleep apnoea in many patients with NMD, a

back-up rate is set to deliver a breath if the patient does not trigger inspiration. To improve tolerance, it may be necessary to begin with sub therapeutic settings and then adjust pressure settings upwards as tolerance improves. Adequate humidification during NIV is necessary. The second major piece of equipment is the interface that connects the ventilator to the patient. Interfaces are available in a wide variety of forms including nasal mask, nasal pillows, full-face mask, and oral interfaces. Evidence that any interface is clearly superior to others is lacking. The type of interface used will depend on the preference of the patient and clinicians, on the patient's facial features and cost [95]. Air leaks are common in NMD because of the presence of facial weakness, the impossibility of complete mouth closure during sleep and because of the particular facial features of some of these children. Air leaks can have a negative effect on comfort and can lead to less effective ventilation particularly affecting patient-ventilator interaction (reducing sensitivity of ventilator trigger and cycle), and can disrupt sleep architecture. An important potential problem with nasal and oro-nasal masks is facial skin breakdown, which most commonly occurs on the bridge of the nose. In some patients needing 24 hour support, the use of mouthpiece ventilation when awake is a reasonable option [96].

Figure 11. *Non-Invasive ventilation (NIV). Devices and interfaces*

The right time to start NIV support is still controversial and should be chosen on an individual clinical basis. Different criteria for starting NIV have been proposed, the most recently by the British Thoracic Society [10]. Fauroux et al. showed however that most clinicians use a number of other criteria by which to start NIV [97]. Recent standards of care for DMD and SMA both focused on a pro-active management of respiratory muscle dysfunction [69, 70]. NIV should be considered if there is altered overnight gas exchange and hypoventilation and hypercapnia and/or clinical symptoms of hypoventilation [98]. Nevertheless, assessment should be on an individual basis. This is mainly due to the different features and timing of onset of SDB and hypoventilation in different diagnostic groups. The cognitive involvement occurring in some NMD can negatively affect their adherence to NIV.

Interestingly, in patients treated with long term NIV for chronic hypercapnic respiratory failure there is a highly occurrence of residual respiratory events during sleep. However, these events were not associated with persistent alteration of gas exchange, and their clinical relevance has yet to be clarified [99].

4. RESPIRATORY AND SLEEP FEATURES IN SPECIFIC NEUROMUSCULAR DIAGNOSTIC GROUPS

All NMD exhibit respiratory decline but with huge variability in the type and severity of respiratory involvement across diagnostic groups and within the same disease. These differences appear highly dependent on genotype.

The following section provides an overview of the respiratory and sleep features of the most common childhood-onset NMD associated with early respiratory impairment.

4. a. Duchenne muscular dystrophy

X-linked Duchenne muscular dystrophy (DMD) is a genetic disorder caused by mutations in the *DMD* gene, which consists of 79 exons encoding the dystrophin protein. The incidence ranges from 1:3802 to 1:6291 live male births [100]. Boys with DMD usually present between the ages of 2 and 5 years because of alteration of gait and developmental delay. The course of the disease follows a predictable clinical pattern with a tendency towards improvement of the child's strength at 6-7 years of age, followed by a 1-2 years plateau in function before a progressive motor decline that leads to loss of independent ambulation and wheelchair dependence. In the absence of corticosteroid or other therapy, the loss of independent ambulation occurs by the age of 12 years. Thereafter, the major determinants of morbidity are progressive cardiac and respiratory failure[101]. Respiratory deterioration in DMD also follows a predictable progression. FVC % and PEF % decline continuously from childhood. The restrictive lung physiology and diaphragmatic impairment are further exacerbated by the progression of scoliosis [38] [39]. Respiratory function decline is well correlated with DMD clinical progression both in ambulant and non-ambulant DMD patients. In the latter group, there is a good correlation between upper limb function and FVC % [102, 103]. Because of the relatively predictable progression of respiratory failure with progressive loss of motor function,

the most recent standard of care for DMD suggest a step-wise approach of respiratory assessments according to patients' age and motor status [69].

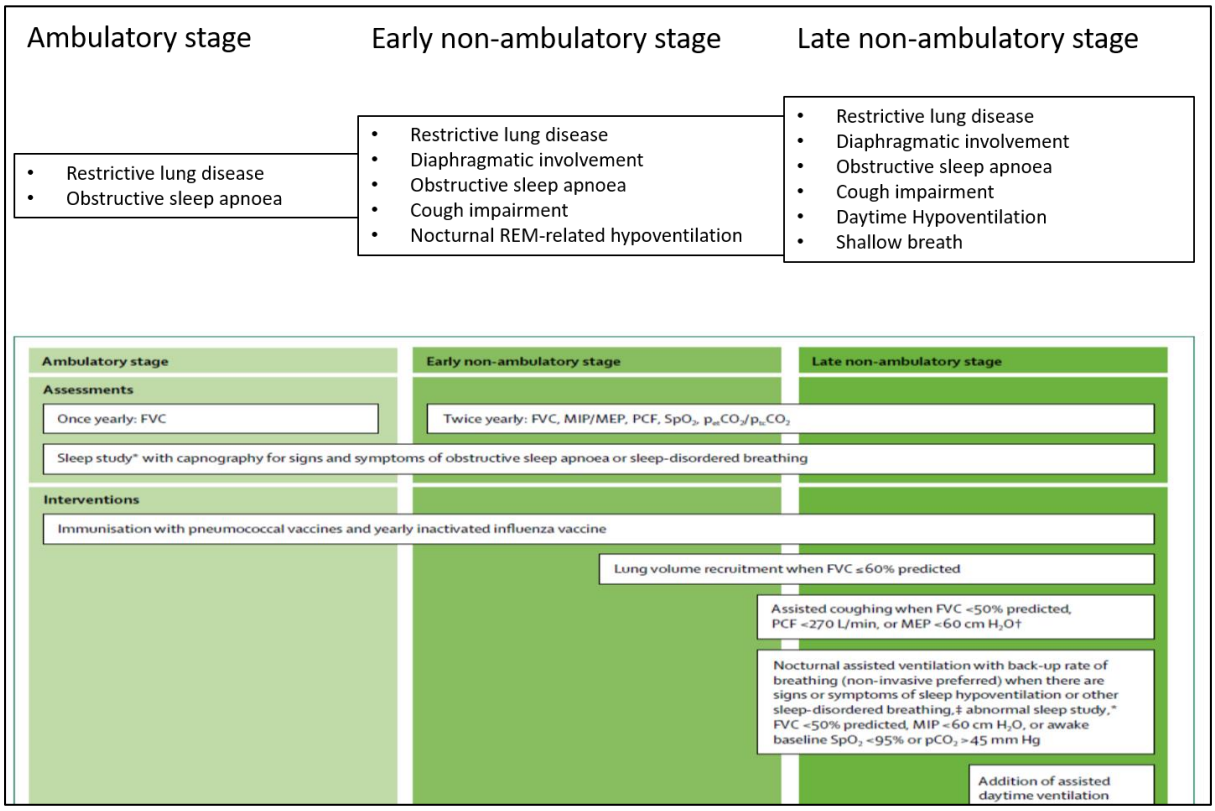


Figure 12. *Progression of daytime and overnight respiratory complications at different stage of motor function in DMD patients. Adapted from Birnkrant et al. 2018 Lancet Neurology*

Without ventilatory intervention, and with the progression of cardiomyopathy with age, death in the absence of corticosteroid therapy typically occurred by the age of 20 years [101]. Ventilatory support has dramatically improved life expectancy of DMD patients over the last twenty years [94, 104]. Additionally, recent work assessing the beneficial role of the regular use of steroids have shown they prolong survival [105] and delay the onset of respiratory failure when compared to untreated DMD [106]. They seem to act at the early stages by improving lung function and delaying the age of decline, but not by reducing the rate of deterioration once it has started.

There is however, increasing interest in finding modifier genes that could explain the variability in the progression of respiratory function amongst individuals with DMD. Recently, different mutations in the β2 adrenergic receptor have been identified in association with an earlier or later NIV requirement. The β2-adrenergic receptor (ADRB2) coupled pathway has been identified as capable of attenuating skeletal muscle degradation in DMD. In animal models, ADRB2 stimulation increases myofibril expression of type IIa myosin heavy chain isoform, promoting bulk muscle size and muscle strength and regeneration. ADBR2 stimulation also increases cross-sectional area, strength, and contractility of the diaphragm, improves mucociliary clearance and inhibits inflammatory pathways, which may be the mechanism affecting respiratory function in DMD [107].

Sleep disordered breathing almost invariably occurs in DMD. Respiratory muscle weakness can lead to reduced total sleep time and efficiency. These patients tend to exhibit marked sleep fragmentation with frequent nocturnal arousals, increased light (stage 1) sleep and reduced REM sleep. The commonest SDB in young DMD boys from the age of 12 years is OSA. The presence and severity of OSA correlates with body mass index, which is further increased by the reduced energy expenditure caused by immobility combined with the chronic treatment with corticosteroids. Hypoventilation appears later on in the course of the disease, and can occur in combination with OSA or in isolation [59]. In end-stage DMD NIV needs to be progressively used during the daytime as increasing ventilatory dependency develops. Daytime NIV relies on the use of a mouthpiece as interface. [108]. Finally, invasive tracheostomy ventilation is often deployed. The early individualised use of NIV and the regular use of cough assist have progressively reduced the requirement for invasive ventilation during acute respiratory failure [109].

New treatments and their role on respiratory function

The number of known genes responsible for many NMD has expanded greatly in the past three decades. The understanding of the genetic basis of NMD and the knowledge of disease-specific complications has helped the development of experimental therapeutic approaches.

These therapeutic approaches can be categorised into two broad strategies. The first strategy relates to the correction of mutant RNA processing, using either antisense oligonucleotides or small molecules that can modify mutant RNA splicing or using drugs that alter the translation of mutant mRNA by inducing a partial read-through of non-sense mutations. The second strategy involves the use of adeno-associated viruses (AAVs) to deliver a functional or partially functional gene copy to the affected cells and tissues [110].

- Eteplirsen, Ataluren

Primary therapies for DMD aim either to repair the *DMD* mutation (using techniques such as exon skipping, stop codon read-through and gene editing) or to restore normal levels of functional dystrophin protein (using treatment with micro dystrophins or cell therapies). Currently, three independent clinical trials of slightly different micro dystrophins, viral vectors and gene promoters are ongoing in patients with DMD[111]. In addition, two gene-based therapies addressing the loss of dystrophin have already been approved: ataluren by the European Medicines Agency (EMA) and Eteplirsen by the FDA. However, these agents are mutation class dependent thus suitable only for around 10-15% of DMD patients. Recent preliminary results showed that DMD patients treated with Ataluren, when compared with patients receiving standard care from the US DMD natural history database (CINRG), had a trend towards a delay in the loss of pulmonary function, measured by FVC % < 60%, FVC % < 50% and FVC < 1 litre [112]. Another recent report showed that DMD boys aged between 10 and 18 years treated with Eteplirsen (skipping exon 51) had a reduced annual reduction of

FVC % compared with the CINRG patients with same genotype used as controls ($p < 0.05$) [113].

- Idebenone

Secondary therapies for Duchenne muscular dystrophy (DMD) act on various pathways that are disturbed as a result of the disease. Idebenone is an anti-oxidant drug that halts the production of reactive oxygen species triggered by the muscle and systemic inflammatory processes occurring in DMD as results of the immune response triggered by the lack of dystrophin. It has been proven to be effective in delaying the progression of respiratory function, when compared to placebo, in DMD steroid naïve patients[114]. A recent trial compared the long-term respiratory function progression during periods On-Idebenone compared to periods Off-Idebenone in the same patients. DMD patients On-Idebenone had a lower rate of decline of FVC % and PEF % when compared to their Off-Idebenone periods. The better pulmonary function was maintained after the Idebenone was stopped [115]. A clinical research trial testing the efficacy of Idebenone in DMD steroid treated is ongoing (*NCT02814019 ClinicalTrials.gov*).

4. b. Spinal Muscular Atrophy

Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder characterized by progressive muscle wasting and loss of function due to severe motor neuron dysfunction. It is caused by mutations in the survival motor neuron 1 (*SMN1*) gene [116, 117]. SMA is classified into different clinical subtypes according to age of onset and maximal motor functional status achieved: weak infants unable to sit unsupported (type 1), non-ambulant patients able to sit independently (type 2), and ambulant patients with childhood (type 3) and adult onset (type 4) [118] [119].

Respiratory impairment is the most frequent complication and the major cause of mortality in children with SMA [70]. It is characterised by the involvement of intercostal muscles with a relative sparing of diaphragm causing a bell-shaped chest, paradoxical breathing and impaired airway secretion clearance [29, 36].

As expected, SMA type 1, type 2 and 3 have substantially different respiratory progression. Respiratory function has been extensively studied in patients with SMA type 1 [120, 121]. There is increasing interest in the respiratory natural history of untreated SMA type 1 children as recently available treatments are leading to a longer life expectancy and new clinical needs [122-124]. SMA type 1 is characterised by involvement of the bulbar muscles causing swallowing dysfunction with risk of aspiration and most children need intragastric feeding by age 12 months. Imbalance between the inspiratory intercostal muscles and the diaphragm causes typical thoraco-abdominal asynchrony ("paradoxical breathing") as the ribcage is not stabilised during inspiration against the negative pull generated by the diaphragm: the upper ribcage is drawn inwardly during inspiration instead of being elevated. Chronic thoraco-abdominal asynchrony often causes chest deformities in young children with SMA type 1 (''bell-shaped" chest and *pectus excavatum*). Other causes of increased respiratory load are micro-atelectasis due to shallow breathing, reduced secretion clearance and reduced elastic recoil of the lung [125]. The outward recoil of the chest wall, coupled via the pleural space to the lung parenchyma helps maintain airway patency[126].

In untreated children with SMA type 1 the median age at reaching the combined endpoint of death or requiring at least 16 hours/day of ventilatory support was 13.5 months (IQR 8.1–22.0 months). Requirement for nutritional support preceded that for ventilatory support. The median survival time for SMA type 1 infants ranged from 8 months (95% CI, 6-17) to more than 24

months according to the time of onset and genetic background (number of copies of the backup gene *SMN2*).

Long-term respiratory data have been assessed in SMA type 2 and 3 [35, 127] without taking into account the ambulatory status. As expected, SMA type 3 have a slower respiratory progression than SMA type 2. The changes in motor and pulmonary function were not correlated and respiratory function was not associated with the number of *SMN2* copies.

Oral salbutamol is used in clinical practice in SMA children as its use has been shown to increase skeletal muscle strength in healthy humans. Furthermore, salbutamol seems to induce a rapid and significant increase in *SMN2* expression and SMN protein in human SMA fibroblasts. Long-term oral salbutamol appeared to increase the strength of the inspiratory muscles in a small cohort of SMA2 patients. On the other hand when administered for >12 months, it did not change motor, upper limb or respiratory function (FVC, FEV1, PCF), breathing pattern and thoraco-abdominal contribution during quiet breathing in seated and supine position when compared to untreated SMA type 2 [29, 128].

Finally, little is known about SDB and sleep architecture in infants with SMA. Compared with control children, SMA patients showed increased sleep latency, AHI and abnormal sleep microstructure, characterized by reduced arousability during non-rapid eye movement sleep [82, 83].

New treatments and their role on respiratory function

- Nusinersen, Risdiplam

In humans there are normally two variants of the *SMN* gene on each allele, a telomeric variant (*SMN1*) and a centromeric variant (*SMN2*). The coding sequence of *SMN2* differs from *SMN1* by one exonic nucleotide, which results in alternative splicing of exon 7, leading to mRNAs lacking exon 7 (90%) causing the transcription of the truncated and unstable proteins. 10%

result in full length transcripts and functional protein. All patients with SMA retain at least one copy of *SMN2*. Generally, the number of copies of SMN2 is inversely related to the severity of the disease. The development of antisense nucleotides is aimed to enhance inclusion of exon 7 in *SMN2* splicing thus increasing the production of full-length transcript. The binding of antisense oligonucleotides to the intronic region between exons 7 and 8 on the SMN2 pre-mRNA induces the inclusion of exon 7 in the transcript [110].

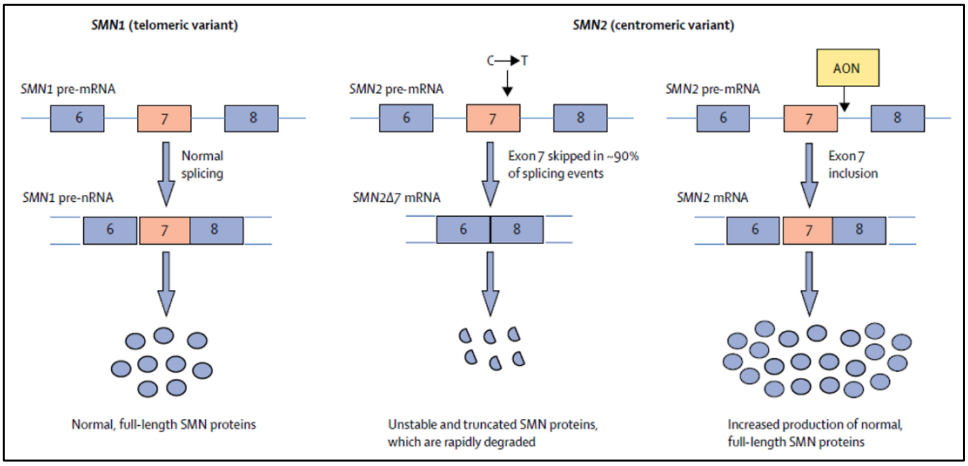


Figure 13. *Mechanism of action of antisense oligonucleotide (AON) in the treatment of Spinal Muscular Atrophy. The binding of AON to the intronic region between exons 7 and 8 prevent the transcription of a truncated SMN protein by promoting the inclusion of exon 7 in mRNA. Adapted from Scoto M. et al. Lanc. Child Adolesc Health 2018.*

Nusinersen is an antisense oligonucleotide that modifies pre-mRNA splicing of *SMN2* to promote increased production of full-length SMN2 protein. The regular intrathecal administration of Nusinersen has changed the landscape of Spinal Muscular Atrophy type 1 [129] and 2-3 [130, 131]. SMA type 1 infants treated had an increased survival compared to the placebo group. However, there are no specific respiratory outcome measures on the effects of the treatment in SMA type 1 except for the number of daily hours of ventilation. A recent paper assessed the differential efficacy of Nusinersen on respiratory and peripheral muscle function in patients with SMA type 1. The responsive to the drug was different according to disease subtypes. SMA type 1 milder subtype C (onset between 3-6 months) were the best responders. Children affected by the most severe subtypes, A and B (onset from birth up to 3

months of age), showed significant improvement in motor but not respiratory muscle function even after a year of treatment [132].

Preliminary data of Nusinersen treated pre symptomatic SMA type 1 infants showed a dramatic improvement, with the achievement of motor milestones close to physiological norms. Twenty-five infants were treated at a median age of 22 (3–42) days. After a median 2.9 years of follow-up, all 25 participants sat without support, 23/25 (92%) walked, of whom 22/25 (88%) could walk independently (ClinicalTrials.gov Identifier: *NCT02386553*) [133].

Orally bioavailable small molecules, which modulate *SMN2* splicing towards an increased expression of stable full-length SMN2 protein are currently being investigated in clinical trials on pre-symptomatic and symptomatic SMA type 1 and SMA type 2 and 3 (*ClinicalTrials.gov Identifier NCT03779334, NCT02913482, NCT02908685, NCT03032172*). Recently (11 Nov 2019) it has been announced that Sunfish, the pivotal part 2 study evaluating oral Risdiplam in people aged 2-25 years with SMA type 2 or 3 met its primary endpoint of change from baseline in the Motor Function Measure 32 (MFM-32) scale after one year of treatment compared to placebo.

- Gene therapy

Currently, intra-venous single-dose gene replacement therapy clinical trials are ongoing for pre-symptomatic patients with SMA type 1 (*ClinicalTrials.gov Identifier: NCT03505099 and NCT03461289*) and intrathecal for SMA type 2 and type 3 (*ClinicalTrials.gov Identifier: NCT03381729*). Preliminary results on AVXS-101–treated infants SMA type 1 compared with SMA type 1 infants enrolled in a prospective natural history study (NCT01736553) showed that the survival rate after 24 months of age was respectively 100% vs 38%. The average motor function score (CHOP-INTEND) improved in treated infants from 28.2 at baseline to 56.5 at

24 months of age while in the natural history group it worsened from 20.3 to 5.3. Only the infants who received AVXS-101 were able to sit and walk unassisted [134].

4. c. Congenital myopathies

Congenital myopathies (CMYO are a group of genetically heterogeneous non-dystrophic muscle disorders. They have highly variable features ranging from profoundly severe presentations within the fetal akinesia spectrum to milder forms with onset in adolescence or even in adulthood. Historically the congenital myopathies have been classified on the basis of the major morphological features seen on muscle biopsy – e.g., rods (nemaline myopathy), cores (central core disease and multiminicore disease), central nuclei (centronuclear/myotubular myopathy) and selective hypotrophy of type 1 fibres (congenital fibre type disproportion). They are mostly disorders of muscle excitation–contraction coupling or of stabilization of the sarcomere [135-137].

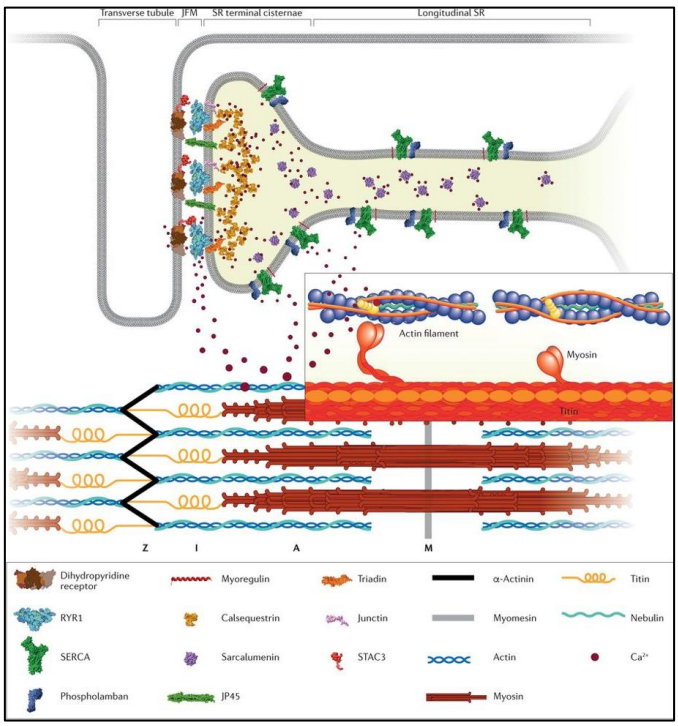


Figure 14. *Main proteins implicated in skeletal muscle excitation–contraction coupling (ECC) and thin–thick filament interaction and assembly that are commonly mutated in congenital myopathies. Adapted from Jungbluth H. et al. Nature Rev Neurol 2018.*

As expected, the respiratory involvement and its progression differs with the underlying genetic mutation. Respiratory muscle involvement leading to respiratory insufficiency at birth can occur in severe nemaline myopathy, *MTM1*-relatedmyotubular myopathy, *MEGF10*-related myopathy and occasionally in *RYR1* and *DNM2*-related myopathies.

- Severe *ACTA-1* (AD) and *NEB* (AR) nemaline myopathies are characterized by infantile onset, hypotonia. *NEB* myopathy has often disproportionate bulbar involvement [138]. Around 60% patients affected by these myopathies required NIV in childhood [139].
- *TNNT1* (AR and AD) mutations cause 'Amish Nemaline Myopathy', characterized by early onset tremors of the jaw and lower limbs, proximal contractures, generalized hypotonia associated with severe pectus carinatum and muscle atrophy, leading to respiratory insufficiency and death in the second year of life [140, 141].
- *LMOD3* (AR) have recently been found to cause a severe form of nemaline myopathy that presents with severe generalized hypotonia, respiratory insufficiency, contractures, and sometimes bulbar weakness [142].
- *CFL2* (AR and AD) myopathies show phenotypic heterogeneity ranging from early-onset and rapid progressive forms to milder myopathy [143].
- *KLHL40* (AR) mutations have been associated with severe/lethal nemaline myopathy [144].
- *MTM1* (X-Linked) myopathy patients may have early and severe disease. In a prospective case-series, out of 45 patients from 3 months to adulthood, thirteen patients had a mild phenotype (no ventilation support), 7 had an intermediate phenotype (ventilation support < 12 hours a day), and 25 had a severe phenotype (ventilation support >12 hours a day) [145].

- *MEGF10* (AR) mutations cause a severe condition called EMARDD (Early Myopathy, Areflexia, Respiratory Distress and Dysphagia) and a milder form with cores in the muscle biopsy [146].
- *DNM2* (AD) Patients may present in the neonatal period through to late adulthood, with inter- and intra-familial variation in age of onset and severity [147].
- *RYR1* (AR and AD) central core disease has been reported to be associated with severe early-onset arthrogryposis, hypotonia, needing permanent tracheostomy ventilation [148].

Respiratory involvement out of proportion to the skeletal muscle weakness, such as nocturnal respiratory insufficiency in ambulant patients, is typically seen in SEPN-1 related myopathy, but also in nemaline myopathy related to NEB or ACTA1 mutations.

- *SEPN-1* (AR) is a multiminicore disease causing respiratory failure with diaphragmatic involvement early in the disease, with associated scoliosis [149].

New treatments and their role on respiratory function

- MTM1 (Gene therapy)

A recent Gene Transfer Clinical Study in X-Linked Myotubular Myopathy (ASPIRO), (*ClinicalTrials.gov Identifier: NCT03199469*) on 16 children with myotubular myopathy showed impressive results in terms of motor and respiratory function (communication at World Muscle Society Conference, 1-5 October 2019). The preliminary data available on 6 children treated more than one year ago showed improvements in respiratory function, with reduction of hours of ventilation in all patients. Four patients achieved complete ventilator independence, and achievement of motor milestones such as head control, sitting unaided, crawling and step taking.

4. d. Congenital muscular dystrophies

Congenital muscular dystrophies (CMD) are early onset muscle disorders in which the muscle biopsy is compatible with a dystrophic process. CMD as a group encompasses great clinical and genetic heterogeneity and making an accurate genetic diagnosis has become increasingly challenging [150]. Early onset hypotonia and muscle weakness are the most typical presentations but onset in adulthood has been reported. At present, around 18 different genes have been shown to be associated with CMD, but in approximately one third of patients no specific genes could be identified.

Respiratory muscles are rarely spared in CMD, even if the type of muscle involvement, severity, and time course varies greatly among the different diseases. Three major categories of CMDs are commonly recognized, each of which has distinct, well described phenotypic features: a. collagenopathies (collagen VI (ColVI)-related CMDs), including Ullrich CMD and Bethlem myopathy; b. merosinopathies (laminin a2 (LAMA2)-related CMDs); and c. dystroglycanopathies (α-dystroglycan–related CMDs), including Fukuyama CMD, muscle–eye–brain disease, and Walker–Warburg syndrome [151].

There is a high level of respiratory morbidity and mortality in CMD usually due to the highly compromised limb and respiratory striated muscle status and the brain involvement which is a frequent feature of this diagnostic group [152]. Children with congenital muscle disorders resulting in nocturnal hypoventilation or daytime hypercapnia should be supported with non-invasive ventilation (NIV). The prevalence of respiratory morbidities in CMD have been assessed in only a few studies [42, 151-153]. These report an overall respiratory complication rate of 12% in CMD. Another study reported that FVC was <80% predicted in all patients with Ullrich CMD by the age of 6 years. One study examined the use of PSG in 2 patients with

CMD and 2 patients with rigid spine syndrome and found that all subjects experienced nocturnal hypoventilation and hypoxemia [151].

Patients at risk of selective diaphragm dysfunction such as those with ColVI–related CMD, must be screened early for sleep disordered breathing [71].

The incidence and prevalence of CMD is unknown. ColVI-related CMD and LAMA2-related CMD together represent the most common CMD subtypes with an estimated prevalence of 0.1 to 0.9 per 100,000[154]. The respiratory and sleep pathologies in these two CMD phenotypes are described below, while a summary of characteristics of all CMD are given in Table 1c.

- ColVI-related CMD (Ullrich and Bethlem muscular dystrophies)

ColVI-related CMD is known to be associated with selective diaphragmatic dysfunction even in the earlier stages of the disease, when ambulation is still preserved [42]. Nevertheless, the decline in respiratory function is related to severity of the disease. In severe muscle weakness either preventing ambulation or resulting in an early loss of ambulation, FVC% declined significantly by 2.6% per year. Intermediate patients had a significant decline in FVC% of 2.3% per year whereas the relationship between age and FVC% in patients with Bethlem myopathy was not significant ($p>0.05$). Nocturnal NIV was initiated in patients with Ullrich CMD by 11.3 years (±4.0) and in patients with intermediate ColVI-related CMD by 20.7 years (±1.5) [153].

In another study, the annual rate of decline of FVC% in the upright (sitting) position was statistically significant only in ambulatory but not non-ambulatory individuals with ColVI-related CMD. In the non-ambulant patients, the use of non-invasive ventilation may potentially stabilize pulmonary function [154].

- *LAMA2*- related CMD

LAMA 2-related CMD is a severe form of CMD. The classical phenotype manifests as hypotonia or muscle weakness during the first months of life and reduced spontaneous movements. Muscle weakness prevents the achievement of normal motor milestones (no head control or ability to sit unsupported) and frequently gives rise to aspiration, recurrent chest infections, and respiratory failure [155]. The weakness of diaphragm and intercostal muscles, the decreased compliance of the chest wall, and the thoracic deformities related to scoliosis all contribute to the progressive decline in pulmonary function and the need for NIV [154].

4. e. Congenital myasthenic syndromes

Congenital Myasthenic Syndromes (CMS) are a group of rare genetic disorders affecting neuromuscular transmission, associated with significant morbidity. They are clinically characterised by symptoms ranging from fatigable weakness and ptosis, episodic respiratory crises, bulbar and respiratory difficulties in neonates with or without concomitant hypotonia, weakness or arthrogryposis, to delayed motor development, and progressive respiratory or bulbar weakness [156].

Currently, mutations in 32 genes are responsible for autosomal dominant or autosomal recessive CMSs. These mutations concern 8 presynaptic, 4 synaptic, 15 post-synaptic, and 5 glycosylation proteins. These proteins function as ion-channels, enzymes, or structural, signalling, sensor, or transporter proteins [157].

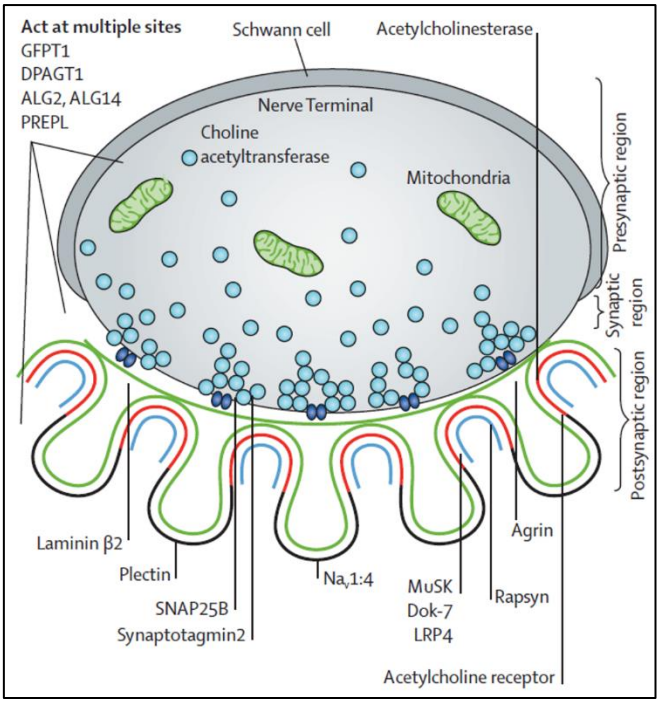


Figure 15. *Neuromuscular junction. Localisation of the proteins involved in the pathogenesis of congenital myasthenic sydromes. Adapted from Engel AG. et al. Lancet Neurol 2015.*

From a respiratory perspective, two pattern of progression have been identified in CMS:

- Progressive ventilatory course (*COLQ*)
- Sudden respiratory apnoeas/crises *(RAPSN*, *CHRNE*, *CHAT*)

Stridor attributable to laryngomalacia or bilateral vocal cord palsy in the context of variable degrees of arthrogryposis and progressive muscle weakness are also features of *DOK7* and *RAPS* mutations [56].

In a small cohort of children with CMS (1 *COLQ*, 1 *RAPSN*, 3 undiagnosed) sleep was characterised by increased AHI with younger infants having the highest AHI, and a normal heart rate. Hypotonia of the upper airway muscles may cause "real" obstructive events, as observed in the two youngest patients. These events may also be falsely scored as "obstructive" if there is paradoxical breathing with thoracic and abdominal components out of phase, due to

diaphragmatic dysfunction or weakness of the intercostal muscles. Patients with respiratory muscle weakness may also present with progressive simultaneous decrease in airflow and thoracic and abdominal movements, which may be accompanied by altered gas exchange, suggestive of a global inspiratory muscle weakness, or a decrease in central drive [81].

4. f. Pompe disease

Pompe disease is an autosomal recessive disorder that belongs to the group of Glycogenoses (it is also called Glycogen storage disease type II). It is caused by deficiency of the lysosomal enzyme acid-a-glucosidase leading to generalized accumulation of lysosomal glycogen, especially in the heart, skeletal and smooth muscle, and the nervous system. Pompe disease is generally classified based on the age of onset as infantile (IOPD) when it presents during the first year of life, and late onset (LOPD) if it presents subsequently. Childhood, juvenile, and adult-onset Pompe disease are examples of the late-onset form.

IOPD associated with cardiomyopathy is referred to as classic Pompe disease and, in the absence of cardiomyopathy, as non-classic Pompe disease [158]. Pompe disease, in addition to progressive respiratory muscle weakness, also causes selective diaphragm dysfunction, which may lead to chronic hypercapnic respiratory failure form alveolar hypoventilation even at early stages of the disease [159].

In LOPD, respiratory muscle weakness is mainly attributable to diaphragm dysfunction, leading to impaired inspiration and hypercapnia as well as weakness of cough. Morphologic changes spare the accessory inspiratory muscles such as the external intercostals. The significance of diaphragm involvement is underlined by the fact that a postural drop in FVC is a clinical hallmark of LOPD. FVC reduction is associated with the presence of (nocturnal) hypercapnia and the need for home ventilatory support as well as clinical disease severity. Interestingly, accumulation of glycogen has also been found in cervical motor neurons and

central nervous system neurons suggesting that neuronal pathology may contribute to respiratory muscle dysfunction [62, 160].

OSA and hypoventilation are common in children with IOPD, even in those with no symptoms of sleep-disordered breathing and should therefore be systematically excluded. A variety of factors may predispose these children to obstructive events. Children with baseline hypotonia may be at heightened risk for airway collapse during sleep due to decreased input to bulbar motor neurons. Facial myopathy, tongue weakness, and macroglossia are also seen in infantile Pompe disease. This combination may contribute to glossoptosis, particularly in the supine position. Hypoventilation is also common due to diaphragmatic involvement [61].

In open studies, enzyme replacement therapy has been shown to improve cardiomyopathy, ventilatory function, and overall survival in 18 children <7 months old with IOPD treated for 52-wks [161]. However, so far no randomized controlled trials have compared the efficacy of treatment vs placebo in changing survival or respiratory status in IOPD [162].

4. g. Congenital myotonic dystrophy

Myotonic dystrophy type 1 or Steinert disease (DM1) is a multisystem disorder historically named for two prominent features, myotonia and muscular dystrophy. It is caused by a CTG (cytosine, thymine, and guanine) repeat expansion in the myotonic dystrophy protein kinase (*DMPK*) gene on chromosome 19. DM1 follows an autosomal dominant inheritance pattern with presentations ranging from a less prevalent, severe congenital form to mild late-onset adult or asymptomatic forms. Due to anticipation (increased CTG repeat number in offspring), successive generations typically have earlier disease onset and increased disease severity.

Congenital myotonic dystrophy (usually >1000 repeats) is the most severe form and may present prenatally with polyhydramnios and decreased fetal movements. At birth, respiratory

failure requiring immediate intubation and ventilation, hypotonia, and feeding difficulties are common. Infants have facial weakness and a characteristic “tented” or “fish-shaped” upper lip. Although myotonia is typically absent in infancy, it may present in early childhood (∼5 years old). In most cases, myotonia is present after age 5 (i.e., 6–11 years old). Mental retardation and behavioural disorders are prevalent in children with congenital DM1 [163].Because of the higher prevalence of the late onset form, respiratory involvement and progression have been better studied in adults. Adult patients with signs of diaphragmatic weakness (exertional dyspnoea, orthopnoea, FVC < 70% predicted) showed a high prevalence (>70%) of nocturnal hypoventilation and almost 80% showed sleep apnoea, either obstructive, central, or “mixed”. Non-invasive ventilation was effective in restoring adequate gas exchange [63, 164].

As expected, respiratory function correlated with number of CTG repeats. Higher CTG repeat number was associated with peak cough flow impairment ($p = 0.007$) and with lower values for maximal inspiratory pressure ($p < 0.0001$) and upright vital capacity. [165].In children with myotonic dystrophy, the literature on respiratory and sleep involvement is scanty. A recent study showed that children with congenital DM1 had a higher prevalence of recurrent lower or upper respiratory tract infections (20.0%), asthma (13.3%), recurrent pneumonia (16.7%), positive airway pressure assistance for OSA (10.0%) and restrictive lung disease (3.3%). Patients with milder forms of DM1 with onset in the teenage years had a prevalence of pneumonia and recurrent infections of 10.0% [166].

Table 1. Respiratory and sleep involvement in different neuromuscular diagnostic groups

a. Duchenne Muscular Dystrophy (DMD) and Spinal Muscular Atrophy (SMA)

Diagnosis	Inheritance	Onset	Respiratory features	Sleep features	Progression	Disease-specific features	Ref
DMD - **Ambulant** - **Non ambulant**	X-linked		Reduction of lung volumes and peak flows from five years of age	SDB and Hypoventilation by teens after loss of ambulation	Proportional to motor function	Diaphragmatic involvement, obesity, macroglossia	[106]
SMA 1	AR	All by 2 years	-Bell-shaped thorax for intercostal muscles atrophy -Paradoxical breathing -Impaired management of secretions	Early onset SDB, early ventilator support	Rapid		[120, 121]
SMA 2	AR	~40% childhood	-Intercostal muscles affected. -Reduction of lung volumes since childhood	30% ventilator support before 18 years	Slow		[127]
SMA 3	AR	Rare in childhood	Normal lung volumes in childhood	3% ventilator support before 18 years	Slow		[127]

b. Congenital myopathies

Diagnosis	Inheritance	Onset	Respiratory features	Sleep features	Disease-specific features	Ref
Central core disease						
- **RYR1**	AD	Variable	Scoliosis		Ptosis	
Multi-mini core disease						
- **SEPN1**	AR	Variable	Diaphragmatic involvement while ambulant	Hypoventilation	Rigid spine	[149]
- **RYR1**	AR	Frequent ranging from mild to moderate	Respiratory muscle spared but restrictive respiratory disorder. Scoliosis		Ptosis	
- **MYH7**	AD	Variable			Cardiomyopathy	[167]
- **TTN**	AR	Variable	Early onset severe phenotype reported		Cardiomyopathy	
Nemaline						
- **NEB**	AR	Severe depending on motor function	Severe depending on motor function		Bulbar involvement, facial weakness	[139]
- **ACTA1**	AD	Severe depending on motor function	Severe depending on motor function			[139]
- **TPM3**	AD, AR	Variable				
- **TPM2**	AD	Mild				
- **TNNT1**	AR	Severe	Early onset hypotonia, pectus carinatum &,respiratory insufficiency			[141]
- **CFL2**	AD, AR	Severe depending on motor function	Severe depending on motor function			[143]
- **KLHL40**	AR	Severe	Respiratory failure 90%			[144]
- **KBTBD13**						
- **LMOD3**	AR	Severe	Joint contractures, severe muscle weakness respiratory failure			[142]
Centronuclear						
- **MTM1**	X-l	Severe	Severe depending on motor function. Ventilatory dependence in 80% children <18 years	Severe sleep disordered breathing		[145]
- **DNM2**	AD	Onset from neonatal period to late adulthood	Mild involvement progressing with motor function		Eye and facial involvement	[147]
- **BIN 1**	AR	Variable				

Fibre type disproportion			
-	**TPM3**	AD	Variable depending on motor function
-	**RYR1**	AR	Variable depending on motor function
-	**ACTA1**	AD	Variable depending on motor function

RYR 1: Ryanodine Receptor 1, **SEPN1:** Selenoprotein N 1, **MYH7:** Myosin Heavy Chain 7, **TTN:** Titin, **NEB:** Nebulin, **ACTA1:** Actin α 1, **TPM3: T**ropomyosin 3, **TPM2:** Tropomyosin 2, **TNNT1:** Troponin T1, **CFL2:** Cofilin 2, **KLHL40:** Kelch like family member 40, **KBTBD13:** Kelch repeat and BTB domain containing 13, **LMOD3:** Leiomodin 3, **MTM1:** Myotubularin 1, **DNM2:** Dynamin 2, **BIN 1:** Amphiphysin 2.

Adapted from KN North et al. Approach to the diagnosis of congenital myopathies. *Neuromuscular Disorders* 2014.

c. Congenital muscular dystrophies (CMD)

Diagnosis	Inheritance	Onset	Respiratory features	Sleep features	Disease-specific features	Ref
Collagen VI-related CMD						
- **Ullrich CMD**	AR	Birth	Early respiratory involvement Diaphragmatic weakness early in the disease (while ambulant)	Early hypoventilation		[153]
- **Bethlem CMD**	AR	Birth	Mild involvement according to motor function			
Merosin (LAMA2)-deficient CMD	AR	Birth	Frequent, due to the severity of the motor involvement (ambulation not achieved)	Sleep disordered breathing, early ventilatory support	Brain involvement	[155]
α-dystroglycan–related CMDs						
- **Fukuyama CMD**	AR	Birth	Frequent, due to the severity of the motor involvement (ambulation not achieved)	Sleep disordered breathing, early ventilatory support	Brain involvement	[168]
- **Muscle-eye-brain disease**	AR	Birth	Frequent, due to the severity of the motor involvement (ambulation not achieved)	Sleep disordered breathing, early ventilatory support	Brain involvement	
- **Walker-Warburg syndrome**	AR	Birth	Frequent, due to the severity of the motor involvement (ambulation not achieved)	Sleep disordered breathing, early ventilatory support	Brain involvement	

Adapted from Kang PB *Evidence-based guideline summary: Evaluation, diagnosis, and management of congenital muscular dystrophy* Neurology 2015

5. HYPOTHESES, AIMS AND OBJECTIVES OF THE THESIS

There are exciting new treatments becoming available for some of the most severe neuromuscular disorders such as SMA type 1 and MTM1 myopathy. Children are living longer with modern support, and new therapies may significantly improving respiratory and motor function. However, data on the respiratory progression in children naïve to experimental therapies are incomplete. In an era when new phenotypes are emerging, establishing the extent of improvement is difficult, particularly in the chronic long-term progressing disorders such as DMD.

Additionally, particularly for young, non-cooperative children, good clinical respiratory outcome measures are often missing or inadequate. The threshold and definitions used in the detection and grading of SDB such as OSA are tailored for otherwise healthy children and may not be applicable to a neuromuscular population.

As longevity increases, new comorbidities may emerge. It is possible that SDB in NMD could play a role in worsening cardio vascular and infective complications. The presence of OSA is associated with a systemic and airway pro-inflammatory state in otherwise healthy children. Serum pro-inflammatory cytokines such as interleukin 6 were found increased in children with OSA. Since NMD children have more complex SDB, not merely OSA, we have explored their correlation with airway and systemic pro-inflammatory status in NMD children.

In summary, the aims of this thesis are:

- To identify the natural history of decline in respiratory function in the commonest paediatric neuromuscular disorder (DMD) and the role of treatment and co-morbidities (Chapter 2)

- To define the reliability of standard and novel outcome measures for the assessment of respiratory and sleep abnormalities in paediatric neuromuscular disorders (Chapter 3)

- To investigate whether the presence and severity of sleep disordered breathing in paediatric neuromuscular disorders is associated with a pro-inflammatory airway and systemic status that can play a role in the development of co-morbidities (Chapter 4)

Chapter 2

RESPIRATORY NATURAL HISTORY/TRAJECTORIES OF THE MOST FREQUENT PAEDIATRIC NEUROMUSCULAR DISORDERS – DUCHENNE MUSCULAR DYSTROPHY

1. BACKGROUND

1. a. Natural history of Duchenne muscular dystrophy, role of genetic and therapeutic modifiers

Motor progression in patients with Duchenne muscular dystrophy

DMD presents in early childhood with delayed motor milestones, including delays in walking independently and standing up from the floor. The mean age of walking is approximately 18 months (range 12-24 months). The mean age of diagnosis of boys with DMD without a family history was reported to be 41 months [169]. Proximal weakness causes progressive waddling gait and difficulty climbing stairs, running, jumping. Boys use the Gower's manoeuvre to rise from a supine position, using the arms to supplement weak pelvic girdle muscles. Between 3 to 6 years of age there may be some evidence of transient improvement, known as the "honeymoon phase" related to normal motor development outpacing the progressive muscle weakness. Between age 6 and 11 years, muscle strength decreases almost linearly with a sequential loss of motor milestones: the ability to climb stairs, to stand from a supine position, then rising to all fours from supine, and finally walking even a short distance. The majority of DMD patients (70%) develop contractures of the heel, iliotibial bands, and hip, causing toe

walking and further affecting ambulation. The natural history of DMD without the regular treatment with coticosteroids, is rapidly progressive, with affected children losing ambulation (LoA) by age 12 years [170]. Respiratory complications and progressive cardiomyopathy are common causes of death. Even though the ventilator support has increased the life expectancy of these patients, they rarely survived beyond the third decade [171-173].

Despite an overall homogenous pattern of disease progression with subsequent identifiable loss of motor milestones, the pace at which function is lost may vary substantially among children with DMD. Several different factors have been indicated as affecting the prognosis of DMD. They can be classified as intrinsic i.e. genetic and extrinsic, related to the beneficial role of common therapeutic approaches such as corticosteroids.

Genetic modifiers

Different genotype subgroups within DMD have shown differences in their motor progression from the overall DMD population. The stratification of these subgroups has gained interest because of the emerging treatment options with personalised medicines, such as antisense oligonucleotides. In a large cohort of DMD ambulant patients amenable to skip exons 44, 45, 51 and 53 the 6 minutes walking distance (6MWT) progressed differently in each subgroup over three years. Mutations amenable to skip exon 53 had lower baseline values and more negative changes while those amenable to skip exon 44 had a longer walking distance at baseline and a slower decline [174]. Patients amenable to skip exon 44 have already been described as having better long-term motor prognosis expressed as age at loss of ambulation (LoA) [175] or North Star Ambulatory Assessment score (NSAA)[176].

The mutation of *DMD* gene leading to DMD phenotype are those affecting the expression of the full-length *DMD* muscle isoform. The *DMD* gene contains at least seven additional independent tissue-specific promoters that produce dystrophin isoforms named according to

their length and splicing patterns [177]. Patients carrying mutations in exons 1 to 79, lack the full length Dp427. Dp427 is the main isoform produced in skeletal and cardiac muscle and is affected by all mutations; the remaining shorter dystrophin isoforms, produced by additional promoters spread along *DMD* gene, are expressed in various tissues. Patients respectively carrying mutations in the exons 30 to 79, 45-50 to 79, 56 to 79 and 63 to 79 lack Dp260, Dp140, and Dp116 and Dp71 isoforms respectively. The shorter Dp260 isoform is expressed primarily in the retina [178]. The Dp140 isoform is expressed in the cerebral cortex only in foetal life stages and in the cerebellum postnatally [179]. Dp116 is expressed in cardiac muscle and peripheral nerve [178, 180, 181] and Dp71 in lung, skeletal and cardiac muscle besides brain and kidney [182-184]. Because of the brain and nerve localisation, most of the existing studies have focused on the role of dystrophin isoforms on cognitive aspects of DMD [185]. So far, only the analysis of cardiac progression was analysed in DMD and was found slower in patients lacking Dp116 isoform [181] but nothing is known about motor and respiratory aspects of the disease.

In the last years, the role of other genes called "modifiers" has emerged. They seem to affect the progression of disease in addition to the site-specific mutations within the *DMD* gene [186]. Osteopontin (SPP1), an inflammatory marker expressed in muscle fibres and inflammatory cells in DMD mouse models, has been suggested as modifier of disease severity in DMD. While less SPP1 in mice leads to improvement, in DMD it leads to greater weakness. It was suggested that SPP1in humans has a positive role in muscle regeneration. The association with deflazacort, can lead to the expression of osteopontin via the induction of its promoter. This can explain partially the differential response of DMD patients to corticosteroids [187-189]. Finally, both the reduced expression of CD40, a co-stimulatory molecule for T cell polarization, and a particular haplotype of LTBP4, involved in modulation of the TGF-b signalling pathway, were associated with earlier LoA [190].

Corticosteroids

There are increasing supporting evidences that the introduction of corticosteroid (CS) therapy and the systematic implementation of the standards of care [69] have shifted LoA and have prolonged DMD patients' survival [105, 191]. A Cochrane review concluded that there is moderate-quality evidence from randomised clinical trials showing that glucocorticoid therapy in Duchenne muscular dystrophy improves muscle strength or function, or both, in the short term for up to 2 years [192].

The time to all disease-related milestones was significantly longer in patients CS-treated for>1 year than in patients CS-naïve ($p<0.0001$). CS treatment was associated with increased median age at LoA by 2.1–4.4 years and upper limb milestones by 2.8–8.0 years compared with no treatment. Nine percent patients CS treated vs 19% of patients CS naïve were reported in a long-term prospective study [105].

CS regimens most commonly used are daily and intermittent (most patients on 10 days on/10 days off; in the past also 10 days on/20 off). The intermittent regimen was proposed to limit the severity of chronic CS-related side effects. A randomized study comparing efficacy and side effect profile of different regimens ("Finding the Optimum Regimen for Duchenne Muscular Dystrophy, FOR-DMD" NCT01603407) is currently ongoing [8]. This trial is aimed to compare the three most frequently prescribed corticosteroid regimes and to standardize their side effects. DMD children aged 4 to 7 years are randomly allocated to prednisone 0.75mg/kg/day, prednisone 0.75mg/kg/day switching between 10 days on and 10 days off treatment or deflazacort 0.9 mg/kg/day and followed for 3 to 5 years. Data on disease progression and corticosteroids multisystemic side effects will be collected and compared across regimens and treatments. So far, the comparison of intermittent and daily CS regimens in the progression of DMD and in the associated risks of side effects showed that daily CS

when compared to intermittent, delayed the median age at LoA of 2.5 years (14.5 vs 12) and the motor decline assessed by North Star Ambulatory Assessment (NSAA) in DMD boys. The beneficial effects of daily treatment in motor function are blunted by the increased occurrence of side effects. Patients treated with daily CS had an increased occurrence of cushingoid features, adverse behavioural events and hypertension [193].

Deflazacort, when compared to prednisolone, prolonged ambulation in a natural history study [194], and delayed the progression of 6MWT and 4 stairs climb (4SC) over one year in relatively small cohorts of DMD patients enrolled in the placebo arms of clinical trials [195, 196]. Long-term deflazacort users showed higher frequencies of growth delay ($p< 0.001$), cushingoid appearance ($p = 0.002$), and cataracts ($p < 0.001$), but not weight gain [194].

1. b. Respiratory progression in Duchenne muscular dystrophy

Natural history and role of corticosteroids

The progressive respiratory milestones in DMD are quite well recognized throughout the course of the disease and correlated with the motor progression [103, 197-199]. The lung function tests assessed by spirometry correlates with the risk of nocturnal hypoventilation in DMD. A FVC < 60% is correlated with increased risk of REM-related hypoventilation and a FVC < 40% with increased risk on REM and NREM hypoventilation [6]. This underlies the stepwise approach proposed by the latest DMD standard of care guidelines [69] as previously described in the introductory chapter.

The annual progression of the respiratory function and the role of genetic factors has not been explored so far. It is unknown whether patients' *DMD* mutations and the subsequent amenability to exon skipping or to the lack of dystrophin isoforms and the expression of gene modifiers are associated to different ages to respiratory endpoints.

Similarly, the role of currently available treatments such as corticosteroids on respiratory progression is only partially known and quite controversial.

McDonald et al followed 162 CS-naïve DMD patients and found that the decline in FVC % pred. over time varied by age. Boys ages 7-10 years declined by 0.3%/year, while boys aged 10-20 years declined by 8.5%/year [200].

In keeping with this finding, the thirty–tree patients on the placebo arm of the DELOS clinical trial with Idebenone (*NCT01027884*), mean age 15.0 (±2.5) years had a decline of FVC % pred. ~8% over one year. In addition, this rate of progression was maintained whether or not (58% vs 42%) they had been treated with corticosteroids prior to the enrolment. Interestingly 94% of them were already non-ambulant [114]. Connolly et al. followed 51 DMD boys, mean age 16.8 (±4.5) years for up to two years. Despite those taking corticosteroids performed better at baseline, the rate of annual decline were similar irrespective of the treatment [201].

The assessment of 340 DMD boys aged 2-28 years showed that FVC % pred. in CS treated DMD boys was higher than naïve patients and those on CS past treatment across age ranges. The higher overall FVC % pred. for CS-treated boys compared to naïve and past treatment was observed across the age 10–12 and 13–15 years [202].

A recent study focused on the respiratory function of 53 CS-naïve and 322 Cs-treated DMD boys followed up to 10 years and provided further information. CS slowed the yearly rate of FVC % pred. in an age-related fashion and only in patients aged 7–9.9 years. They confirmed that CS treatment resulted in higher peak absolute FVC and peak expiratory flow (PEF%) values with a later onset of peak values and respiratory decline. Progression to an absolute FVC < 1 litre was delayed by CS [106].

Lastly, a retrospective multicenter study compared 170 CS-treated, 5 with past CS-use and 50 CS-naïve DMD patients. Again, the rates of decline for both groups varied with age with maximal rate of decline between 12-20 years in CS-treated and 11-20 years in CS-naive. The peak of FVC % was, as expected, significantly higher in CS-treated patients but it was achieved at significantly earlier ages in CS-treated patients than CS-naïve (11.9±2.9 years vs 13.6±3.2 years) [203].

Taken together, these results show that a. the respiratory decline in DMD start from a young age b. the respiratory rate of decline in DMD is likely age-related, and most probably, this is highly associated with patients' ambulatory status; c. steroids have a beneficial role on the lung function at all age range when compared to no treatment; d. steroids delay the onset of clinically meaningful respiratory endpoints by increasing baseline lung function but not reducing the rate of annual decline once it has started.

Role of new treatments

- Eteplirsen, Ataluren

A recent communication of preliminary results showed that DMD patients treated with Ataluren (skipping of *DMD* null mutations) when compared with patients receiving standard of care alone from the US DMD natural history database (CINRG), had a trend towards a delay in the loss of pulmonary function, measured by FVC %< 60%, FVC %< 50% and FVC < 1 litre [112]. Another recent report showed that DMD boys aged between 10 and 18 years treated with Eteplirsen (skipping exon 51) had a reduced annual reduction of FVC % compared with the CINRG patients with same genotype used as controls ($p < 0.05$) [113].

- Idebenone

DMD patients On-Idebenone had a lower rate of decline of FVC % and PEF% when compared to their Off-Idebenone periods. The better pulmonary function was maintained after the

Idebenone was stopped [115]. A clinical research trial testing the efficacy of Idebenone in DMD steroid treated is ongoing (*NCT02814019*).

2. HYPOTHESES AND AIMS

We hypothesized that different corticosteroids administered at different regimens would have a differential impact in slowing down the progression of respiratory decline and the achievement of respiratory endpoints when compared to CS-naïve patients.

We aimed to compare the impact of the two mostly used CS, deflazacort and prednisolone, and their regimens, intermittent and daily with CS-naïve patients and to describe the long-term respiratory progression and time-to- meaningful respiratory thresholds in a large UK cohort of paediatric DMD.

3. MATERIALS AND METHODS

3. a. Study design and population

Retrospective study of paediatric DMD patients (aged <18years) followed at the Dubowitz Neuromuscular Centre (Great Ormond Street Hospital London) from May 2000 to June 2017. We included patients whose parents consented to the North Star database. The study was registered as audit at Great Ormond Street Hospital. TheNorth Star UK Network for data collection and the conduct of research studies within the Network received Caldicott guardian approval.

Patients enrolled in the FOR-DMD trial and in any other interventional clinical trials were excluded. Patients in the Heart Protection Trial ("A double-blind randomised multi-centre, placebo-controlled trial of combined ACE-inhibitor and beta-blocker therapy in preventing the development of cardiomyopathy" *EudraCT2007-005932-10*)[204] were excluded from the cardiac analyses. All information was collected from medical records. The first visit recorded for each patient was defined "baseline". Height was assessed standing for ambulant, or calculated from arm span in non-ambulant patients. Ambulatory status was recorded at each visit. LoA was the inability walking independently for 10 meters. Scoliosis was defined as a Cobb angle >20° [69] from spine x-ray. Age of scoliosis surgery was collected.

3. b. Corticosteroids

CS-naïve patients had never received CS therapy. CS-treated patients took either daily or intermittent CS (10 days on/10 days off) for >1 month. CS consisted of prednisolone 0.9 mg/kg or deflazacort 0.75mg/kg. The boys who had mixed steroids or regimens were defined "switchers". For them we explored two CS and regimens definitions to compare daily vs intermittent. We considered either patients' initial or majority CS regimen defined as ≥ 60%

treatment duration from baseline to latest visit [193]. Results were similar and we have presented the most clinically relevant majority treatment, defined in the manuscript "CS-daily" and "CS-intermittent". Patients' treatment were labelled "deflazacort" or "prednisolone" based on the majority CS. Patients whose CS information was missing were called "not known". They were excluded from the CS regimens comparison. For patients who stopped CS during the study, only data prior to stopping was included.

3. c. Genotyping

Dystrophin (*DMD*) gene mutations were analysed by multiplex ligation-dependent probe amplification, polymerase chain reaction or direct sequencing.

We stratified patients based on their lack of dystrophin isoforms. Dp427, produced in skeletal and cardiac muscle, is affected by all mutations. Because of the recent finding about the localization of Dp71 and Dp116 respectively in lung and heart, the respiratory progression was analysed in DMD patients lacking Dp71 and Dp116 compared to remainders.

Patients were also stratified according to their mutations within *DMD* and their amenability to the skip of the most common exons potentially treatable with antisense oligonucleotides. Patients were classified as amenable to exon 44, 45, 51 or 53 skipping and the respiratory function of each group was compared with that of the remainder patients in the study population.

3. d. Respiratory function

Spirometry was performed in seated position according to ERS/ATS guidelines [18]. Absolute FVC in litre (L) was collected and FVC % pred. calculated according to reference data [19]. The FVC and FVC % pred. yearly progression was compared between CS regimens. Time to FVC % pred. <50% was the main endpoint of respiratory impairment [69]. Time to absolute

FVC<1L and the age of requirement of non-invasive ventilation (NIV) were secondary endpoints. Absolute FVC< 1 L predicts nocturnal hypoventilation [10, 17].

3. e. Statistical analysis

Characteristics of the sample are presented as mean (SD), median (range or interquartile range) for skewed data and frequency (percentage) for categorical data.

For FVC % pred. we describe the longitudinal trajectories and estimate the mean annual change using mixed effects regression models, accounting for the longitudinal data and age at baseline. Models were fitted including patient as a random effect and CS regimen (intermittent or daily) and treatment (deflazacort or prednisolone) as fixed effects, using an unstructured correlation matrix. For FVC % pred. we considered the decline after the age of 9 years onwards, as respiratory capacity continues to increase until up to this age. We compared rates of decline between steroid regimens in a separate set of models according to patients' amenability to exon- 44, 45, 51 and 53 skipping, using appropriate interaction terms. Results are presented as mean annual change, or difference in mean annual change between subgroups, with 95% confidence intervals.

Using Kaplan Meier analysis and Cox regression analysis we estimated the median age at which clinically meaningful endpoint occurred: loss of ambulation, scoliosis, NIV, FVC % pred. <50% and FVC <1L. We investigated whether the average age at which these events occurred varied according to majority steroid and regimen through the inclusion of an interaction term and hazards ratios with 95% confidence intervals are presented. We compared the estimated age at respiratory endpoint by Dp71 and Dp116 isoform-deficiency. All analyses were conducted in Stata v15 with significance level of $p < 0.05$.

4. RESULTS

4. a Study population

There were 293 patients for 2363 visits (mean 8/patient), mean age at baseline 6.0 (± 2.3) years, mean follow-up 5.6 (± 3.5) years. Seventy-seven boys (26%) transitioned to adult care, 36 (12%) were lost to follow-up. The remaining 180 (61%) were followed throughout the whole study. Of them 7 (4%) died, mean age 16.5 (± 3.8) years, 1 for cardiomyopathy, 1 after general anesthesia, no information for 5. At the time of death, 3 patients had stopped CS and 4 were still CS-treated (2 CS-daily, 2 CS-intermittent) (See Table 1).

At baseline 285 boys (97%) were ambulant, mean age 5.8 (±2.1) years. Eight (3%) were non-ambulant, mean age 11.5 (±2.7) years. At last assessment, 162 (55%) patients were ambulant. Median (IQR) age at LoA was 12.1 (10.0-14.5) years in the whole population, 12.5 (10.0-15.7) years in CS-daily, 12.0 (10.0-14.0) years in CS-intermittent, 10.5 (9.1-11.2) years in CS-naïve patients. CS-naïve lost ambulation at similar age of CS-daily (p= 0.09) and CS-intermittent (p= 0.34). Fifty-eight patients (20%) had scoliosis. Five (2%) had scoliosis already at baseline, 53 (18%) developed scoliosis throughout the study. The median age (50%) of scoliosis was 17.1 years in the whole population, 17.1 years in CS-treated, 13.9 years in CS-naïve (p=0.18) (See Table 2 and Figure 1).

CS duration and regimens

Sixty-six of 293 (23%) patients were CS-daily, 182 (62%) CS-intermittent, 22 (8%) CS-naïve, 23 (8%) not known. In the cardiac cohort, 52 of 252 (21%) patients were CS-daily, 156 (62%) CS-intermittent, 21 (8%) CS-naïve, 23(9%) not known.

Thirty-eight boys (13%) stopped CS, median age 10.1 (5.5-15.5) years. Five were CS-daily prior to stopping and their reasons were unavailable, 32 were CS-intermittent. One stopped due

to behavioral issues, one to weight gain and one to blood pressure increase, information was missing for the remainder. One patient belonged to the "not known" group. In the cardiac cohort, 33 patients stopped CS.

Two-hundred four of 293 (69.6%) were on prednisolone, 36 (12.3%) were on deflazacort for ≥60% of treatment. Twenty-five switched compound, all from prednisolone to deflazacort .

	Total population	
	N=293(%)	Mean age (SD)
Age diagnosis	278	4.3 (2.4)
Age first visit	293	6.0 (2.3)
Age last visit	293	11.6 (4.4)
Age of starting CS		6.2 (1.7)
CS[a] regimen (≥60% treatment)		
- **Daily**	66 (22.5)	
- **Intermittent[b]**	182 (62.1)	
- **Naïve**	22 (7.5)	5.8 (1.4)
- **Not Known[c]**	23 (7.9)	6.4 (1.8)
Deflazacort (≥60% treatment)	36 (12.3)	
Prednisolone (≥60% treatment)	204 (69.6)	
CS regimen and compound (n=240)		
- **Daily Deflazacort**	14 (4.8)	
- **Intermittent Deflazacort**	22 (7.5)	
- **Daily prednisolone**	50 (17.1)	
- **Intermittent prednisolone**	154 (52.6)	
Stopped steroids	38 (13)	
- **Daily**		
- **Intermittent**	5	
- **Not Known**	32	
	1	
Steroid switchers		
- **Daily to Intermittent**	0	
- **Intermittent to daily**	39 (12.5)	8.9 (2.2)
Amenable to exon skipping		
Exon 44	20 (6.8)	
Exon 45	23 (7.9)	
Exon 51	29 (9.9)	
Exon 53	21 (7.2)	
Mutations leading to lack of Dys Isoforms		
Dp427	293 (100)	
Dp116	28 (9)	
Dp71	18 (6)	

Table 1. Clinical and genetic features of study population (N=293) and cardiac cohort (N=252).

	N=293 (%)	Mean age (SD)
Ambulatory status		
Ambulant at baseline	285 (97.3)	5.8 (2.1)
Not ambulant at baseline	8 (2.7)	11.5 (2.7)
		Median age at LoA[a](IQR)
Not ambulant at last follow-up	129/291 (44.3)	12.1 (10.0, 14.5)
- Daily	28/65 (43.1)	12.5 (10.0, 15.7)
- Intermittent	88/181 (48.6)	12.0 (10.0, 14.0)
- Naïve	12/22 (54.5)	10.5 (9.1, 11.2)
- Other	1/23	
Scoliosis		
Scoliosis at baseline	5 (1.7)	12.8 (1.1)
		Median (IQR)
Scoliosis	58 (19.8)	17.1 (13.7, *)
- Daily	7/66 (10.6)	
- Intermittent	44/182 (24.2)	
- Naïve	6/22 (27.3)	13.9
- Regimen not known[b]	1/23 (4.3)	
Scoliosis surgery	16/293 (5.5)	
- Daily	1/66 (1.5)	
- Intermittent	15/182 (8.2)	
- Naïve	0/22	
- Regimen not known	0/23	

***** not possible to estimate

Table 2. Ambulatory status and scoliosis of study population.

Corticosteroid treatment: regimen used for ≥60% total CS treatment duration. [a]LoA: loss of ambulation.

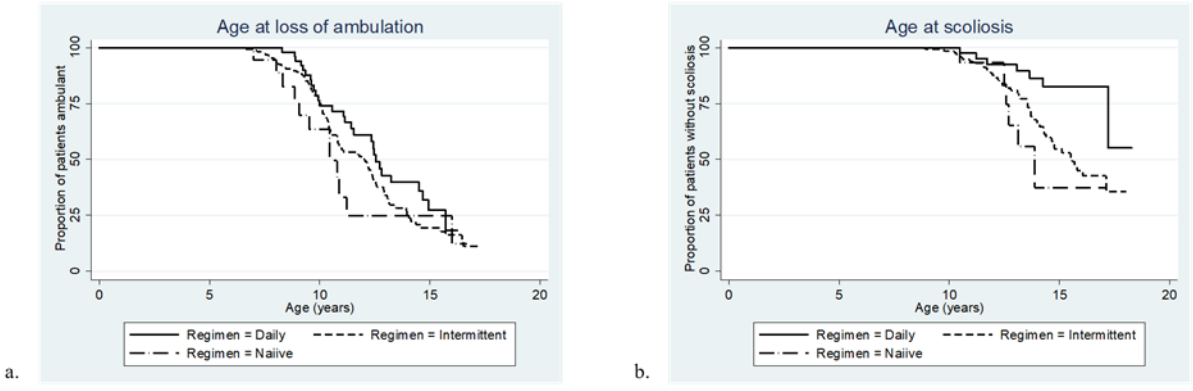


Figure 1. *Time to loss of ambulation (a.) and time to scoliosis (b.) according to CS regimens.*

Loss of ambulation (LoA) was the inability walking independently for 10 meters. Scoliosis was defined as a Cobb angle >20° from spine x-ray.

4. b. Respiratory decline according to corticosteroid treatment

FVC % pred. linearly declined in the whole population from age 9 years by 6.1%/year, 95%CI (5.6, 6.6). In CS-naïve FVC % pred. declined by 4.7%/year, 95%CI (2.8, 6.6). There was no difference between either CS-naïve or CS-treated (p=0.15) or between regimens (p=0.27) (See Figure 2).

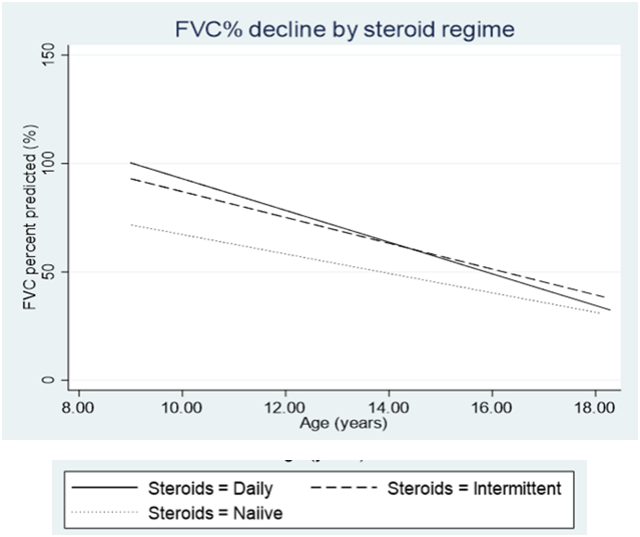


Figure 2. *FVC % pred. decline in CS-daily, CS-intermittent, CS-naïve DMD patients*

Linear population average model of respiratory function progression expressed as FVC % pred. according to CS regimen after the age of nine years. In the whole population FVC % pred. declined linearly by 6.1% per year, 95% CI (- 6.6, - 5.6). FVC % pred. declined by 4.7% per year, 95% CI (- 6.6, -2.8) in CS-naïve. There were no differences in the yearly rate of decline with between CS-naïve and CS-treated patients (p=0.15).

Mean age at peak FVC % pred. before declining was 9.7 (±3.4) years in the whole population. It was similar between CS-naïve and CS-treated and between regimens. The peak value FVC % pred. before the decline was significantly higher in patients on CS-daily and CS-intermittent than CS-naïve ($p<0.01$ and $p<0.05$). Patients on CS-daily had the higher FVC % pred. peak 90.8 (±22.7)% than CS-intermittent 83.9 (±21.8)%, (p=0.05) and CS-naïve 68.9 (18.6)%, ($p<0.01$) before the decline.

Mean age at absolute FVC peak was 11.2 (±3.4) years and was similar in CS-naïve and CS-treated irrespective of regimen. Absolute FVC value was not different according to CS

treatment vs no treatment and between the two regimens. It peaked up to 2.0 (±0.5) L in CS-daily, 2.2 (±0.6) L in CS-intermittent, 1.9 (±0.8) L in CS-naïve before declining. Patients on CS-daily are shorter than those on CS-intermittent due to known CS side effects on growth. The higher FVC % pred. observed in CS-daily is likely due to their lower denominator (height).

4. c. Time to events according to corticosteroid treatment

Fifty-two patients fell to FVC % pred. <50%. Twelve were CS-daily, 34 CS-intermittent, 6 CS-naïve. Median age (50%) at FVC % pred. <50% was 13.2 years in CS-naïve, lower than CS-daily (16.1 years, p<0.01) and CS-intermittent (16.3 years, p=0.001) but not different between regimens (p=0.86).

The median age (50%) at FVC % pred. <50% was significantly lower (p=0.04) in those treated with deflazacort compared to prednisolone (15.4 vs 16.8 years) HR 2.3, 95%CI (1.03, 5.31) (Figure 3).

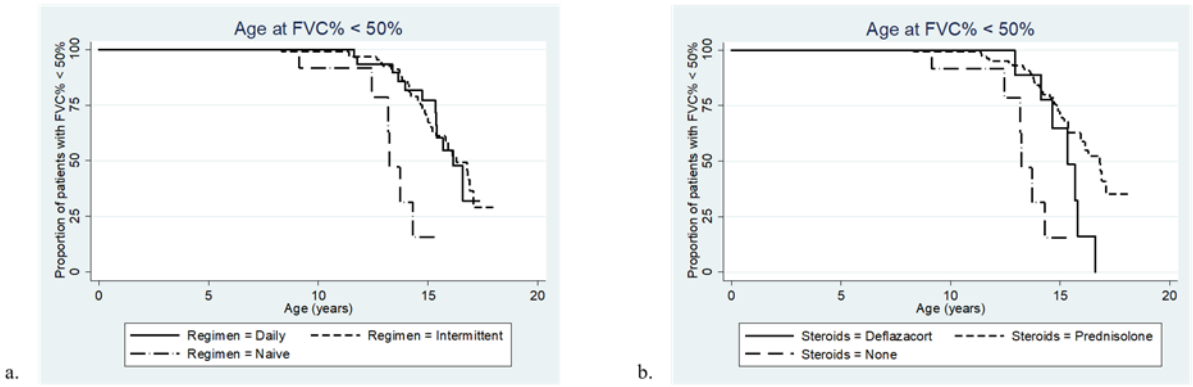


Figure 3. *Time to respiratory failure defined as FVC % pred. < 50% according to CS regimen and compound*

a. Time to reach FVC % pred. <50% according to regimen. Median age at FVC % pred. <50% was 13.2 years in CS-naïve patients. It was lower than CS-daily (16.1 years, p<0.01) and intermittent (16.3 years, p=0.001). Age at FVC % pred. <50% was similar between the two CS regimens (p=0.86).

b. Time to reach FVC % pred. <50% according to steroid compound. The median age at FVC % pred. <50% was significantly lower (p=0.04) in those treated with deflazacort compared to prednisolone (15.4 vs 16.8 years) HR 2.3, 95%CI(1.03, 5.31)

Subgroup analyses of respiratory failure combining regimen and steroid are presented. Prednisolone treatment, irrespective of its regimen, consistently has a stronger beneficial impact on respiratory function when compared to naïve patients.

Patients treated with deflazacort were less and this might explain the differences observed in its impact on respiratory function. Intermittent deflazacort had the lowest effect in delaying respiratory failure in our cohort (See Figure 4).

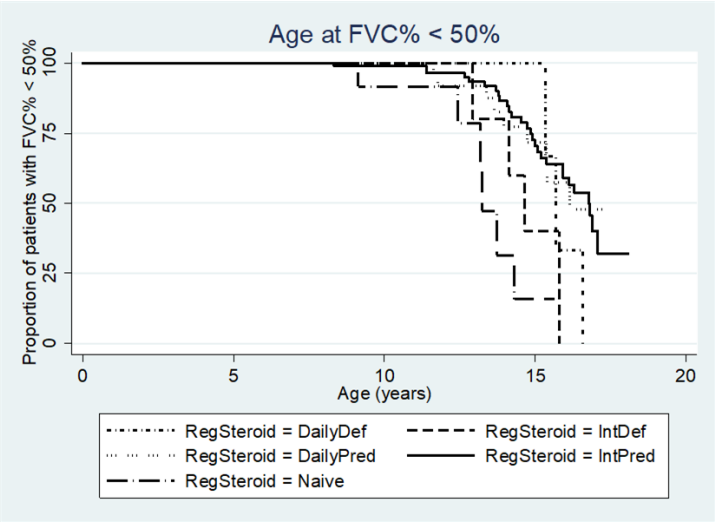


Figure 4. *Subgroup analyses of respiratory and cardiac time to respiratory failure and cardiomyopathy combining CS regimen and compound.*

The main respiratory endpoint was defined as FVC % pred. < 50%.

Absolute FVC fell <1 L in eleven of 293 patients (4%), two of 66 (3%) were CS-daily, 6 of 182 (3%) CS-intermittent and 3 of 22 (14%) were CS-naïve. CS-naïve patients reached absolute FVC<1 L at a median age of 17 years, earlier than those on CS-daily (p=0.04) and CS-intermittent (p=0.01) who fell below 1L after 18 years.

Twenty-one of 293 (7%) required NIV. Five of 66 (8%) were CS-daily, 12 of 182 (7%) CS-intermittent and 3 of 22 (14%) were CS-naïve. CS-naïve boys required NIV at a median age of

15.7 years, while less than 25% patients on any CS regimen required NIV by 18 years (Figure 5).

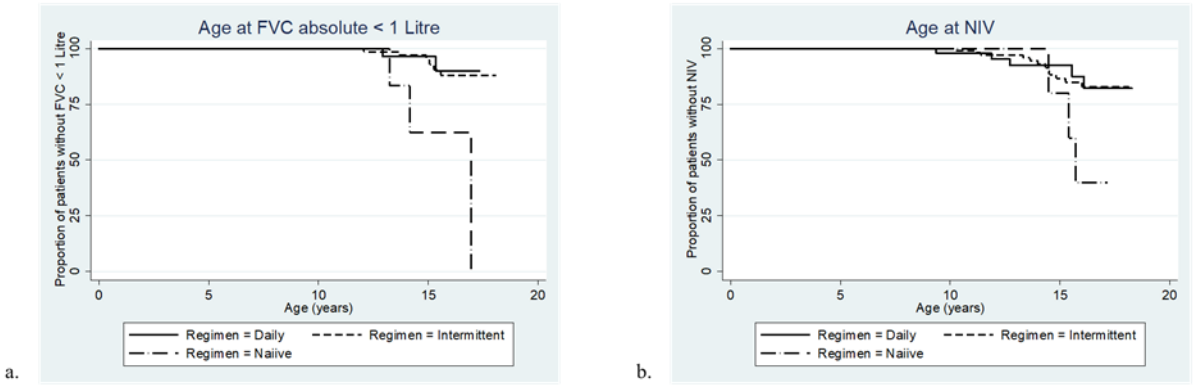


Figure 5. *Time to respiratory clinically meaningful endpoints, absolute FVC < 1 litre and non-invasive ventilation (NIV) requirement according to CS regimen.*

a. Time to reach absolute FVC < 1L. Eleven of 293 patients (4%) had absolute FVC <1 litre. Two of 66 (3%) were CS-daily, 6 of 182 (3%) CS-intermittent and 3 of 22 (14%) were CS-naïve. CS-naïve patients reached absolute FVC<1 L at a median age of 17 years, earlier than those on CS-daily (p=0.04) and CS-intermittent (p=0.01) who fell below 1L after 18 years.

b. Time to Non-Invasive ventilation (NIV) requirement. Twenty-one of 293 (7%) required NIV. Five of 66 (8%) were CS-daily, 12 of 182 (7%) CS-intermittent and 3 of 22 (14%) were CS-naïve. CS-naïve boys required NIV at a median age of 15.7 years, while less than 25% of patients on any CS regimen required NIV at 18 years of age.

4. d. Respiratory progression according to genotype

Children amenable to exon 44 skipping had a slower respiratory decline (4.5% per year) than patients not amenable to skip of exon 44 ($p<0.05$). Respiratory decline was not different in patients respectively amenable to skip 45, 51 and 53 compared to patients not amenable to skip of any of these exons.

Eighteen (6%) and 28 (9%) patients had mutations causing Dp71 and Dp116 isoform deficiency. FVC % pred. <50%, absolute FVC<1L, age at NIV and cardiomyopathy were similar in patients lacking Dp71 and Dp116 isoforms compared to the children expressing them.

5. DISCUSSION

Corticosteroids are the current standard mutation-independent treatment for DMD. They have shown a positive role on motor, cardiac and respiratory function when compared to steroid naïve patients. We have followed 293 patients over more than 5 years observation and up to the age of 18 years to understand the impact of each CS and its regimen on long-term respiratory function, which is currently unknown.

Firstly, our results have confirmed the beneficial effect of CS in delaying the onset of respiratory failure when compared to CS-naïve patients. The age at FVC % pred < 50% was 13.2 in CS-naïve and 16.1 CS-treated. The median (50%) age when CS-naïve had FVC fell <1 L was 17 years and when required NIV was 15.7 years. By the age of 18 years, none of CS-treated patients fell <1 L or required NIV.

The positive effect of CS, in keeping with previous observations [201], seems not to lie in the reduction of the yearly progression of lung function but in the increase of its baseline values, stressing the importance of early initiation of this therapy, before the initiation of the decline in function. In our work, the peak value FVC % pred. before the decline was significantly higher in patients on CS-daily and CS-intermittent than CS-naïve ($p<0.01$ and $p<0.05$). FVC % pred. in CS daily was ~10% higher than CS-intermittent and ~22% higher than CS-naïve. Importantly, when looking at the actual absolute FVC, i.e. removing the effect of height, patients CS-treated had higher peak volumes than CS-naïve, and CS-intermittent > CS-daily, yet not significantly. We can argue that one of the greater effects of steroids on respiratory function is the increase of lung function and it could be due to the positive effect they have in increasing the strength of diaphragm. We can postulate that CS positive effect on diaphragmatic function led to greater lung function [38] as at these early stages the effect of steroids in delaying scoliosis cannot explain the higher baseline lung volumes in CS-treated.

As opposed to previous works [106, 203], we have found that the age at peak of FVC % pred. and FVC did not differ between CS treated and CS naïve patients.

We confirmed that CS did not act by reducing the yearly decline of FVC % pred. when compared to CS-naïve patients. Most likely, the respiratory progression in DMD patients is affected by clinical variables only partially treated for by CS, as age, ambulatory and disease status when additional co-morbidities (poor swallowing, ineffective cough) affect lungs. This might explain the contrasting results obtained so far. Whilst in the age range 7-10 years FVC % pred. declined slower in CS-treated than CS-naïve (0.69% vs 5.9%), it was similar in boys aged 10-18 years (5.44% CS-treated vs 6.06% CS-naïve) [106] and in older, non-ambulant DMD young men [114, 201, 205].

As results of these factors, despite declining at a similar rate every year, steroid treated patients reached clinically meaningful endpoint later than naïve (without differences in age between CS-daily and CS-intermittent). CS-treatment led to a delay of FVC % pred. < 50% of 3 years and of NIV requirement of over 2 years irrespective of CS-regimen. CS delay the onset of respiratory decline and the achievement of respiratory milestones, but do not slow down its progression once decline is started [105, 106].

Interestingly, deflazacort, previously reported to have a better effect on ambulation in a long-term study [194] and on timed motor function outcomes in one-year observation studies [195, 196], seemed to not be able to maintain lung function as long as prednisolone in our cohort. We have shown for the first time that patients on deflazacort reached FVC % pred. <50% more than one year earlier than those on prednisolone (15.4 vs 16.8 years). It can be argued that the fact that most of the patients in these studies were on daily while most of ours were on intermittent deflazacort could partially explain the different results. We have shown that the CS regimen does not affect the progression. Our data suggests that deflazacort is effective on

short/medium term motor function but that the long-term effect on lung function could be affected by the more significant growth restriction caused by this corticosteroid compared to prednisolone [194].

In light of the differences we have observed in the differential response to CS treatment, we have explored underlying DMD genetic factors potentially able to predict different trajectories of respiratory impairment. The long-term respiratory progression according to patients' exon skipping amenability have implications in ongoing trials and will help the design of future studies. Patients amenable to skip of exon 44 had better walking distance and slower decline than others patients [174] and were shown to lose ambulation later [3, 176]. We add that they also have a slower respiratory function decline ($p<0.05$). Patients amenable for skipping exons 45, 51, 53 have a respiratory decline similar to the other patients. There were no significant differences in time to cardiomyopathy or respiratory failure in boys lacking Dp71 or Dp116 compared to those expressing them. We had hypothesized that mutations also affecting Dp71 could potentially have a protective role in dystrophic heart. The overexpression of AAV mediated-Dp71 worsened *mdx* mouse phenotype by competing with utrophin in its binding to dystrophin-associated protein complex [206]. Regarding the role of another shorter isoform, Dp116, on cardiac function, we could not replicate the previously reported protective role of Dp116 deficiency on heart function [181].

In our population 10.6% CS-daily, 24.2% CS-intermittent and 27.3% CS-naïve developed scoliosis. The small numbers of events did not allow a time-to-event analysis. The percentage of scoliosis in our CS-naïve is lower than previously reported [207] due to our more stringent definition, furthermore we did not consider scoliosis severity.

This work is the first describing the long-term cardiorespiratory progression in DMD and the role of two CS and their regimens. Previous studies have focused on motor function either

comparing deflazacort vs prednisolone or intermittent vs daily regimens. Some followed patients over one year, while our long-term real world data were collected within the North Star UK database. Local differences in CS dosage and regimens affects the conduct of large, long-term double-blind-controlled studies. The only ongoing randomised trial, the FOR DMD will address the question whether intermittent or daily CS regimens is more effective over five years. Patients included in the FOR DMD are 4-7 years of age thus they could still be too young to reach clinically meaningful cardiorespiratory endpoint. In this study, patients were followed over more than 5 years observation and up to the age of 18 years.

The main limitations of this study are its retrospective and monocentric design. We have only included assessments conducted in a single tertiary site by the same highly skilled operators to limit the risk of bias, which is however inevitable. We acknowledge the imbalance in cohorts sample size, with lower numbers of CS-naïve boys, as well as the presence of switchers might have affected our results.

6. CONCLUSIONS AND FUTURE STUDIES

Our work provides new insights on the long-term effect of corticosteroids on respiratory function in DMD. Irrespective of regimen, CS-treated boys had significantly higher FVC % pred. than CS-naïve and a similar yearly FVC % pred. decline. CS-treated boys reached clinically meaningful respiratory thresholds (FVC % pred. <50% and NIV requirement) later than CS-naïve patients. Further work is needed to evaluate the role of CS in older non-ambulant patients, particularly in view of the evidence for their positive effects on cardiac function, and to evaluate whether it is possible to find early predictors of early/late respiratory decline.

In light of the results of this and previous works, the evaluation of the rate of respiratory decline should take into account not only patients' age range but also their clinical motor status and the height and lung volumes. DMD standard of care have identified different respiratory morbidities according to the ambulatory status (early ambulant – late ambulant, early non-ambulant, late non-ambulant), and we feel further works on wide cohorts should focus on the mean peak FVC % pred. and FVC absolute and on the yearly respiratory progression within each of these phases of the disease. Similarly, since motor and respiratory progression match and are strictly related throughout the course of the disease, early motor predictors of increased risk of clinically meaningful respiratory milestones and of sleep disordered breathing (i.e. hypoventilation) would be clinically relevant.

Despite the respiratory progression in DMD is variable and affected by multiple clinical factors, we have been able to identify pattern of decline according to the site of mutation within DMD gene. Therefore, it is likely that gene modifiers for respiratory progression does exist and play an additional role and should be investigated in wide, homogeneous DMD cohorts.

Chapter 3

STANDARD AND NOVEL OUTCOME MEASURES FOR THE ASSESSMENT OF RESPIRATORY AND SLEEP ABNORMALITIES IN PAEDIATRIC NEUROMUSCULAR DISORDERS

1. BACKGROUND

1. a. Standard and new daytime respiratory assessments

Despite marked heterogeneity in severity and in their progression; patients with NMD almost invariably suffer from respiratory morbidity at some time. The British Thoracic Society (BTS) has issued general guidelines for the respiratory management of all children with NMD [10]. The following measurements are recommended at least yearly:

- Lung function. When lower than critical thresholds, FVC predicts the susceptibility to respiratory infections [17] and the occurrence of nocturnal hypoventilation [27]
- Respiratory muscle strength. MIP is a measure of inspiratory muscle strength. It is highly correlated with FVC, and thus predictive of SDB [27, 208, 209].
- Cough. PCF is measured during a cough manoeuvre using a facemask. A PCF > 270–300 L/min is considered sufficient to ensure a PCF>160 L/min during intercurrent infections. A PCF > 160 L/min is considered the critical threshold at which patients are able to effectively clear secretions [22, 23]. This PCF value is quite arbitrary and will vary depending on patient's general health and underlying NMD diagnosis. There are normal data available only for healthy children as presented in the introduction [24].

Different standards of care individualised for each group of condition (DMD, SMA, CMYO, CMD and CMS) take into account the specific features of each group [56, 69-71, 152, 210]. They are summarised below.

DMD guidelines suggest a more thorough assessment of respiratory function after the loss of ambulation, this milestone being strongly correlated with a more rapid decline in FVC [69] [211].

- In the ambulatory stage, annual monitoring of spirometry from 5-6 years of age is recommended
- In the non-ambulatory stage, seated absolute and predicted FVC (related to arm span [212] or ulnar length [213]), PCF, respiratory muscle strength tests and daytime SpO_2 are recommended six monthly

Respiratory management in SMA is strongly dependent on its subtype [70].

- SMA type 1. Clinical status, daytime SpO_2 (and CO_2) monitoring should be carried out every 3 months. Manual chest physiotherapy combined with assisted cough should be introduced pro-actively.
- SMA types 2 and 3. All patients able to perform spirometry should be tested every 6 months.

Currently there are no guidelines specifically tailored for CMYO, CMD and CMS due to their heterogeneity and lack of data [71, 152]. In these group of patients experts suggested assessing upright and supine spirometry, and cough and respiratory muscle strength tests every 6 months. Chest x-ray, spot SpO_2 with or without CO_2, are indicated at diagnosis and every 12-24 months or earlier if clinical symptomatic with intercurrent respiratory infections. Patients with CMS, especially those caused by *CHAT* and *RAPSN* mutations have the additional risk of sudden rapid decompensation. Parents need to be aware of the warning signs indicating respiratory crises such as desaturations and apnoeas.

The assessment of respiratory function in weak non-cooperative children in clinical settings is difficult. Multiple measures have been explored [152].

MIP and MEP can be performed in infants during crying. There are normative data from healthy children 1 month to two years old (MIP 118±21 cmH_2O and MEP 125±35 cmH_2O), this is not a test usually performed in clinical settings [214].

Opto-electronic plethysmography (OEP) is a system capturing breath-by-breath, three-dimensional, real time assessment of absolute chest wall volumes, and the variations in the compartments of the chest wall (rib cage and abdomen) using markers placed on the skin and special cameras. It is highly reliable in assessing breathing pattern in infants when compared to pneumotachograph [215] The assessment of breathing pattern recorded by OEP was feasible in infants with SMA type 1. It showed that children with SMA type 1 treated with intrathecal nusinersen had a differential extent of improvement in their breathing volumes and a reduction of thoraco-abdominal asynchrony according to their clinical severity. SMA type 1 A-B patients (onset within the third month of life) did not significantly differ from untreated patients while SMA type 1 C patients (onset between three and six months of life) were the best responders [29, 132].

Forced Oscillation technique (FOT) is based on the application of pressure oscillations via a mouthpiece during quiet breathing. It measures the changes in airway pressures and flows and calculates airway resistance amongst other parameters. It was feasible in children with SMA type 1 as young as 3 years [216].

The above techniques, despite being promising, are not easily applicable on a regular clinical basis in the assessment of infants or very weak children.

1. b. Standard overnight assessments

Indications for sleep studies

When to perform a sleep study in children with NMD is currently still unresolved. There is also no consensus about which sleep study to perform for maximal information and minimal invasiveness.

BTS guidelines suggest oximetry as the first step. Scoring systems to stratify the severity of oximetry have been mostly utilised in healthy children [217, 218].

NMD children with abnormal overnight oximetry should undergo more detailed sleep monitoring with at least capnography to look for nocturnal hypoventilation.

Either a full PSG or a CR Polygraphy should be performed, according to availability, in case of doubt about the cause of SDB [10].

BTS guidelines suggest starting sleep assessments and carry them out at least annually in

- children with FVC<60% who have lost ambulation or who never walked
- infants with muscle weakness
- children with signs and/or symptoms of obstructive sleep apnoea or hypoventilation
- children with clinically apparent diaphragmatic weakness
- children with rigid spine syndrome

Sleep studies should be carried out more frequently in

- young children whose rate of disease progression is uncertain
- children who have shown clinical deterioration
- children suffering from recurrent respiratory infections
- children with symptoms of SDB

These patients should be referred to a respiratory physician early and interventions are started based on multidisciplinary assessment of nutrition, swallowing, risk of respiratory infections and pattern of breathing [210].

BTS guidelines and standards of care for DMD and SMA type 2 and 3 suggest starting NIV either in presence of symptomatic nocturnal hypoventilation such as morning headache, morning nausea, daytime sleepiness or in case of daytime hypercapnia [69, 70]. For young non-verbal children and/or for those belonging to other, rarer, diagnostic groups the indications for a sleep assessment are mainly based on the clinical judgement. Low SpO_2, increased work of breathing, apnoea and increased secretions are usually red flags for clinicians.

Hypoventilation and indications for NIV requirement

Hypoventilation is defined as shallow and slow breathing, resulting in a retention of CO_2.

As discussed in the Introduction there is no consensus about the definition of hypoventilation and its severity. The prevalence of hypoventilation ranges from 10.3 % to 61.2 % according to its definition and to patients' age and underlying disease [66, 219]. The current definition of hypoventilation much widely used in children is $PaCO_2$ 50 mmHg > 25% total sleep time [65].

The right timing to start treating hypoventilation with NIV in NMD children is also still unclear [77]. A recent retrospective study evaluated the criteria that prompted clinicians to start CPAP/NIV in children with different underlying conditions. In those who were initiated irrespective of daytime symptoms the most frequent overnight gas exchange abnormalities were heterogeneous (minimum SpO_2<90%, maximum $tcPaCO_2$ >50mmHg, SpO_2 <90% or $tcPaCO_2$ >50mmHg ≥2% total sleep time, ODI>1.4/hr) and were generally less stringent than those suggested by guidelines [97].

The existing criteria to start NIV are summarised in Table 1.

Diagnostic group	Criteria to start NIV	Ref
DMD	Daytime tests - FVC <50% predicted - MIP < 60 cmH_2O - Et/tc $PaCO_2$>45 mmHg - Blood pCO2 >45 mmHg - SpO_2 < 95% in room air or Overnight tests - Et/tcPaCO2 >50 mmHg ≥2% TST - Et/tcPaCO2 10 mmHg above the awake baseline ≥2% TST - SpO2 ≤88% ≥2% total sleep time or ≥5 minutes consecutively - AHI≥5/h In the presence of symptoms of nocturnal hypoventilation	[69]
SMA	Daytime respiratory failure Or Sleep disordered breathing In presence of symptoms of nocturnal hypoventilation	[70]
CMYO, CMD	Overnight tests - Minimum SpO_2 <90% > 2% TST and/or >5 minutes of TST - Mean SpO_2 <94% - ODI (desaturations>3%) >5 events/hour. - Maximum $tcPaCO_2$ > 50 mmHg > 2% TST or > 5 minutes TST - Et/$tcPaCO_2$ >10 mmHg from baseline awake level - Repeated elevated et/$tcPaCO_2$>5 mmHg between NREM - $EtPaCO_2$ >45 mmHg > 25% of TST - AHI >5 events/hour - REM AHI >10 events/hour Alteration of Sleep structure - Sleep efficiency <75% - REM sleep <15% TST ± symptoms of nocturnal hypoventilation	[71]
CMS	Nocturnal hypoventilation High risk of recurrent apnoea	[56]

Table 1. Criteria for starting NIV in different NMD diagnostic groups according to the current standard of cares or to opinion of experts.

TST: total sleep time; **$EtPaCO_2$:** end tidal partial pressure of carbon dioxide; **$TcPaCO_2$:** trans-cutaneous partial pressure of carbon dioxide

The following sections of this chapter are made of three separate projects.

The first is aimed to assess the feasibility of a novel assessment of breathing pattern and respiratory muscle strength in SMA type 1.

The second and third assess whether existing assessment of overnight altered gas exchange would be similarly applicable in children with associated medical conditions and in healthy children.

2. PROJECT 1 - NON-INVASIVE ASSESSMENT OF RESPIRATORY PATTERN IN SPINAL MUSCULAR ATROPHY TYPE 1 BY STRUCTURED LIGHT PLETHYSMOGRAPHY

2. a. Introduction

SMA type 1, as described in the introduction, is characterized by severe early respiratory failure classically leading to death by two years of age [120]. Currently, the natural history of the disease has changed thanks to new treatments that have improved motor and respiratory function leading to a prolonged survival [70, 122-124, 129, 220]. However, the actual rate of improvement after treatment has been calculated only indirectly based on patients' ventilator requirement. The young age and disease severity of children with SMA type 1 have limited the availability of respiratory outcome measures. They would be useful to measure longitudinally the improvement of respiratory muscle strength in children with SMA type 1.

Structured Light Plethysmography (SLP) is a portable non-invasive, light-based method of assessing patients' breathing pattern. It measures thoraco-abdominal wall movements by projecting a grid of light onto the anterior thoraco-abdominal wall. The axial displacement of the light grid, recorded by two digital video cameras in its scanning head, measures the thoraco-abdominal movement during tidal breathing over a set time (up to 5 minutes). Post-processing analyses of the SLP built-in software provides the following outputs: respiratory rate and inspiratory, expiratory and total breathing times and breathing pattern indexes (i.e. contribution of chest and abdomen to each breath and degree of thoraco-abdominal synchrony) [221]. SLP is feasible in non-cooperative young children [222] and was able to detect different respiratory pattern features in children with asthma when compared to healthy controls [223]. A recent study has demonstrated the feasibility of SLP in children with neuromuscular disorders. SLP was able to detect disease specific features of the breathing pattern of some group of disorders,

such as a reduced abdominal contribution to tidal volume in Duchenne Muscular Dystrophy [40].

2. b. Hypothesis and aims

The hypothesis of this work was that the breathing pattern of infant and children with SMA type 1, captured via SLP, would reflect their clinical status.

Main aim was to determine whether SLP would capture the classical respiratory features of young children with SMA type 1. The secondary aim was to correlate the breathing pattern grade of abnormality captured by SLP and the motor severity assessed by standardized clinical scales.

2. c. Patients and methods

Study population

Prospective cross-sectional study on consecutive children with SMA type 1 referred to the Paediatric Neurology and Muscle Disease Unit, Istituto Giannina Gaslini, Genoa from June 2016 to May 2017. The study protocol was approved by the Regional Ethic Committee – San Martino Hospital, Genoa (REC 377REG2015) and was carried out according to the Helsinki Declaration. Verbal consent was obtained from parents/caregivers of all children and documented in the patients' clinical note before enrolment.

Children (age <18 years) with a confirmed genetic diagnosis of SMA and consistent clinical features, onset of symptoms before 6 months of age and inability to sit unsupported, who were on ventilatory support less than 24 hours/day were included. Patients who were acutely unwell at the time of recording were excluded. We also excluded children with severe scoliosis, which could have affected the SLP partitioning of thoraco-abdominal quadrants.

Study protocol

All patients underwent a standardised protocol of assessments to ensure consistency. Anthropometrics (height, weight, chest circumference) were collected first by the same paediatric nurse. Secondly, the same operator carried out a five-minute SLP recording on supine patients during spontaneous breathing. Finally, the same trained and certified neuromuscular physiotherapist performed motor assessments consisting of Hammersmith Infant Neurological Examination (HINE) and Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND).

Anthropometrics and clinical features

Patients' length in the supine position with a stadiometer and weight were measured as per routine clinical assessment. Length (cm), weight (kg) and BMI (m/kg2) were expressed as absolute value and percentiles based on the World Health Organization (WHO) infant charts for 0-2 year old and for >2 year old children. Chest circumference was measured at the level of the nipples.

All patients were asked what regular respiratory physiotherapy were performing at home, whether the cough assist device was used at home, the type of respiratory support (invasive vs non-invasive) and hours of respiratory support per day. Feeding support information, namely use of gastrostomy, was collected.

The respiratory rate for each patient was assessed manually and by SLP and was compared to the normal respiratory rate published on age-matched healthy children [224].

SLP recording of respiratory pattern

The assessment of the breathing pattern was carried out with SLP PneumaCare Thora3DI® device (Cambridgeshire, UK) combined with the light projector Thora 3DI®. The child's breathing pattern was measured using a grid of light, tracked by a digital vision system of two high-speed cameras. All recordings were carried out with children bare chested. The grid

pattern projected could be adjusted to accommodate the size of the thoraco-abdominal region for all patients. Three SLP grid sizes were available (small, medium and large) according to the number of squares projected on the chest and abdomen of the patients (14 × 10, 12 × 8, 10 × 6). Each square of the grid contributed equally to the signal. In order to ensure consistency of recording, we used the medium SLP grid size. This size best covered the thoraco-abdominal area of all patients. The SLP grid was centred on the patients' xiphoid process and its position was maintained throughout the recording. We adjusted the SLP projector in order to consistently capture the same regions in all patients. We considered "chest", the region from the clavicles to the xiphoid process and "abdomen" the region from the xiphoid process to the anterior iliac crests.

Patients were laid at a distance of up to 1 metre from the SLP projector. Sampling rate was 30 Hz. (Figure 1).

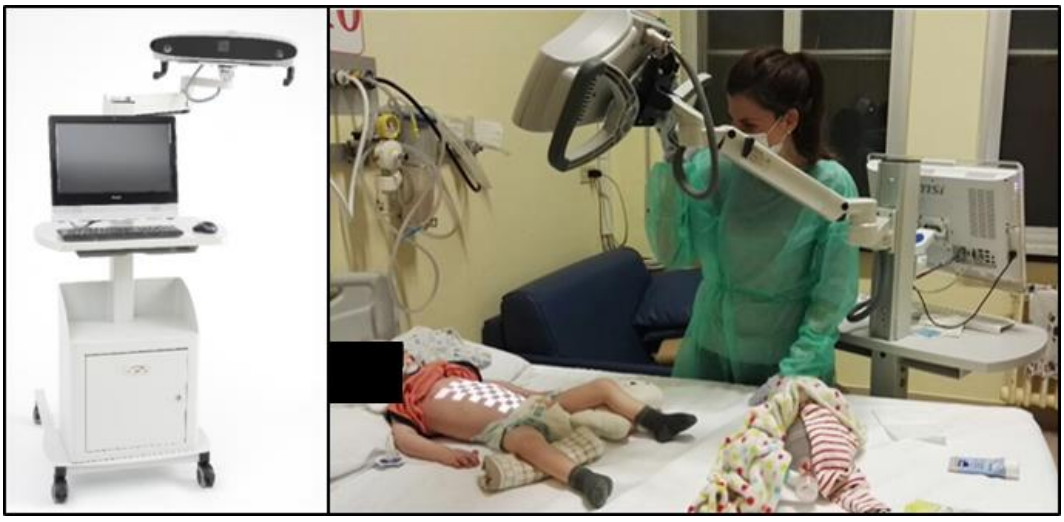


Figure 1. *PneumaCare Thora3DI System device and acquisition of respiratory pattern in a child with SMA type 1. SLP projects a grid of light onto the thoraco–abdominal (TA) wall of a participant. The grid is centred on the patients' xiphoid process. Changes in the grid pattern are recorded by video cameras in the scanning head.*

Immediately after the recording, the quality of traces was checked. If the recording was deemed suitable for analysis, it was saved and stored in the system for processing. The sixty seconds better representative of each patient breathing pattern were selected.

The data gathered was analysed via the built-in software (PneumaView™ 3D). PneumaView™ 3D provides a virtual static and dynamic reconstruction of the surface of the thoraco-abdominal wall of the patient. The average axial displacement of the light grid on patient's chest and abdomen within the recorded period provided a graphical representation of breathing pattern with chest and abdomen breathing movements represented as sine waves (Figure 2).

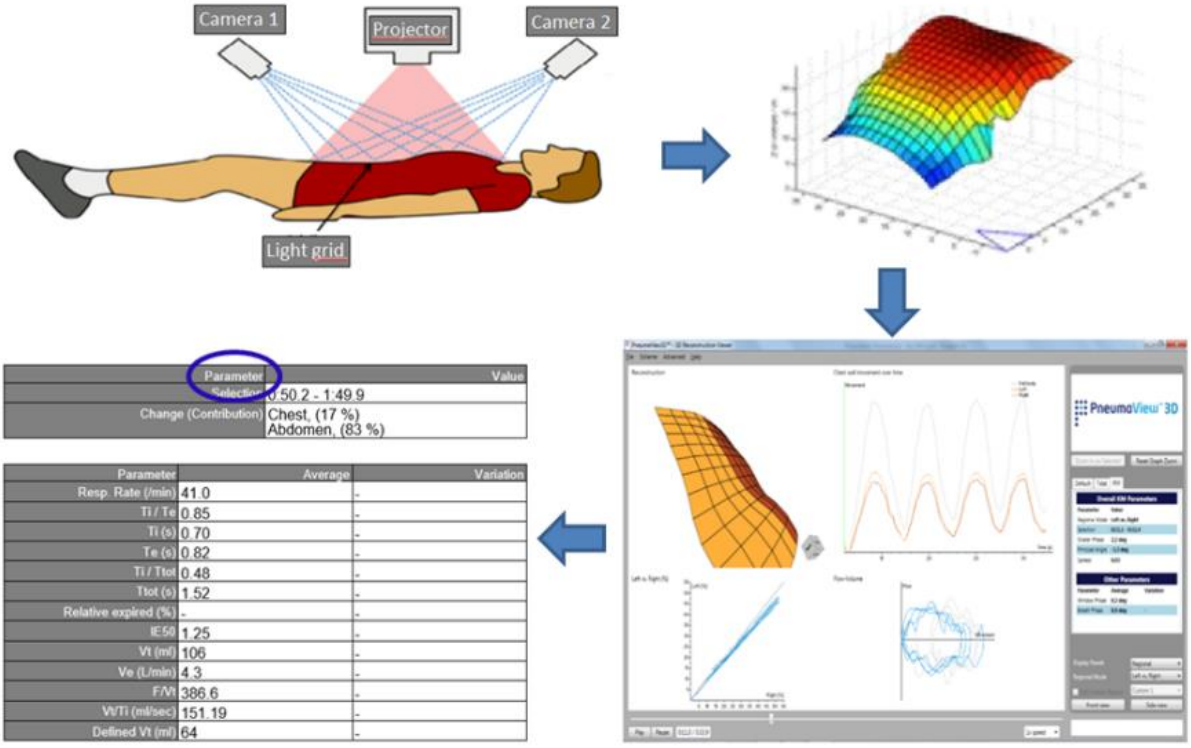


Figure 2. *Data acquisition, recording and processing via PneumaView 3D.*

The PneumaCare Thora3DI projected a light grid on the chest of the patient lying in supine position (top left). The displacement of the thoraco-abdominal light grid was three-dimensionally reconstructed by the PneumaView 3D (top right). After the recording, the PneumaView 3D provided a visual (bottom right) and numeric (bottom left) output of the information available for the analyses.

The synchrony of chest and abdomen during each breath within the recorded time was expressed as the phase angle (overall phase). This was defined as the phase difference between the chest and abdominal traces. The displacement of each region (either chest or abdomen) is plotted against that of the other to measure asynchrony. The shape of the graph, called Konno-Mead (KM) is used to indicate the magnitude of asynchrony (see Figure 3 a). It expresses the extent of synchrony of the movements of the chest and abdomen during each breath throughout the recording. When chest and abdomen move in perfect synchrony the KM loop is flat and the phase angle is zero (i.e. there is no difference in the movement phase of chest and abdomen and both are in inspiration or expiration at the same time). As asynchrony appears the KM loop opens, while complete asynchrony (paradoxical movements) results in a flat KM loop and a phase angle of 180° (i.e. the movements of chest and abdomen are completely out of phase and one region is in expiration when the other is in expiration and vice-versa)[225].

Thoraco-abdominal asynchrony was defined as 0°<Phase Angle<180° and paradoxical breathing as Phase Angle =180°.

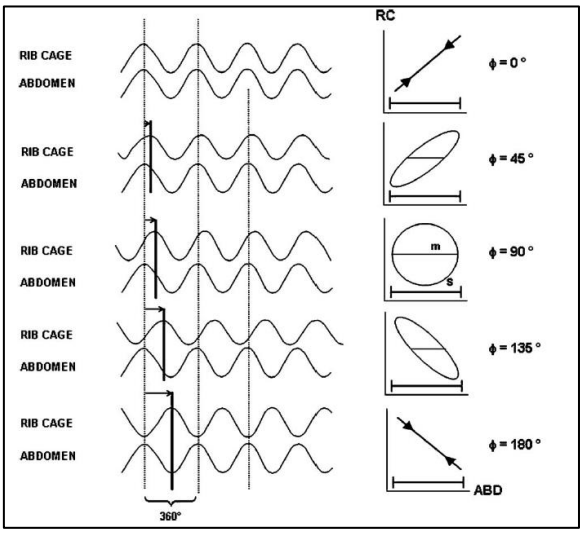


Figure 3. *Phase angle analysis and rib cage and abdominal synchrony.*

Rib cage (RC) and abdominal (ABD) motions are treated as sine waves. When RC and ABD move in synchrony (top) the phase angle is zero. As asynchrony appears, the loop opens (middle). Complete asynchrony (paradoxical movements) results in a phase angle of 180° (bottom). Adapted from Hammer J. et al. Paed. Resp. Rev. 2009.

The contribution of chest and abdomen to the breathing movements within the recorded time was expressed as:

a. The relative expired chest % and relative expired abdomen %. Considering the thoraco-abdominal region as being composed of two compartments i.e. chest and abdomen, the relative contribution of any compartment can be quantified as percentage of the total displacement. Relative expired chest % and relative expired abdomen % are defined as the ratio of respectively chest and abdominal range of movement during expiration to the total thoraco-abdominal range of movement during expiration, averaged over all breaths recorded.

b. The principal angle (PA). It was defined as the angle of the principal, dominant, axis of the KM plot relative to the 45-degree line. It provides information similar to relative expired %, but compares the contribution of two regions to the whole breath, not just in expiration. It has the advantage of being graphically summarised by the KM plot, therefore it provides immediate information to the operator regarding the main contribution of each region to each breath (Figure 4 a). In our work we have considered the PA between chest and abdomen contribution to breath. In this case, a positive angle indicates chest dominant breathing [225].

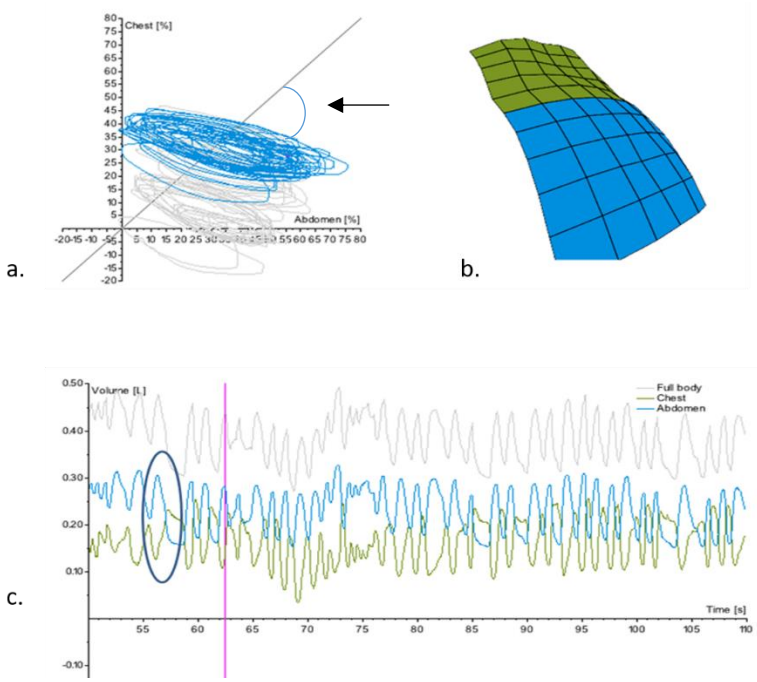


Figure 4. *Visual output of a patient's breathing pattern provided by PneumaView™ 3D*

a. Konno-Mead loop showing the prevalent contribution (percentage) of abdomen versus chest to each recorded breath. When the contribution is equally distributed, the main axis of the loop is parallel to 45° and the Principal angle is 0°.

In the loop represented here the Principal angle (the angle between the 45° line and the principal axis of the Konno-Mead curve) is negative (arrow)

b. Bi-dimensional reconstruction of the patient's chest (green) and abdomen (blue) during quiet breathing.

c. Sine wave representation of chest (green) and abdominal (blue) motion during quiet breathing. The breathing pattern is characterised by a thoraco-abdominal phase shift and paradoxical breathing (blue circle).

The absolute numerical values of respiratory times and rates were expressed as

a. The respiratory rate (RR) was expressed as breaths per minute.

b. The Ti/Te (inspiratory-expiratory breathing time ratio) was defined as the ratio of Ti, the inspiratory time of a breath, to Te, the expiratory time of a breath, averaged over all breaths in the selected recording.

c. The Ti/Ttot (inspiratory-total breathing time ratio) was defined as the ratio of Ti, the inspiratory time of a breath, to Ttot, the total cycle time of a breath (from start of inhalation to end of exhalation), averaged over all breaths in the selected time period.

Volumetric measurements were not a direct output of SLP, this being two-dimensional. An estimate Vt of 8 ml/kg based on Vt of healthy children was entered manually based on patient's weight before each recording. The software provided an estimated average patients' Vt within the selected time period. We calculated the Rapid-Shallow Breathing Index (RSBi) as the ratio of RR to SLP Vt per patient' weight (breath/min/ml/kg) [226].

Motor assessments

Motor assessments consisted of the Hammersmith Infant Neurological Examination (HINE) which assesses motor developmental milestones and the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND). HINE adapted for neuromuscular infants is a validated outcome measure taking into account global tone and posture and gives overall information on motor milestones [227]

CHOP-INTEND is a global motor function assessment created *ad hoc* for children with SMA, which was validated in 2011. It consists of 16 motor items (upper and lower limbs movements, rolling, head control, axial tone) which can be scored from 0-4. A separate score for right and left side is recorded for all the items. CHOP-INTEND overall score, the maximum of which is 64, consists of the sum of the best scores for each item [228, 229].

Statistical analyses

Statistical analyses was performed using IBM SPSS® version 24. Descriptive statistics were used to describe study the population features and anthropometrics. Given the relatively small sample size, non-parametric analyses were used. Descriptive data were expressed as median and interquartile range (25^{th} – 75^{th} centile). We reported the median (IQR) value of Respiratory rate, Ti, Te, Ttot, Ti/Te, Ti/Ttot, Vt, Relative Expired Abdomen (%), Relative Expired Chest (%), Principal angle (°) and Phase Angle (°) and RSBi of our study population.

Wilcoxon test was used to compare the patients' respiratory rates with those of age-matched normal data. Finally, the correlations between respiratory and both clinical variables/anthropometrics and motor function assessments were performed by Spearman rank correlation.

2. d. Results

Patients' characteristics

Nineteen children with SMA type 1 were evaluated, 13 male (68%).

N=19	**N (%)**	**Median (IQR)**	**WHO percentile**
Male	13 (68%)		
Age (years)		2.3 (1.3-7.9)	
Age of onset (years)		0.3 (0.2-0.4)	
Age at diagnosis (years)		0.6 (0.3-0.9)	
Height (cm)	16	89.5 (75-118.3)	9^{th} – 25^{th} percentile
Weight (kg)	16	10.2 (7.2-17.9)	< 3^{rd} percentile
BMI (m/kg2)	16	13.2 (11.5-14.7)	< 3^{rd} percentile
Chest circumference (cm)	11	47.5 (41-57.5)	
Use of cough assist (y)	19 (100%)		
Age starting ventilatory support (months)		10.5 (5.5-24)	
Ventilatory support	17 (89%)		
- **Invasive**	4		
- **Non-Invasive**	13		
Ventilation requirement			
- **1-8 hours/day**	6		
- **9-16 hours/day**	6		
- **17-23 hours/day**	5		
Orthopaedic features			
- **Mild scoliosis (y)**	10 (53%)		
- **Joint contractures**	14 (74%)		
Feeding			
- **Oral**	6		
- **Nasogastric tube**	3		
- **Age at NG (months)**		9 (5-14.5)	
- **Gastrostomy**	10		
- **Age at gastrostomy (months)**		23 (11-26)	
Comorbidities			
- **Interatrial septum defect**	2		
- **Pre-term birth**	2		
- **Adenotonsillar hypertrophy**	1		
Concomitant oral salbutamol	4		
CHOP-INTEND at assessment	15	32 (20-34)	
HINE at assessment	16	52 (46-57)	

Table 2. Clinical features of the study population

Respiratory pattern acquired by SLP

The breathing pattern captured by SLP in this cohort was consistent with that previously described in SMA type 1. The three main features observed were a median increased respiratory rate with preserved inspiratory/expiratory time, an increased contribution to the breathing of abdominal compartment relative to the chest and chest-abdomen asynchrony during breathing. A summary of the output recorded from one-minute post-recording is presented in Table 4.

Increased respiratory rate.

The median RR was higher than reference value for age-matched healthy children [224]. The ratio between Ti and Te was normal.

Increased abdominal contribution to breathing.

The abdominal contribution to whole breathing in age-matched healthy children was found to be 59% [224]. In our cohort, the median (IQR) relative expired abdomen % was higher, being 77% (68 - 90).

There was a strong negative correlation between the relative expired abdomen % and the principal angle ($r=-0.8$, $p<0.01$). A negative principal angle is indeed the expression of an increased abdominal contribution to each breath (Figure 6). In light of their good correlation, only one of the two needs to be used to determine regional contribution to breathing.

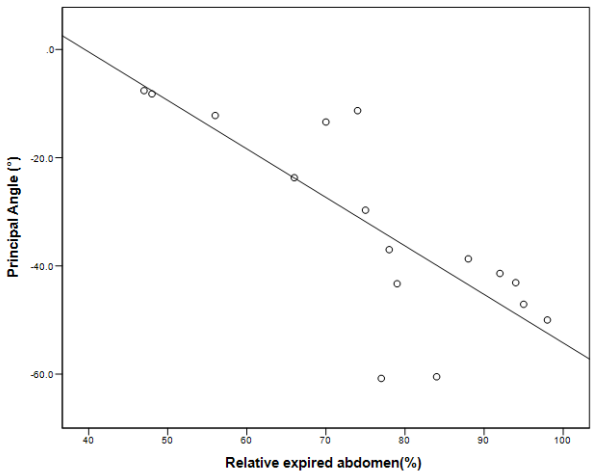


Figure 5. *Negative correlation between relative expired abdomen % and principal angle r=-0.8, p<0.01.*

A negative principal angle, visually described as the main axis of the Konno-Mead loop sitting below the 45° line, is a parameter of abdominal>chest contribution to the breath. In our cohort of SMA 1 children, there was an increase of relative expired abdomen % and a tendency towards a more negative principal angle.

Chest-abdomen asynchrony.

The median phase angle was 48.7° (41.6-63.2), higher than reference values for healthy children (11.8°) [224]. All patients in our cohort had a phase angle above 0°. The phase angle ranged from a lowest of 34.3° to highest of 144.4°. None had full paradoxical breathing.

As expected, there was a negative correlation between phase angle and principal angle ($r=-0.6$; $p<0.05$). This confirms that the increased abdominal contribution to each breath (SLP principal angle absolute value) is associated with a higher chest-abdomen asynchrony, as expressed by SLP phase angle (Figure 7).

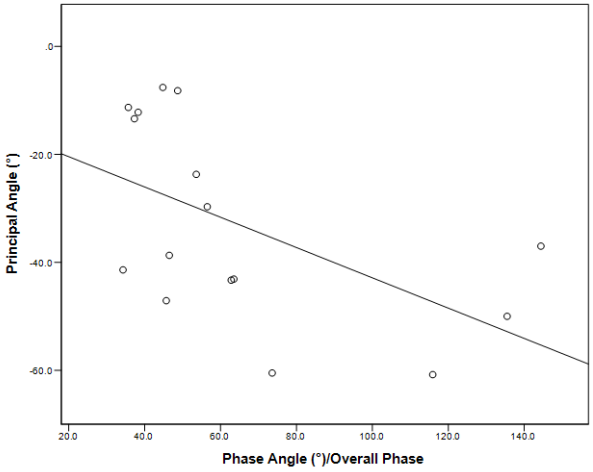


Figure 6. *Correlation between principal angle and phase angle (overall phase) r=-0.6, p=0.02.*

A negative phase angle, associated with a higher abdominal contribution to the breathing, was associated with a higher chest-abdomen asynchrony. This confirms the known breathing pattern in SMA type 1 characterised by reduced movements of intercostal muscle with an associated increase of abdominal effort.

SLP Parameters	Median (IQR)	Reference Value (healthy subjects)	Respiratory Pattern
Respiratory rate (bpm)	33.5 (26.6 - 41.7)	27 CI 95% (26-28) age 2.3 [224]	Increased RR
Ti (s)	0.8 (0.6 - 1)		
Te (s)	1.1 (0.9 - 1.4)		
Ttot (s)	1.9 (1.5 - 2.3)		
Ti/Te	0.4 (0.4 - 0.8)		Normal inhalation/exhalation time ratio
Ti/Ttot	0.4 (0.4 - 0.5)	Ti:Te=1:2	Normal inhalation/total time ratio
Vt (ml) pre-imposed (8ml/kg)	79.0 (57 - 140.5)		
Vt (ml)*	95.5 (56.2 - 120.3)		
Vt (ml)/Vt (ml) pre-imposed (%)	94 (75.6 - 117.6)		
RSBi (RR/Vt (l))*	342.1 (236.5 - 457.9)		Rapid shallow breathing
RSBi (RR/ml/kg)*	3.9 (3.2 - 5.4)		
Relative Expired Abdomen (%)	77 (68 - 90)		Prevalent diaphragmatic component physiological in supine position
Relative Expired Chest (%)	23 (10 - 32)		
Chest contribution to Vt (%)		59 CI 95% (45.4-72.6) age 2.3 [224]	
Principal angle (°)	-37 (-43.2 - -12.8)		
Phase Angle (°)	48.7 (41.6 - 63.2)	11.8 CI 95% (10.2 – 13.4) age2.9 [224]	Increased thoraco-abdominal asynchrony

*Vt (l) is the output of SLP calculated on the average breaths within the recorded time, after entering a pre-imposed standard Vt of 8ml/patients'weight in kg.

Table 3. Summary of respiratory parameters acquired by one-minute SLP recording, reference values per age range (Balasubramaniam et al. Paed Resp Rev 2019) and their clinical meaning

Correlation between respiratory pattern and clinical status

The three main features of of the breathing pattern captured in our patients were correlated with their clinical and motor status.

The rapid shallow breathing index, RSBi, strongly negatively correlated with patients chest circumference ($r=-0.7$, $p<0.01$). This correlation demonstrates the compensatory ventilatory mechanism for rib cage narrowing and restrictive lung impairment typical of SMA type 1.The RSBi did not correlate with the ventilator requirement expressed as hours of ventilation per day.

The relative expired abdomen % was higher in patients with higher RR, higher number of hours of ventilator requirement and with smaller chest circumference. Nevertheless, no significant correlations were observed likely due to the low power of our sample. Respiratory rate, RSBi and relative expired abdomen % obtained by SLP did not correlate-with either CHOP-INTEND or HINE. The phase angle strongly negatively correlated with CHOP-INTEND ($r=-0.8$, $p<0.01$) but not with HINE score likely due to the higher sensitivity of the former to detect changes in the motor function of children with SMA type 1.

2. e. Discussion

This work is the first attempt to use SLP in the assessment of respiratory pattern in a population of infants and young children with SMA type 1. It showed that SLP captured breathing traces of all the children enrolled, as young as 3 months of age. It was well tolerated and allowed the simultaneous recording of multiple respiratory parameters. Rapid, predominantly abdominal breathing and chest-abdomen asynchrony characterized the respiratory pattern captured by SLP in our patients. All three are well-recognised hallmarks of SMA type 1. We also showed that rate of thoraco-abdominal asynchrony captured by SLP in our cohort negatively correlated with motor function assessed via CHOP-INTEND.

This study has shown that a non-invasive, user-friendly tool can be added to the clinical respiratory assessment of young non-cooperative children. The clinical, respiratory and motor aspects of all the children in our cohort were characterised, and SLP outputs were correlated with all those aspects in all patients in the cohort. Despite its novelty and its prospective design, the main weakness of this study is the relatively small sample size and the lack of an age-matched control population. Further longitudinal studies are required to assess the clinical utility of SLP and whether it adds value to standard assessments.

Recent cross-sectional studies have evaluated new tools for the assessment of respiratory function in children with SMA type 1. Lo Mauro et al. [29] assessed the breathing pattern 1 using Opto-Electronic Plethysmograpy (OEP). In keeping with their findings, these results confirmed the shallow and paradoxical breathing with a reduced chest contribution to each breath as respiratory features of SMA type 1. In this work, the patients' respiratory pattern correlated with the extent of ribcage restriction, expressed as chest circumference, and the ventilatory requirement per day. While OEP is a valuable tool for research purposes, it is not feasible in many clinical settings being not portable. OEP set-up requires a dedicated room equipped with infrared cameras in four corners.

Finkel et al. [230] evaluated several invasive and non-invasive respiratory function tests on seven children with SMA type 1 younger than six months of age. They assessed patients' thoraco-abdominal asynchrony via respiratory inductance plethysmography. Consistently with the results of this work, six of the seven subjects had a phase angle above the normal range of 9±3° to 16±10° in healthy infants. Five of the seven had nearly complete paradoxical thoraco-abdominal motion with phase angles of >140°.

Lung Clearance Index (LCI), forced oscillation technique (FOT) and overnight full PSG have been tested as predictors of NIV requirement in children with SMA type 1 [216]. FOT, but not LCI, was feasible in children with SMA (3 patients SMA type 1) as young as 3 years. LCI

reflects lung ventilation inhomogeneity using inert gas washout [231, 232]. In this cohort, 11 out of 19 children were younger than 3 years. PSG is able to assess the presence of SDB and paradoxical breathing in children with SMA type 1. However, it usually requires a hospital overnight stay.

SLP showed a reduction of chest movement and predominant abdominal expansion consistent with the known atrophy of intercostal muscles associated with sparing of the diaphragm in SMA. Children with the narrowest (bell-shaped) chest had the highest shallow breathing rates. This result confirms that the breathing at low tidal volumes in these patients is compensated by increased respiratory rate. Children with a lower abdominal contribution to breathing, in keeping with a better preserved intercostal muscle function, had a trend toward a lower requirement of ventilation per day. The correlation between the SLP phase angle, as an index of chest-abdominal asynchrony, and motor function, measured by CHOP INTEND, confirmed in this cohort that children with better motor function also have stronger intercostal respiratory muscle.

Although the reliability and repeatability of SLP still needs to be tested in a much larger population, we have shown that this new tool is feasible in a clinical setting and can be carried out relatively easily even in severely affected infants and children. SLP could be used both in clinical and in research settings. For example, it could be a useful outcome measure in the context of clinical trials. Prospective studies in a larger cohort of patients with SMA type 1 need to be carried out to assess the effects of the new therapies available (such as Nusinersen) on respiratory function. Additionally, SLP could be added to longer observational studies on intermediate SMA (type 2 and 3) as a tool to compare their differential respiratory patterns and progression over time.

3. PROJECT 2 - THE McGILL SCORE AS A SCREENING TEST FOR OBSTRUCTIVE SLEEP DISORDERED BREATHING IN CHILDREN WITH CO-MORBIDITIES

3. a. Introduction

The McGill score is used to stratify the severity of OSA in children and thus to prioritize their treatment [217]. It ranges from 1 (normal or inconclusive) to 4 (severely abnormal) according to the clusters of desaturation events seen in overnight oximetry. Previous work on otherwise healthy children has shown that oximetry, when compared to PSG, has a high positive predictive value (PPV) and a low negative predictive value (NPV) in the detection of OSA [233]. The current European Respiratory Society and American Academy of Pediatrics guidelines therefore suggest that an abnormal overnight oximetry based on McGill criteria can accurately detect OSA of at least moderate severity but a negative result does not exclude OSA with certainty [78, 234]. These conclusions, however, were based on studies performed in otherwise healthy children being investigated for OSA and there is little data on children with co-morbidities. Of two studies of children with Down syndrome, one found that the McGill score had a sensitivity of 43%, specificity 93%, PPV 94%, NPV 39%, which was comparable to otherwise healthy children [235], whilst the other found that the score overestimated the number of children with OSA, due to central apnoeas causing desaturations [236]. A recent paper compared the oximetry recordings from children with obliterative bronchiolitis or cystic fibrosis, called collectively “obstructive lung disease (OLD)”, otherwise healthy children with SDB due to adenotonsillar hypertrophy and controls. Children with OLD had significantly lower baseline SpO2 when compared to the latter two groups. Whilst they had a higher number of desaturations/h compared with controls, it was lower compared with the adenotonsillar hypertrophy group [237].

3. b. Hypothesis and aims

The hypothesis of this study was that the positive and negative predictive values of the McGill score in detecting OSA would be worse in children with associated comorbidities such as NMD compared with otherwise healthy children.

Main aim was to determine the relationship between the McGill score and CR Polygraphy in the diagnosis of OSA in children referred to a tertiary care paediatric sleep centre.

3. c. Patients and methods

Two-year retrospective analysis of children (aged<18years) who underwent a CR Polygraphy at the Paediatric Sleep and Ventilation Unit at Royal Brompton Hospital for evaluation of OSA. Patients acutely unwell at the time of the overnight sleep study were excluded.

The oximetry traces were obtained via TCM CombiM® monitor, Radiometer, Copenhagen, Denmark and a simultaneous CR Polygraphy montage (SOMNOScreen™ plus, SOMNOmedics, Germany) was used for overnight monitoring. FT calculated the McGill score (1), blinded to CR Polygraphy results. McGill score=1 was considered normal or inconclusive, while >1 abnormal.

The McGill score results was compared with two different diagnostic definitions of OSA, either an Obstructive Apnoea Hypopnoea Index oAHI) ≥1 (mild, moderate and severe OSA) or an oAHI ≥5 (moderate and severe OSA).

Statistical analysis

Data were analysed by GraphPad Prism® software version 7.02 and by IBM SPSS® version 24. Descriptive statistics were generated for each measure. For all the reported variables a test of normality was performed. For non-parametric data, median with interquartile range was reported. McGill sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and accuracy were calculated for the whole cohort, for the otherwise healthy

children and those with associated medical conditions. For all subgroups the results and the 95% Confidence Interval (95% CI) were reported. Sensitivity was the proportion of all children with OSA (at CR Polygraphy) with an abnormal McGill score. Specificity was the proportion of all children without OSA with a normal McGill score of 1. Accuracy was the proportion of all the correct assessment out of all the assessments.

3. d. Results

Three-hundred fifteen children were studies, three patients' data was discarded because of oximetry artefact, thus 312 patients were analysed. The median age was 4.5 (2.4-7.9) years, 190 (61%) were male. 129(41%), median age 4.5 (2.4-7.8) years, were otherwise healthy, while 183 (59%), median age 4.5 (2.4-8.0) years, had associated medical conditions: neurological/neuromuscular syndromes (n=50), upper airway malformations/wheeze (n=75), interstitial lung disease/chronic lung disease (n=40), congenital heart disease (n=15), obesity (n=3). Median age was not significantly different across the diagnostic groups.

One hundred and two of 312 (32%) children had OSA when defined as oAHI≥1, 45/129 (35%) otherwise healthy and 57/183 (31%) with medical conditions. Forty-eight of 312 (15%) had OSA when defined as oAHI≥5, 23/129 (18%) otherwise healthy and 25/183 (14%) with medical conditions.

Median (IQR) SpO_2 was 97.6 % (96.6-98.4), median (IQR) minimum SpO_2 was 90% (86-93) and median (IQR) oxygen desaturation index (ODI>4%) was 3.0 (1.8-5.2)/h in the whole study population. Otherwise healthy children had median (IQR) SpO_2 97.9% (96.9-98.7) and median (IQR) minimum SpO_2 91% (88-93). The median (IQR) ODI in otherwise healthy children was 2.8 (1.6-4.2)/h. Children with medical conditions had median (IQR) SpO_2 97.5% (96.2-98.3) and median (IQR) minimum SpO2 90% (86-92). The median (IQR) ODI was 3.2 (1.9-6.4)/h.

Median (IQR) McGill score in the whole population was 1 (1-2). It was 1 (1-1) in otherwise healthy children and 1 (1-2) in children with medical conditions. Median (IQR) McGill in neurological/neuromuscular syndrome children was 1 (1-3), in upper airway malformations/wheeze was 1 (1-1), in children with interstitial lung disease/chronic lung disease was 1 (1-2), in those with congenital heart disease was 2 (1-2) and in children with obesity was 1 (1-1).

For OSA defined as obstructive oAHI ≥1, McGill' score sensitivity was low in the whole cohort (47%) and was similarly poor in the two groups of otherwise healthy children and those with medical conditions (40% and 52% respectively). The specificity was lower in the group of children with medical conditions compared with the otherwise healthy group (79% vs 92%). The PPV was also lower in the children with medical conditions (52% vs 72%). The NPV was similar between the two groups. In children with neurological/neuromuscular disorders the McGill score had the highest PPV and NPV (70% and 83% respectively). Conversely, children with upper airway malformations/wheeze had the lowest PPV (43%) and children with interstitial or chronic lung disease had the lowest NPV (75%).

When OSA was defined as oAHI ≥5, McGill's sensitivity increased in the overall population (74%) and in the two groups of otherwise healthy children and those with medical conditions. Nevertheless, it was still too low to be satisfactory for a screening test. The specificity and PPV were once again lower in children with associated medical conditions than otherwise healthy children. There was little difference in the NPV between the two groups. Results are summarised in Tables 4 and 5.

	Sensitivity 95% CI	Specificity 95% CI	PPV 95% CI	NPV 95% CI	Accuracy 95% CI
Total cohort (n=312)	47/101 (47%) (36.6-56.7)	177/211 (84%) (78.2-88.6)	47/81 (58%) (48.8-66.7)	177/231 (77%) (73.0-79.9)	224/312 (72%) (66.5-76.7)
Otherwise healthy (n=129)	18/45 (40%) (25.7-55.7)	77/84 (92%) (83.6-96.6)	18/25 (72%) (53.7-85.1)	77/104 (74%) (69.0-78.5)	95/129 (74%) (65.2-81.0)
Medical conditions (n=183)	29/56 (52%) (38.0-65.34)	100/127 (79%) (70.6-85.5)	29/56 (52%) (41.4-62.0)	100/127 (79%) (73.6-83.1)	129/183 (70%) (63.3-77.0)
- **Neurological/neuromuscular syndromes (n=50)**	14/19 (74%) (48.8-90.9)	25/31 (81%) (62.5-92.6)	14/20 (70%) (52.0-83.4)	25/30 (83%) (69.8-91.5)	39/50 (78%) (64.0-88.5)
- **Upper airway diseases/wheeze (n=75)**	6/19 (32%) (12.6-56.6)	48/56 (86%) (73.8-93.6)	6/14 (43%) (23.0-65.3)	48/61 (79%) (72.8-83.6)	54/75 (72%) (60.4-81.8)
- **Lung diseases (n=40)**	6/13 (46%) (19.2-74.9)	21/27 (78%) (57.7-91.4)	6/12 (50%) (28.5-71.5)	21/28 (75%) (63.6-83.8)	26/40 (67%) (50.9-81.4)
- **Congenital heart disease (n=15)**	3/5 (60%) (14.7-94.7)	3/10 (30%) (6.7-65.3)	3/10 (30%) (15.8-49.4)	3/5 (60%) (26.4-86.3)	6/15 (40%) (16.3-67.7)
- **Obesity (n=3)**	0/0 (0%) N/A	3/3 (100%) (29.2-100)	0/0 (0%) N/A	3/3 (100%) N/A	N/A N/A

Table 4. Sensitivity, specificity, PPV, NPV and accuracy of McGill score when compared to CR Polygraphy in detecting OSA (defined as oAHI≥1) in otherwise healthy and children with medical conditions

	Sensitivity 95% CI	Specificity 95% CI	PPV 95% CI	NPV 95% CI	Accuracy 95% CI
Total cohort (n=312)	35/47 (74%) (59.7- 86.1)	219/265 (83%) (77.5 87.0)	35/81 (43%) (35.8-51.01)	219/231 (95%) (91.8- 96.8)	254/312 (81%) (76.6-85.6)
Otherwise healthy (n=129)	15/23 (65%) (42.7-83.6)	96/106 (91%) (83.3- 95.4)	15/25 (60%) (43.6- 74.4)	96/104 (92%) (87.2-95.5)	111/129 (86%) (78.9-91.5)
Medical conditions (n=183)	20/24 (83%) (62.6- 95.3)	123/159 (77%) (70.1-83.6)	20/56 (36%) (28.4- 43.8)	123/127 (97%) (92.6- 98.7)	143/183 (78 %) (71.5- 83.9)
- **Neurological/neuromuscular syndromes (n=50)**	12/14 (86%) (57.2- 98.2)	28/36 (78%) (60.9- 89.9)	12/20 (60%) (44.0-74.1)	28/30 (93%) (79.3-98.1)	40/50 (80%) (66.3-90.0)
- **Upper airway diseases/wheeze (n=75)**	4/5 (80%) (28.4- 99.5)	60/70 (86%) (75.3- 92.9)	4/14 (29%) (16.3- 45.2)	60/61 (98%) (91.2-99.7)	64/75 (85%) (75.3-92.4)
- **Lung diseases (n=40)**	1/2 (50%) (1.3- 98.7)	27/38 (71%) (54.1-84.6)	1/12 (8%) (2.0-28.4)	27/28 (96%) (86.9-99.1)	28/40 (70%) (53.5-83.4)
- **Congenital heart disease (n=15)**	3/3 (100%) (29.2-100.0)	5/12 (42%) (15.2-72.3)	3/10 (30%) (21.0-41)	5/5 (100%) N/A	8/15 (53%) (26.6-78.7)
- **Obesity (n=3)**	0/0 (0%) N/A	3/3 (100%) (29.2-100.0)	0/0 (0%) N/A	3/3 (100%) N/A	N/A N/A

Table 5. Sensitivity, specificity, PPV, NPV and accuracy of McGill score when compared to CR Polygraphy in detecting OSA (defined as oAHI≥1) in otherwise healthy and children with medical conditions

3. e. Discussion

In this cohort of children referred for investigation of obstructive sleep disordered breathing, the McGill score had a lower PPV in the group of children with medical conditions than the group of otherwise healthy children due to a higher number of false positives. The NPV was similar between the two groups.

This low PPV, particularly in children with underlying lung diseases, may be because they are more likely to desaturate following a brief hypopnoea or apnoea than healthy children. Desaturations have also been shown to occur during periods of phasic partial respiratory muscle inhibition in rapid eye movement sleep, resulting in decrease in functional residual capacity, airway closure in the dependent lung regions and ventilation-perfusion mismatch [1]. Moreover, children with neurological conditions or syndromes may have non-obstructive events such as central apnoeas resulting in desaturations (Figure7) which may also result in a false positive McGill score.

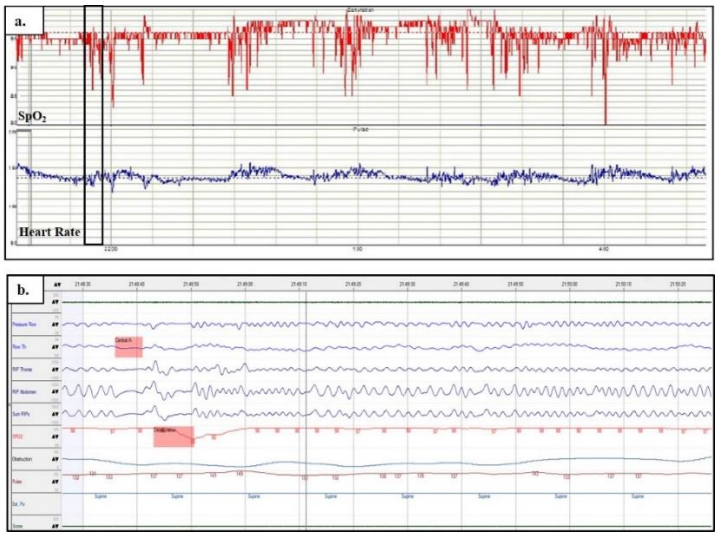


Figure 7. *Example of overnight oximetry (a.) coupled with cardio-respiratory polygraphy (b.) of a 6 month-old patient with congenital severe hypotonia*

a. Overnight oximetry, scored as McGill score 3
b. Example of a 2-minute epoch from cardio-respiratory polygraphy showing a central apnoea resulting in a desaturation to 88%

These results are in keeping with the findings of Coverstone et al who also found that central apnoeas were the cause of some children with Down syndrome having positive McGill scores [236]. In this work NPV was higher than that reported by Brouillette et al. which may be due to the different thresholds of referral and different patient populations – 60% of the children in that study had OSA confirmed on PSG, compared with 35% in otherwise healthy group and 31% in the group of children with co-morbidities in this work [233]. Interestingly, whilst the majority of the patients in that study were otherwise healthy, they reported that the three false-positive oximetry results they had were all in the subgroup of children who had diagnoses other than adenotonsillar hypertrophy that might have affected breathing during sleep, which would be consistent with our results. The use of CR Polygraphy instead of PSG in the diagnosis of OSA could in part explain why in this work even in otherwise healthy children PPV was lower (72% for oAHI≥1 or 60% for oAHI≥5 vs 93%) than previously reported [234]. CR Polygraphy is commonly used in the diagnosis of OSA in the UK but it can potentially underestimate OSA severity when compared to PSG.

This is the largest study to examine the use of the McGill score in children with co-morbidities. The main limitations of this work are its retrospective nature and the small size of some of the subgroups of medical conditions, such as obesity.

4. PROJECT 3 - REDUCING THE NEED FOR CO_2 MONITORING IN THE INVESTIGATION OF PAEDIATRIC SLEEP DISORDERED BREATHING

4. a. Introduction

There are no current clear recommendations as to which subset of paediatric patients would benefit from CO_2 monitoring in the overnight assessment of SDB. CR Polygraphy montage consists of nasal cannula and/or mouth thermistor for flow and snoring, thoracic and abdominal bands for respiratory effort, SpO_2 and pulse rate sensor, body position detector but often without a CO_2 sensor, particularly in home studies.

4. b. Hypothesis and aims

The hypothesis of this work was that CO_2 data did not change management in otherwise healthy children who are being investigated for obstructive sleep apnoea (OSA), but was important in those with pre-existing medical conditions or co-morbidities, when added to cardio-respiratory polygraphy data.

Main aim of this work was to determine how often the addition of overnight CO_2 monitoring changed the clinical management of patients when added to CR Polygraphy data.

4. c. Patients and methods

Two year, retrospective analysis of children age<18years who were referred to the Paediatric Sleep and Ventilation Unit at Royal Brompton Hospital for investigation of sleep disordered breathing and who underwent a baseline CR Polygraphy with measurement of transcutaneous CO_2.

The management recommendations were firstly made blinded to the patient details and CO_2 data, and then re-assessed after considering transcutaneous CO_2. Age, gender, underlying disease and OSA symptoms were recorded. The same CR Polygraphy equipment

(SOMNOScreen™ plus, Polygraphy set, SOMNOmedics, Germany) was used throughout the study period.

Mean CO_2 was obtained through transcutaneous capnography (TCM CombiM® monitor, Radiometer, Copenhagen, Denmark) and was considered abnormal if >6.7kPa (50mmHg). Hypoventilation was defined as CO2>6.7 kPa (50mmHg) >25% of recorded night as per American Association of Sleep Medicine recommendations [65]. As per European Respiratory Society guidelines, OSA was defined as an Apnoea Hypopnoea Index (AHI)> 1 in the presence of OSA symptoms and OSA severity was classified as mild, moderate or severe according to AHI [78]. The presence of symptoms of OSA such as snoring or witnessed apnoea was also recorded.

Patients' pre-existing medical conditions or co-morbidities were classified as: craniofacial abnormalities, chronic cough, laryngomalacia, asthma/wheeze, interstitial lung diseases, cystic fibrosis, congenital heart diseases, neurological/neuromuscular disorders and other syndromes, and obesity (Table 1).

Statistical analysis

Data were analysed by GraphPad Prism® software version 7.02. Descriptive statistics were generated on each measure. For all the reported variables a test of normality was performed. For non-parametric data, median (IQR 25th-75th centiles) was reported. Multiple comparisons were performed via ANOVA or Kruskall-Wallis test according to data distribution.

4. d. Results

There were 513 patients, 311 (61%) male, median age 4.5 years (IQR 2.3-7.9). Thirteen of 513 were prescribed overnight oxygen (O_2), 1/513 Continuous Positive Pressure (CPAP) and O_2, and 1/513 non-invasive ventilation (NIV).

One hundred and thirty of 513 (25%) were otherwise healthy children being investigated for OSA. Three hundred and eighty three of 513 (75%) had pre-existing medical conditions or co-morbidities. They were craniofacial abnormalities (n=7), chronic cough (n=38), laryngomalacia (n=14), asthma/wheeze (n=80), interstitial lung diseases (n=63), cystic fibrosis (n=15), congenital heart disease (n=48), obesity (n=6) and neurological/neuromuscular syndromes (n=112). Of this last group, 17 were affected by primary myopathies.

One hundred and eighty nine of 383 (49%) had clinical symptoms of OSA. They were 5/7 (71%) with craniofacial abnormalities, 23/38 (61%) with chronic cough, 7/14 (50%) with laryngomalacia, 43/80 (54%) with asthma/wheeze, 31/63 (49%) with interstitial lung diseases, 9/15 (60%) with cystic fibrosis, 16/48 (33%) with congenital heart diseases, 52/112 (46%) with neurological/neuromuscular syndromes and 3/6 (50%) with obesity. One hundred and seven of 130 (82%) otherwise healthy patients were diagnosed with OSA from the baseline CR Polygraphy results; 23 had a normal study.

In these children without comorbidities with respectively mild, moderate and severe OSA the prevalence of high mean CO_2 was 0/73 (0%), 2/14 (14%) and 5/20 (25%), the prevalence of hypoventilation was 13/73 (18%), 7/14 (50%) and 12/20 (60%), and the presence of REM-related CO_2 elevation was 8/73 (11%), 7/14 (50%), 13/20 (65%). The addition of CO_2 data did not change management in any of these 107 children.

Conversely, in 20/383 (5%) children with pre-existing medical conditions or co-morbidities, either abnormally high mean CO_2 levels (17/20) or elevated CO_2 during REM sleep (3/20)

changed management when these results were interpreted in conjunction with the CR Polygraphy data. Median overnight CO_2 levels did not differ significantly across diagnostic groups (p=0.73) and did not significantly differ between children with or without pre-existing medical conditions or co-morbidities (p= 0.44).

In the group of children with pre-existing medical conditions or co-morbidities, there were changes in recommendations after including CO_2 data to information from the CR Polygraphy in 20 children. CO_2 data changed management respectively in 1/7 (14%) patients with craniofacial abnormalities, 0/38 with chronic cough, 1/14 (7%) with laryngomalacia, 1/80 (1%) with asthma/wheeze, 1/63 (2%) with interstitial lung diseases, 0/15 with cystic fibrosis, 2/48 (4%) with heart disease, 2/6 (33%) with obesity and 12/112 (11%) with neurological/neuromuscular syndromes.

Overall, in 18/20 (90%) children, CPAP or NIV was established. NIV was started in 1/1 with laryngomalacia, 1/1 with asthma/wheeze, 1/2 patients with heart diseases, 12/12 patients with neurological/neuromuscular syndromes; CPAP was established in 1/1 patient with craniofacial abnormalities and 2/2 patients with obesity. Finally, the addition of CO_2 to CR Polygraphy prompted the safe up-titration of O_2 flow in 1 patient with heart disease and low baseline saturations. There were concerns that increasing oxygen therapy might result in an increase in his CO_2 levels, therefore the study was performed on increased oxygen levels. Since CO_2 did not increase significantly, the child's home oxygen could be safely increased. In one patient with interstitial lung disease, unexpectedly high CO_2 levels lead to the instigation of further investigations.

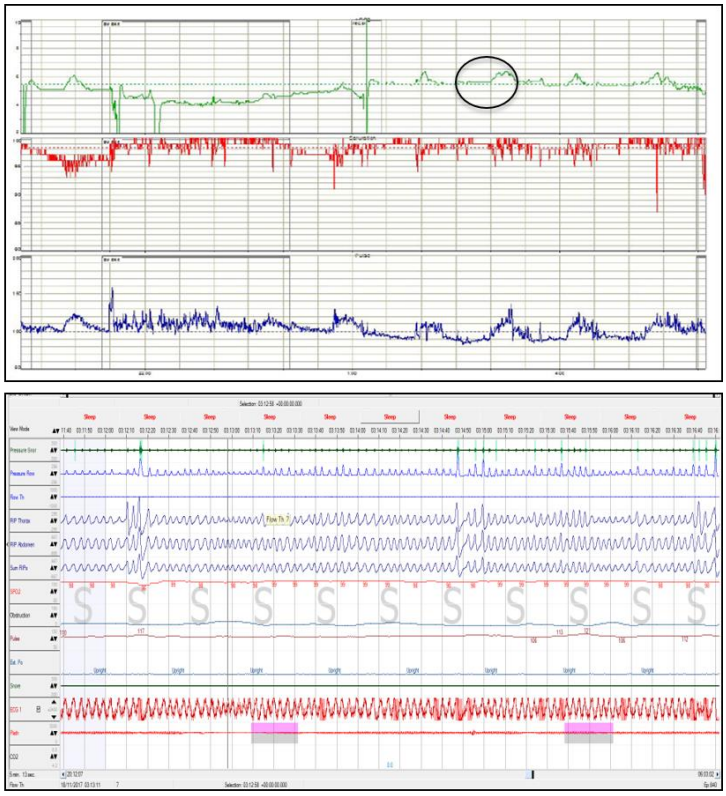
	OSA symptoms (n)	Median CO_2 kPa (IQR)	Changes after adding back CO_2 data (n)	Outcome after adding back CO_2 data
Otherwise Healthy	130/130	5.9 (5.6-6.2)	0/130	N/A
Craniofacial abnormalities	5/7	5.7 (5.4-6.5)	1/7	CPAP (1/1)
Chronic Cough	23/38	5.8 (5.6-6.0)	0/52	N/A
Laryngomalacia	7/14	5.9 (5.4-6.2)	1/14	NIV (1/1)
Asthma/Wheeze	43/80	5.8 (5.5-6.2)	1/80	NIV (1/1)
Interstitial lung diseases	31/63	5.9 (5.6-6.1)	1/63	Further investigation (1/1)
Cystic Fibrosis	9/15	5.8 (5.7-6.3)	0/15	N/A
Congenital heart disease	16/48	5.9 (5.5-6.2)	2/48	Start NIV (1/2) Change O_2 (1/2)
Neurological/neuromuscular syndromes	52/112	6.0 (5.6-6.4)	12/112	Start NIV (12/12)
Obesity	3/6	5.9 (5.7-6.3)	2/6	Start CPAP (2/2)
Total	319/513		20/513	

Table 6. Clinical characteristics of study population. Patients are classified as "otherwise healthy" or "having pre-existing medical conditions or co- morbidities". A breakdown of patients' pre-existing medical conditions or co-morbidities, their overnight CO_2 values, cardio-respiratory polygraphy data and management post-sleep study are outlined.

4. e. Discussion

In otherwise healthy patients with suspected OSA, none had changes in clinical management with additional CO_2 data. Conversely, CO_2 abnormalities, either high mean CO_2 values or elevation during REM, can occur in patients with pre-existing medical conditions or co-morbidities (20 out of 383, 5%), particularly neurological/neuromuscular, even without significant changes in the CR Polygraphy. See Figure 8.

a.



b.

Figure 8. *O_2CO_2 showing CO_2 elevation during REM sleep with no respiratory events at cardio-respiratory poygraphy. Three-year old boy with history of repaired congenital diaphragmatic hernia referred for investigation of OSA*

In 18 out of 20 patients, respiratory support was initiated as a result. A strength of this study is the large sample size (>100). The main limitation of this study is the retrospective nature, and we acknowledge that confirmatory prospective studies are needed.

According to these results, overnight CO_2 monitoring is not necessary for the diagnosis of SDB in otherwise healthy children, but is crucial for the management of paediatric patients with pre-existing medical conditions or co-morbidities. This is important, because it means home CR Polygraphy, without transcutaneous CO_2 measurement, can safely be used to diagnose OSA in otherwise healthy children thus saving resources and reducing the need for admission. On the other hand, this data confirm that overnight oximetry alone is not sufficient to rule out nocturnal hypoventilation the in children at risk for it such as NMD.

5. CONCLUSIONS

The projects presented in this chapter provide novel additions to the current literature.

Firstly, SLP is a promising tool for the respiratory assessment of young, non-cooperative children with SMA type 1. Acquisition of thoraco-abdominal traces during quiet breathing using SLP was feasible in all 19 children in the study cohort (median age 2.3 years). The analysis of SLP data allowed the quantification of the selective involvement of chest and abdominal muscle groups showing rapid shallow breathing, predominant abdominal contribution to breathing and chest-abdomen asynchrony. In the study cohort, the rate of thoraco-abdominal asynchrony measured by the Phase Angle negatively correlated with motor function assessed via CHOP-INTEND. More work is needed to ascertain the place of SLP in the clinic.

Our results on oximetry and capnography both demonstrate that the diagnosis of OSA solely by oximetry and the management of SDB without CO_2 are not suitable for children with underlying conditions, including neuromuscular disease. These results are clinically relevant and merits inclusion in the guidelines for the diagnosis and management of children with NMD.

The use of McGill score in the diagnosis and grading of OSA in otherwise healthy children and in those with co-morbidities have shown that the PPV of the McGill score is significantly lower in children with medical conditions. This lower PPV in the group of children with medical conditions was due to a higher number of false positives. REM-associated clustered central apnoea in NMD children can result in desaturations and in a false positive McGill score.

The results of the second work showed that children with co-morbidities who have a positive McGill score must not be assumed to have OSA, and require more detailed sleep studies to determine the physiological reason for their desaturations.

Capnography is often neglected in the overnight assessment of SDB in NMD. The high costs of CO_2 equipment usually mean it is omitted from home sleep studies or in the community where the centres might not be experienced in the management of NMD children. Moreover, the information provided by CO_2 when added to CR Polygraphy and its role in changing patients' management had not previously been fully clarified. The third work showed that capnography changed treatment in 12 out of 112 (11%) NMD children when added to CR Polygraphy. All the 12 patients had critical levels of CO_2 that required establishment of NIV (12/12).

CO_2 monitoring is therefore not necessary for the diagnosis of SDB in otherwise healthy children, but is crucial for the management of paediatric patients with pre-existing medical conditions or co-morbidities, particularly in NMD children.

6. SUMMARY OF PROJECTS

	Project 1. **"Non-invasive assessment of respiratory pattern in Spinal Muscular Atrophy type 1 by Structured Light Plethysmography"**	**Project 2.** **"The McGill score as a screening test for obstructive sleep disordered breathing in children with co-morbidities"**	**Project 3.** **"Reducing the need for CO_2 monitoring in the investigation of paediatric sleep disordered breathing"**
Background	The young age and the severity of children with SMA type 1 have limited the respiratory outcome measures available. Structured Light Plethysmography (SLP) is a non-invasive, light-based method of assessing patients' breathing pattern.	The McGill score is used to stratify severity of oximetry in children referred for investigation of obstructive sleep apnoea (OSA) to identify those with more severe disease and prioritize treatment.	There is increasing interest in the use of home sleep studies to diagnose paediatric sleep disordered breathing. The majority of home sleep study equipment do not include measurement of CO_2.
Hypotheses and aims	The breathing patterns of infant and children with SMA type 1, captured via SLP, will reflect their clinical status. AIMS: to determine whether SLP would capture the classical respiratory features of young children with SMA type 1. To correlate the grade of breathing pattern abnormality captured by SLP and the motor severity assessed by standardized clinical scales.	McGill's score Positive predictive value (PPV) and Negative predictive value (NPV) in detecting OSA differs significantly between children with medical conditions and otherwise healthy children. AIM: to determine the relationship between the McGill score and CR Polygraphy in the diagnosis of OSA in children referred to a tertiary care paediatric sleep centre.	CO_2 data would not change management in healthy children with OSA, unlike in those with pre-existing medical conditions or co-morbidities, when added to CR Polygraphy. AIM: to determine how often overnight CO_2 monitoring changed management of patients when added to CR Polygraphy.

Methods	One-year prospective cross-sectional study of consecutive SMA1 children. All children underwent one-minute tidal breathing recording in supine position off ventilation by Structured light Plethysmography (SLP). Motor function (CHOP-INTEND and Hammersmith Infant Neurological Examination, HINE) and anthropometrics were collected on the same day.	Two-year retrospective analysis of children referred for investigation of OSA who underwent a CR Polygraphy study. McGill score was calculated from the oximetry trace blinded to CR Polygraphy results. Two definitions of OSA were considered: oAHI ≥1 and ≥5. McGill sensitivity, specificity, PPV and NPV were calculated. McGill score=1 was considered normal or inconclusive, >1 abnormal.	Retrospective analysis of two-year data on paediatric patients referred for a first CR Polygraphy + tcCO2 study. Patients were classified as otherwise healthy or as having pre-existing medical conditions or co-morbidities Management recommendations were firstly made blinded to CO_2 data, then after considering $tcCO_2$. Mean CO_2 was abnormal if>6.7kPa5
Conclusions	SLP has proven to be a feasible and reliable non-invasive tool in capturing the respiratory features typical of SMA type 1 in a clinical setting	The positive predictive value of the McGill score is significantly lower in children with co-morbidities than otherwise healthy children. Children with co-morbidities who have with an abnormal McGill score should not be assumed to have OSA and need more detailed sleep studies to delineate the problem	Overnight CO_2 monitoring is not necessary for the diagnosis of OSA in otherwise healthy children Overnight CO_2 monitoring is crucial for the management of patients with pre-existing medical conditions or co-morbidities

Chapter 4

INFLAMMATION IN CHILDREN WITH NEUROMUSCULAR DISORDERS AND SLEEP DISORDERED BREATHING

1. BACKGROUND

1. a. Pathophysiology of inflammation in obstructive sleep apnoea (OSA)

The correlation between chronic intermittent hypoxia and systemic and airway inflammation in children and adults with OSA has been extensively studied. As described in detail in the thesis introduction, OSA is characterised by episodes of repeated prolonged increased upper airway resistance culminating in partial or complete intermittent obstruction during sleep. This leads to intermittent snoring, repetitive hypoxemic and hypercapnic events and sleep fragmentation due to repeated arousals. Much evidence strongly supports the concept that paediatric OSA is a chronic, low-grade inflammatory condition [238, 239]. Different mechanisms are responsible of initiating and maintaining the inflammatory cascade.

The acute phase response initiates the inflammatory process. The acute phase response is the rapid and early activation of an immune cascade in response to injury or infection. The toll-like receptors (TLRs), a class of pattern recognition receptors that recognize self and non-self elements, are one of the major components of the innate immune system. TLRs are expressed on tissue-resident macrophages, and induce the production of inflammatory cytokines, chemokines and prostaglandins. The outer lipopolysaccharide component of bacterial cell membranes classically activates these pathways. Sleep deprivation and hypoxia can also stimulate the TLR pathways, especially TLR-4, and induce cytokine production [240, 241]. In

consequence, OSA may cause elevation in the levels of systemic inflammatory mediators, such as C-Reactive protein (CRP), Tumour Necrosis Factor α (TNF-α), Interleukin 6 (IL-6), and Interferon γ (IFN-γ) [242], and the reduction of anti-inflammatory substances, such as Interleukin 10 (IL-10), tilting the balance toward a heightened pro-inflammatory state [243].

Secondly, there is increasing evidence that OSA leads to a polarization of immune-effector cells such as monocytes-macrophages and lymphocytes from a regulatory anti-inflammatory to a pro-inflammatory and cytotoxic status. This leads to a further enhancement of the release of inflammatory cytokines [242, 244-247]. Children with OSA have an imbalance of T-lymphocytes with reductions in circulating Th2 regulatory and a shift toward a Th1 pro-inflammatory phenotype. An increased ratio of pro-inflammatory Th1 /Th2 regulatory was found in children with OSA [244]. The degree of hypoxia in children with OSA was also associated with an altered balance of (Th17)/T regulatory toward a predominance of pro-inflammatory Th17 [247]. Pro-inflammatory CD8+ T-lymphocytes of patients with OSA seemed to have an increased cytotoxicity against vascular endothelial and smooth muscle cells [242].

Thirdly, immune cells are a source of reactive oxygen species (ROS) and may trigger systemic oxidative stress in OSA. Patients with OSA were found to have markedly enhanced release of superoxide from stimulated polymorphonuclear neutrophils (PMNs) [248]. ROS can oxidise a variety of biological molecules including lipids, proteins and DNA, and can further alter biological responses. Transcription factors such as NF-κB are oxidative stress-responsive and can be activated in OSA [249]. NF-κB is involved in the transcription of more than 200 genes, including those responsible for the production of cytokines and inflammatory markers [249, 250].

Additionally, the evidence is that there is a reciprocal relationship between sleep and the immune system. On the one hand sleep modulates immune system function, and on the other hand the activation of the immune system and the production of inflammatory cytokines could affect sleep [238]. The most extensively studied inflammatory cytokines in sleep regulation are Interleukin 1β (IL-1β) and TNFα [251]. In patients with inflammatory diseases such as rheumatoid arthritis as well as in patients with OSA, the high systemic levels of TNF-α have been linked to daytime fatigue and excessive sleepiness [252].

Taken together, these changes, mainly hypoxia-re-oxygenation events, lead to increased generation of reactive oxygen species and systemic inflammatory responses [249] which may be mechanistically involved in end-organ injury, for example atherogenesis [253], metabolic [254], and neurocognitive and behavioural disturbances [255].

1. b. Inflammatory cytokines in OSA and their role in co-morbidities

Oxidative stress and increased activation of inflammatory processes have been proposed to be mechanistically involved in the systemic consequences of OSA. OSA is now recognized as a major cause of cardiovascular morbidity [256] and neurocognitive dysfunction in children [257, 258]. Genetic susceptibility and extrinsic factors (i.e. obesity) are also involved [258]. Some of the important pro- and anti-inflammatory cytokines playing a role in the pathogenesis of co-morbidities in OSA are described below [259-261].

Interleukin 6

IL-6 is one of a family of cytokines. IL-6 activates different types of cells via a signalling complex consisting of the IL-6 α-receptor (IL-6R) and the signal-transducing β-subunit glycoprotein 130 (gp130). IL-6R exists as soluble as well as membrane-bound forms, which allows discrimination between IL-6 classic (via the membrane-anchored IL-6R) and IL-6 trans signalling (via soluble IL-6R). IL-6R is predominantly expressed in hepatocytes,

megakaryocytes and several leukocyte subpopulations while Gp130 is expressed in every tissue and cell type of the human body. IL-6 trans signalling via soluble IL-6R accounts for the pro inflammatory properties of IL-6 [262]. The production of IL-6 in OSA is mainly induced by intermittent hypoxia, via polarization of macrophages to an activated pro-inflammatory state.

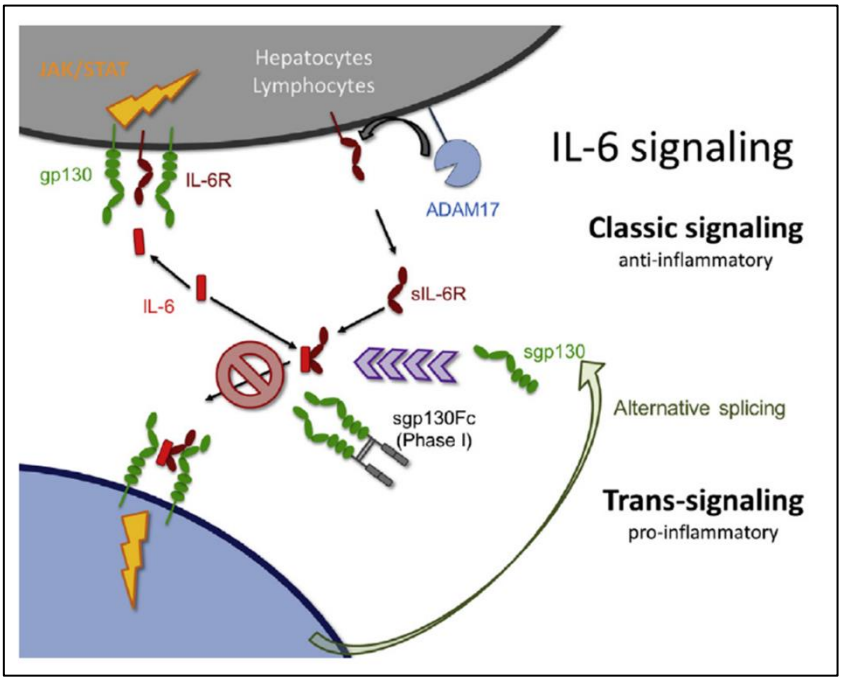


Figure 4. *Interleukin-6 classic and trans signalling. The Interleukin-6 receptor (shown in dark red) is expressed on hepatocytes and lymphocytes, where IL-6 (shown in red) can activate Jak/STAT signalling via a gp130 (shown in green) homodimer (classic signalling). The metalloprotease ADAM17 can cleave the IL-6R, and the resulting sIL-6R can form an agonistic complex with IL-6 that can activate other cells via trans signalling. Adapted from Wolf J. Cytokines 2014.*

There is a wide range of serum levels of IL-6 in children with OSA. This may be related to the presence of at least two IL-6 gene polymorphisms causing differential susceptibility to OSA, but also to the presence and severity of environmental factors such as obesity, concurrent cardiovascular or metabolic disease [263]. IL-6 plasma levels were variably reduced after adenotonsillectomy [263, 264] and CPAP treatment [265].

IL-6 is recognised to be a mediator of metabolic co-morbidities induced by intermittent hypoxia and by chronic sleep fragmentation by enhancing adipose tissue inflammation [266]. The production of IL-6 from adipocytes can further promote endothelial dysfunction via the NF-

κB-dependent pathway [267]. IL-6 furthermore induces the production of the pro inflammatory hs-CRP in liver. Elevations in hs-CRP were associated with higher risk of cardiovascular disease, diabetes and cognitive deterioration [268].

Interleukin 10

IL-10 is a cytokine synthesis inhibitory factor, released from epithelial cells, macrophages (resident in microglia and heart), monocytes, dendritic cells, neutrophils, mast cells, eosinophils, and natural killer cells, in addition to CD4 and CD8 T cells and B cells in response to a variety of stimuli. IL-10 is a cytokine with important effects on the development of an immune response. An understanding of how IL-10 expression is regulated in different innate and adaptive immune cells is of importance for the development of immune intervention strategies in various pathologies. The recognition of pathogens by dendritic cells and macrophages activates the production of IL-10. The subsequent activation of innate immunity triggers the production of IL-10 by lymphocytes Th1 and Th2 and Th17. Th1 cells producing IL-10 can be generated by incubating T cells in the presence of IL-12 [269, 270]. In turn, IL-10 acts as a key mediator of the anti-inflammatory response. IL-10 reduces the antigen-presenting capacity of monocytes and inhibits cytokine production by activated macrophages and dendritic cells and Th1 lymphocytes. Reduced levels of serum IL-10 were associated with OSA [243]. In non-obese children with OSA, plasma levels of IL-6 were higher and IL-10 lower when compared to controls. Both IL-6 and IL-10 were restored to normal after adenotonsillectomy [243]. Another study found no differences in IL-10 pre- and post-CPAP in a population of patients with OSA [271]. In severely obese subjects, IL-10 serum levels were lower than in lean controls. When considering the obese patients only, those with OSA had a significantly lower serum IL-10 than those without OSA and IL-10 levels were inversely related to the severity of OSA [272].

Tumour Necrosis Factor α

TNF-α is a pro-inflammatory cytokine with a role in sleep regulation [273]. The TNF-α receptor has two forms, a 26 kD trans-membrane protein expressed predominately in brain and adipose tissue and a soluble 17 kD protein which is more abundant in muscle and liver. In mice, the inhibition of TNF-α receptors in the hypothalamus inhibited spontaneous NREM sleep. Injection of TNF-α in the anterior hypothalamus and locus coeruleus in murine brain resulted in the suppression of NREM sleep [251].

High levels of circulating TNF-α were associated with sleep fragmentation and intermittent hypoxia, typical features of OSA. Mice in whom sleep fragmentation was induced had up-regulated TNF-α expression in the cerebral cortex and a reduced ability of NREM and REM sleep to recuperate after previous exposure to sleep fragmentation, as well as cognitive and mood disturbances [274]. TNF-α production is driven in part by other pro-inflammatory cytokines via NF-κB and via its own positive feedback loop [275]. Studies in mice made chronically intermittently hypoxic suggested that increased cellular and extracellular levels of TNF-α were highly associated with the recruitment of TLR-4-NF-κB pathway. The activation of NF-κB pathways in turn activate nitric oxide synthase, cyclooxygenase 2, and adenosine A1 receptors, all of which are implicated in sleep regulation [275] and also atherogenesis [276, 277]. In addition, a single nucleotide polymorphism-308 in the TNF-α gene in patients with OSA was associated with the presence or otherwise of concurrent excessive daytime sleepiness [252].

C Reactive Protein

CRP is a highly soluble member of the pentraxin family, consisting of a discoid configuration of five identical globular subunits organized in a cyclic pentamer. Pentameric CRP (pCRP) is primarily synthesized in the liver, but also in the kidney, atherosclerotic lesions, neurons and

tissue resident (adipose tissue, lung) macrophages. CRP production from macrophages is induced by IL-6 during increased oxidative stress and infection and, to a lesser degree, by IL-1β and TNF-α. A monomeric isoform of CRP (mCRP) accumulates in atherosclerotic lesions, prolongs neutrophil survival and induces leukocyte recruitment via monocyte chemoattractant protein-1 (MCP-1) and IL-8. In endothelial cells, mCRP but not pCRP directly facilitates the expression of intercellular adhesion molecule-1 (ICAM-1), E-Selectin, and vascular adhesion molecule-1 (VCAM-1) [278]

CRP has also been shown in adults to be increased in the presence of obesity and OSA [279, 280]. In children CRP levels were correlated with the level of obesity but not with SDB [281]. CRP levels were found to be independent biomarkers of future cardiovascular events in OSA. Children with OSA had higher high sensitivity hsCRP levels than primary snorers and controls. Patients with severe OSA and higher hsCRP had significantly more frequent left ventricular diastolic dysfunction than control group [268].

Interleukin 1β

IL-1β is released by monocytes and macrophages in response to many stimuli including sleep loss, tissue injury, and infections [282]. IL1-β increases the duration of NREM sleep. Sleep loss and subsequent altered IL-1β levels are associated with enhanced sensitivity to pain, fatigue, sleepiness and rebound sleep, but also to metabolic syndrome including type 2 diabetes [283], and impaired cognition and memory.

Interleukin 17 and 23

Th17 lymphocytes are key effectors in the immune response and play a critical role in the development of autoimmunity. Adults and children with OSA have a significant increase in peripheral Th17 number and levels of the Th17-related cytokines IL-17, IL-6 [284, 285] and

IL-23 [245]. It is speculated that IL-17 may be one of the main contributors to the increased cardiovascular morbidity in inflammatory diseases [286].

IL-23 is a pro-inflammatory cytokine that plays a key role in the pathogenesis of many autoimmune inflammatory diseases and malignancies. It consists of the subunits p19 and p40, connected via a disulphide bond. Inflammatory macrophages express the IL-23 receptor and are activated by IL-23 to produce IL-1, TNF-α, and IL-23 itself. IL-23 is involved in the differentiation of Th17 cells in a pro-inflammatory context in the presence of TGF-β and IL6. In adults serum IL-23 was found to correlate with OSA severity (AHI) and to be a useful marker of improvement in OSA following CPAP treatment [287].

1. c. Adhesion molecules in OSA and their role in co-morbidities

Intercellular adhesion molecule (ICAM-1) and vascular cellular adhesion molecule (VCAM-1) are present in their soluble forms in plasma. Their expression on endothelial and lung epithelial cell surfaces is enhanced directly by hypoxia [288-290], reactive oxygen species [291] as well as CRP and pro-inflammatory cytokines such as, TNFα and IL-1β [292]. Once expressed, they promote the adhesion of leukocytes and monocytes to the endothelium. Once bound to the cell surface, the activated leukocytes release cytokines and chemokines. These cascades of events maintain the inflammatory state and the production of ROS ultimately leading to end-organ dysfunction [293]. ICAM-1 and VCAM-1 are the main mediators of atherogenesis and cardiovascular morbidities triggered by hypoxia in OSA [288, 293, 294]. In adults with moderate-to-severe OSA, two years of CPAP prevented the increase in adhesion molecules observed in non CPAP users. For ICAM-1, the largest effect of CPAP was found in the most obese subjects [295]. ICAM-1 and VCAM-1 were also found expressed on alveolar epithelial cells after 2 to 4 hours of hypoxia. Their expression was associated with increased adhesiveness of neutrophils and macrophages. Hence, ICAM-1 and VCAM-1 were suggested as mediators of lung damage caused by hypoxia [290].

Intercellular Adhesion Molecule 1

ICAM-1 is a member of the Ig supergene family, and the full-length isoform is composed of five Ig domains, a transmembrane domain, and a short cytoplasmic tail with multiple threonine residues. The cytoplasmic tail interacts with the actin cytoskeleton and results in the activation of several intracellular signalling pathways that contribute to cytokine production and cellular trafficking events. On most cell types and under non-inflammatory conditions, ICAM-1 expression is constitutively low and generally detectable only on endothelial cells. TNF-α, IL-1β, IFN-γ, and other cytokines elicit increased ICAM-1 expression in a cell- and cytokine-specific fashion. ICAM-1 upregulation is a signature event during inflammation, particularly on endothelium where expression may remain elevated for extended periods of time [292]. Culture cells stimulated by IL-1β expressed VCAM-1 on their outer membranes for up to 4-8 hours and ICAM-1 for up to 6-72 hours [296].

The alternative splicing of ICAM-1 generates six membrane-bound forms and one soluble form. The soluble form of ICAM-1 is in part cell specific (endothelial and peripheral blood mononuclear cells) and its secretion is modulated by IFN-α. It is also generated by proteolytic cleavage of the membrane bound form by neutrophil elastase, cathepsin G and bacterial-derived enzymes [292].

ICAM-1 does not only bind other β2-integrins (Mac-1 and p150, 95), but acts as a receptor for virus and other micro-organism. ICAM-1 was found to mediate the bond of *Plasmodium falciparum* to the membrane of red blood cells. Membrane-bound ICAM-1 is also a receptor for Rhinoviruses [297]. Influenza virus infection trigger the expression of ICAM-1 in airway epithelial cells. In turn, the expression of ICAM-1, via NF-κB indices apoptotic and inflammatory effects that induce the clearance of infected cells [298].

Vascular cellular adhesion molecule 1

VCAM-1 is also member of the immunoglobulin super family and it binds to α4β1-integrin on leukocytes. VCAM-1 is a transmembrane protein containing either seven immunoglobulin-like domains or a protein with just domains 1–3 and 5–7. Domains 1 and 4 of VCAM-1 are the ligand binding domains. VCAM-1 regulates leukocyte migration from blood into tissues [291]. VCAM-1 is induced on endothelium in inflammatory sites in patients with inflammatory bowel disease, atherosclerosis, allograft rejection, infection, and asthma. VCAM-1 expression is triggered by ROS or cytokines such as TGF-1β or IFN-γ. Once activated, VCAM-1 promotes endothelial cells contraction to open an "endothelial cell gate" for leukocytes to move between endothelial cells [291].

1. d. Markers of inflammation in serum and exhaled breath condensate

Exhaled breath condensate (EBC) was introduced more than two decades ago as a novel, non-invasive tool to assess airway inflammation. The detection of volatile and non-volatile components is quite challenging due to their small and variable concentrations in the exhaled breath. Applying highly sensitive technology to analyse exhaled breath enabled the evaluation of different molecules in these samples. Its possible applicability has been more extensively studied in children in airway diseases such as asthma [299]. The ERS recently issued guidelines on EBC collection and storage, in order to ensure greater consistency of analysis [300]. Leukotrienes and prostaglandins can be quantified in children with OSA. The concentration in EBC of leukotrienes LTB4, LTC4, LTD4 and LTE4 but not of prostaglandin E2 (PGE2) increased with worsening severity of OSA [301]. In adults, EBC and serum IL-6 and TNF-α correlated with AHI better than EBC and serum IL-10 and isoprostane 8 [302]. However, to date it is unclear if EBC has a clinical role in the assessment of airway inflammation.

Comparisons between airway and systemic inflammation in children and adult with OSA have not been performed [260, 261, 303].

2. HYPOTHESIS AND AIMS

Children with NMD are at risk of SDB, including nocturnal hypoventilation and OSA. Main hypothesis of this project is that SDB in NMD would be associated with systemic and airway inflammation. We aimed to relate systemic (serum) and airway (EBC) levels of inflammatory cytokines and adhesion molecules to the severity of SDB.

Primary endpoint

- To determine whether there is any evidence of systemic and airway inflammation in children with NMD with SDB

Secondary endpoint

- To determine whether any systemic and airway levels of inflammation present relate to the severity of SDB

3. PATIENTS AND METHODS

3. a. Study design and population

Prospective study on consecutive children (age 5-18 years) affected by genetically confirmed neuromuscular conditions referred to Sleep and Ventilation Unit, Royal Brompton Hospital. The study was approved by Health Research Authority London - Brent Research Ethics Committee (15/LO/1883). Informed consent and age-appropriate assent were obtained from parents and children. Patients acutely unwell or with significant co-morbidities were excluded. Height and weight were collected and Body mass index (BMI) was calculated as kg/m^2. BMI was plotted on the WHO growth reference charts for boys and girls aged 5-19 years. Patients were defined overweight if BMI > +1standard deviation (SD) (equivalent to BMI 25 kg/m2 at 19 years), obese if BMI > +2SD (equivalent to BMI 30 kg/m2 at 19 years) and thin if BMI <-2SD [304].

3. b. Polysomnography

Children underwent overnight full polysomnography (PSG) (SOMNOscreen™ plus; Somnomedics), including eight channels EEG, bilateral EOG, chin and anterior tibial EMG, and analogue output from body position sensor. Chest and abdominal wall movement were measured by inductance plethysmography, heart rate by electrocardiogram, airflow by nasal pressure cannula. Sleep architecture was assessed by standard techniques as per AASM guidelines [65]. AHI was scored from the total number of apnoeas and hypopnoeas per hour of TST as conventionally defined [65]. OSA was defined as oAHI≥1 [78].

SpO_2 was recorded by pulse oximetry (Nellcor N 100; Nellcor Inc). Mean SpO_2 and ODI (the number of falls in SpO2 by ≥ 4% / hour TST) were recorded. The reference values of ODI in otherwise healthy children are 2.2/h (95^{th} centile) and 2.4/h (97.5^{th} centile) [75]. An ODI≥3 was considered abnormal.

$TcCO_2$ was recorded by TCM CombiM monitor and mean overnight $tcCO_2$ recorded. Abnormally elevated CO_2 was defined as $tcCO_2$ levels>6.7 kPa (>50 mmHg) for >2% TST as were reported as a criteria for starting NIV in clinical settings [97].

3. c. Serum and exhaled breath condensate

The following morning, while the child was fasting, blood and EBC were collected [300]. Children wore a nose-clip and breathed for 10 minutes at tidal volume into an RTube™ (Respiratory Research, Inc.) refrigerated in a -20C metallic sleeve. After ten minutes rest, 1.2 ml blood was collected by venepuncture in EDTA tubes. Within thirty minutes of collection, blood and EBC were brought in a refrigerated container to the lab to be aliquoted and stored.
Blood samples were firstly centrifuged for 15 min at 3000 RPM and the serum was then collected. Sixty µL were aliquoted into individual 2mL polypropylene vials (Eppendorf tubes) and immediately frozen at ≤−80 °C until analysis.
EBC was aliquoted in 60µL and transferred into Eppendorf tubes and immediately frozen and then frozen at ≤−80 °C until analysis. The total EBC obtained from each patient was recorded as per ERS guidelines [300].

3. d. Cytokines analysis

EBC and serum cytokines were analysed on three MesoScale Discovery (MSD) (Rockville, MD) multiplex electro-chemilluminescent immunoassays: V-Plex Proinflammatory 1 (IL-1β, IL-6, IL-10, TNFα), VPlex Th17 (IL-17, IL-23) and V-Plex vascular injury (hsCRP, VCAM-1, ICAM-1) in duplicate. The minimum volume of sample required for the assay was 25µl.

The 96-well plates were supplied by MSD in a prepared format and were ready to use without further preparation. Reagents for the assay were prepared according to the MSD literature. Prior to analysis, all samples were removed from −80 °C storage, thawed in an ice bath for 30–45 minutes, and then vortexed for five seconds to achieve uniform consistency. The calibration

standards for the immunoassay were supplied in a single mix at 2500 ρg/mL and required a series of 4-fold dilutions for V-Plex Proinflammatory 1 and V-Plex Th17 Panel and a series of 5-fold dilutions for V-Plex vascular injury. Next, duplicate 25 µL aliquots of each calibration standard (a total of 16 wells) and appropriate samples were then added to the plate. Samples were diluted in twice the volume for Pro-Inflammatory Panels, 4 times for Th17 Panel and 1000 times for vascular injury Panel. Next, the plates were washed three times with a phosphate buffered saline-0.05% Tween (PBS-T) solution, then a 50µL sample volume (of both EBC and serum) was added to V-Plex Proinflammatory 1 and V-Plex Th17 Panel wells and a sample volume of 25µL to the vascular injury Panel well. The 96-well plate was sealed and incubated on a shaker at room temperature for two hours. Then, the plate was washed three times with a phosphate buffered saline-0.05% Tween (PBS-T) solution. 25 µL of a detection antibody solution was added into each of the 96 wells followed by sealing and incubating the plate on a shaker at room temperature for two additional hours (V-Plex Proinflammatory 1 and V-Plex Th17 Panel) or one hour (V-Plex vascular injury). The plate was washed three times with PBS-T and 150 µL of Read Buffer (V-Plex Proinflammatory 1 and V-Plex Th17 Panel) or 75 µL of Read Buffer (V-Plex vascular injury) was added to each well. Finally, the plate was analysed on a MSD SECTOR Imager 2400.

The MSD Discovery Workbench® analysis software (Rockville, MD) was used to estimate the concentration data for each target cytokine. These data were based on an internally calculated signal-concentration 4-paramter logistic calibration curve. When a result was below the instrument-derived LLOQ, the software would frequently report the signal but not a calculated concentration [305].

3. e. Statistical analysis

Due to the exploratory nature of the study the sample size was opportunistic. Data were analysed by SPSS® v.24. Non-parametric statistics were used for all the analyses due to the small sample size and median and interquartile range (IQR 25th-75th centiles) were calculated. Serum and EBC cytokine levels were compared according to the presence of SDB, i.e. ODI≥3 or $tcCO_2$≥6.7kPa>2%TST with non-parametric tests for unpaired variables. Serum and EBC cytokines levels were correlated with ODI, mean overnight $tcCO_2$ and AHI via Spearman rank correlation.

4. RESULTS

4. a. Study population and sleep architecture

There were 23 patients, 13 male. Median age was 12.6 years (IQR 8.7-14.6). Nine patients, median age 11 years (9.1-15.5) were using overnight NIV support. One patient with CMD was overweight while two patients were thin, one with SMA 2 and one with DM1. All the remaining 20 patients had a BMI within normal range per age. See Table 1.

	SMA 2	CMD	CMYO	CMS	DM1	HMSN
N	7	5	3	3	4	1
Median Age (IQR)	13.4 (9.7-14.4)	12.6 (8.9-14.1)	13.2 (8.6-14.7)	13.6 (10.4-14.1)	13.4 (8.6-14.1)	15.4
NIV (yes)	4	3	1	0	1	0
AHI	0.6 (0.2-1.4)	1.6 (0.0-3.8)	0.1 (0.0-0.5)	0.2 (0.0-0.4)	2.1 (0.9-3.4)	2.7
oAHI	0 (0.0-0.2)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.2)	0.1
cAHI	0.3 (0.0-0.9)	1.6 (0.0-3.8)	0.1 (0.0-0.5)	0.2 (0.0-0.4)	1.5 (0.6-2.0)	2.1
Mean SpO2 (%)	97.7 (97.1-98.9)	97.3 (94.9-98.3)	98 (94.5-99.1)	98.5 (94.7-99.8)	97.5 (91.9-98)	97.8
ODI	1.6 (1.1-2.5)	8.2 (1.7-11.1)	0.9 (0.3-5.5)	0.9 (0.5-3.4)	2.8 (1.4-7)	4.1
Mean TcCO2 (kPa)	5.9 (5.3-6.1)	5.7 (5.4-6.6)	6.5 (5.6-8.4)	6.1 (5.8-6.7)	6.3 (6.0-7.0)	6.1
Sleep efficiency (%)	79.5 (66.6-84.0)	90.4 (80.3-92.4)	82.8 (77.3-86.3)	87.5 (83.6-91.3)	83.8 (46.4-94.8)	91.7
Stage 1 (%)	8.6 (4.0-12.4)	2.9 (2.0-7.9)	3.1 (3.1-4.3)	5.7 (1.5-9.9)	4.2 (2.8-4.8)	3.7
Stage 2 (%)	42.1 (38.6-47.2)	48.4 (42.6-52.7)	48.6 (40.4-55.5)	59 (50.8-67.1)	46.3 (31.7-49.5)	54.6
Stage 3 (%)	32.4 (27.9-34.5)	26.7 (23.6-32.1)	30.6 (22.6-39.7)	22.3 (19.8-24.7)	29 (26-56.2)	19
REM (%)	20 (15.2-22.3)	19.8 (18.3-23.2)	16.8 (16.4-18.8)	13 (6.6-19.4)	20.2 (7.7-21.9)	22.6
Arousal/TST	12.2 (6.9-13.1)	6.4 (3.6-16.0)	6.1 (3.5-15)	8.4 (3.1-13.6)	5.8 (5.0-7.1)	8.9

Table 1. Clinical and sleep features of study population

SMA 2: Spinal Muscular Atrophy type 2, **CMD:** Congenital Muscular Dystrophy, **CMYO:** Congenital myopathy, **CMS:** Congenital Myastenic Syndrome, **DM1:** Congenital myotonic dystrophy, **HMSN:** Hereditary motor sensory neuropathy.

NIV: non-invasive ventilation, **AHI**: apnoea-hypopnoea index, **oAHI:** obstructive apnoea-hypopnoea index, **cAHI:** central apnoea-hypopnoea index, **SpO2(%):** oxygen saturation, **ODI:** oxygen desaturation index, **REM:** rapid-eye movement sleep, **Arousal/TST:** EEG arousal/total sleep time

Of the nine patients who were on NIV, four still had abnormal $tcCO_2$. Seven children had ODI≥3/h, fourteen had ODI<3/h and data were missing in two. Ten children had abnormally elevated CO_2, eleven normal, two had missing CO_2 data. Of the 10 patients with high CO_2, 3 had elevated ODI.

Mean SpO_2 was within the normal range for all the patients. None had significant OSA (oAHI=0 (IQR 0-0)). Sleep architecture was normal in the overall cohort and in the underlying diagnostic subgroups. See Table 2.

	N=23
Age (y)	12.6 (IQR 8.7-14.6)
Sleep architecture	
Sleep efficiency	83.7 (IQR 77.4-90.7)
Stage 1 (%)	4.1 (IQR 2.7-8.9)
Stage 2 (%)	47.3 (IQR 41.9-51.4)
Stage 3 (%)	28.2 (IQR 24.9-34)
REM (%)	19.3 (IQR 16.5-22.0)
Arousal/TST	7.4 (IQR 4.0-12.2)
Arousal/REM	8.3 (IQR 5.2-11.7)
AHI	0.7 (IQR 0.1-2.1)
oAHI	0 (IQR 0-0)
cAHI	0.4 (IQR 0-1.5)
Overnight gas exchange	
Mean SpO_2 (%)	97.8 (IQR 95.5-98.4)
ODI	1.9 (IQR 1.1-4.8)
Mean $TcCO_2$ (kPa)	6.0 (IQR 5.7-6.4)

Table 2. Polysomnographic results of study population

NIV: non-invasive ventilation, **AHI**: apnoea-hypopnoea index, **oAHI:** obstructive apnoea-hypopnoea index, **cAHI:** central apnoea-hypopnoea index, **SpO2 (%):** oxygen saturation, **ODI:** oxygen desaturation index, **REM:** rapid-eye movement sleep, **Arousal/TST:** EEG arousal/total sleep time

4. b. Cytokine levels in EBC and serum

Four patients were unable to perform EBC collection, two generated EBC volumes below the threshold for analysis. In seventeen EBC samples, TNFα and IL-1β levels were below the limit of detection. Sera were available for nineteen patients. See Table 3.

	Serum	EBC
IL-10 (pg/ml)	0.23 (0.17 - 0.49)	0.01 (0.01-0.02)
IL-6 (pg/ml)	0.80 (0.59 - 1.26)	0.80 (0.59-1.26)
IL-1β (pg/ml)	0.19 (0.08 - 19.51)	-[a]
TNF-α (pg/ml)	1.58 (1.38 - 1.94)	-[a]
IL-17 (pg/ml)	-[a]	-[a]
IL-23 (pg/ml)	1.67 (0.78-4.1)	-[a]
hsCRP (ng/ml)	450.76 (114.54 - 884.38)	0.92 (0.34 - 12.01)
ICAM-1 (ng/ml)	350.42 (281.29 – 466.73)	0.29 (0.19 – 0.52)
VCAM-1 (ng/ml)	414.34 (310.71 – 481.21)	1.04 (0.79 - [b])

Table 3. Levels of cytokines in serum and EBC in study population (n=23), expressed as median and IQR.

IL-10: Interleukin 10, **IL-6:** Interleukin 6, **IL1-β:** Interleukin 1 beta, **TNF-*α*:** Tumour Necrosis Factor alpha, **IL-17:** Interleukin 17, **IL-23:** Interleukin 23, **hsCRP:** high sensitivity C Reactive Protein, **ICAM-1:** Intercellular Adhesion Molecule 1, **VCAM-1:** Vascular Cellular Adhesion Molecule 1, **EBC:** Exhaled Breath Condensate.

[a] In seventeen EBC samples, TNFα, IL-1β IL-17 and Il-23 levels were below the limit of detection. IL-17 serum levels were below the limit of detection

[b] Not possible to estimate

4. c. Comparison of cytokine levels according to the presence of sleep disordered breathing

Median (IQR) serum IL-6 value was 1.2 pg/ml (0.9-1.9) in the seven children with ODI≥3, higher (p=0.02) compared with 0.6 pg/ml (0.4-0.8) in the remainder. See Figure 1.

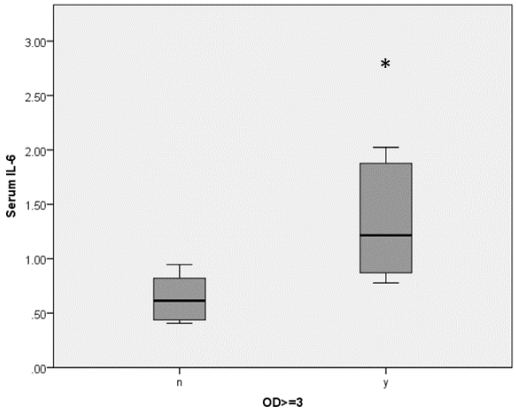


Figure 5. *Comparison of IL-6 levels according to the presence of sleep disordered breathing. The serum levels of the pro-inflammatory cytokine IL-6 were significantly higher (p=0.02) in children with higher Oxygen desaturation index ODI≥3/h.*

Median (IQR) serum ICAM-1 was 410.9 ng/ml (361.7-541.9) in children with high CO_2 compared with 333.2 ng/ml (246.6-349.7) in the remainder (p=0.04). Median serum VCAM-1 was 471.6 ng/ml (384.4-542.9) in children with high CO_2 compared with 315.8 ng/ml (271.9-361.6) in the remainder (p=0.01). See Figure 2.

There were no other significant differences. Relating cytokine levels to the presence of oAHI≥1 was not feasible as no patient had OSA.

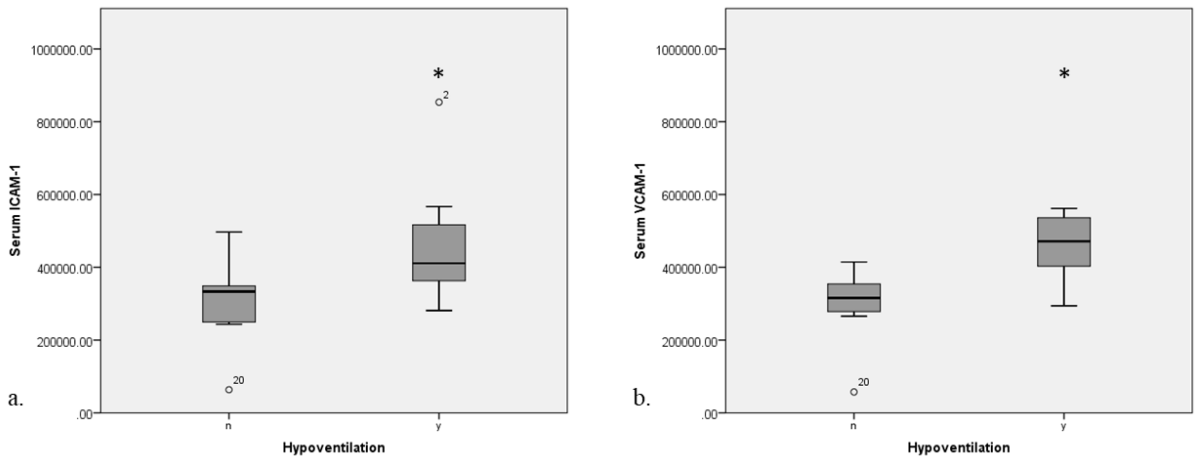


Figure 3. *Relationship of ICAM-1 levels and sleep disordered breathing. Overnight elevated CO2, defined as mean tcCO2≥6.7kPa>2% sleep, was associated with higher serum adhesion molecules ICAM-1 (p=0.04) and VCAM-1 (p=0.01) (a. and b.).*

4. d. Correlation between cytokine levels and CO_2 levels, oxygen desaturation index (4%)

Mean overnight $tcCO_2$ levels positively correlated with serum levels of ICAM-1 (r=0.570, p=0.026) and VCAM-1 (r=0.76, p=0.001). See Figure 2. ODI did not correlate significantly with any measurement.

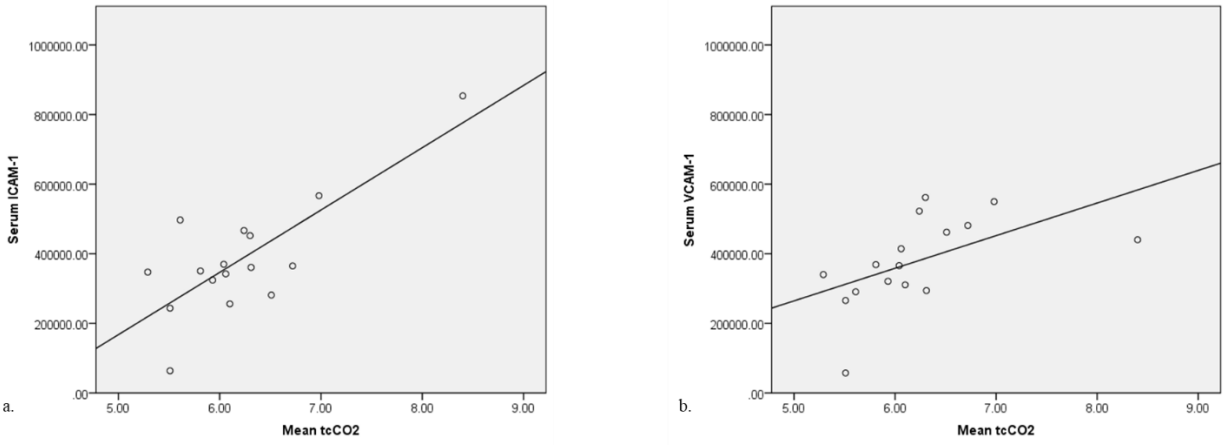


Figure 4. *Mean overnight $tcCO_2$ levels positively correlated with serum adhesion molecules ICAM-1 (r=0.570, p=0.026) (a.) and VCAM-1 (r=0.76, p=0.001) (b.).*

5. DISCUSSION

There is extensive work on inflammation in otherwise healthy patients with OSA, but much less is known about whether inflammation is present in NMD patients with SDB not related to OSA. The results of this pilot study suggest that SDB in NMD patients is indeed associated with a pro-inflammatory state. Children with an abnormally elevated number of desaturations per hour have significantly higher serum IL-6 (p=0.02). The median serum IL-6 level in this study was 1.2 pg/ml in children with ODI≥3, similar to levels reported in otherwise healthy children with OSA and higher than children without OSA (mean 1.1 ± 0.2 pg/ml)[245].

Additionally, children with a mean transcutaneous CO_2 above 6.7kPa (50 mmHg) for more than 2% of their sleep time, which some clinicians have used as a criteria for starting NIV [97], had raised serum levels of VCAM-1 (p=0.01) and ICAM-1 (p=0.04). Moreover, the mean overnight CO_2 correlated with serum VCAM-1 (r 0.76, p=0.001) and ICAM-1 (r 0.570, p=0.026). This association has not been described before.

ICAM-1 and VCAM-1 have been found to be significantly increased in adult patients with OSA when compared to controls [294]. One study has described elevated ICAM-1 levels in children with OSA compared with age-matched healthy controls [306], but another study found that their elevation was associated with obesity rather than OSA [75]. None of the patients in this cohort was defined as obese as per WHO guidelines. All except one had BMI within the normal range for age, and only one was considered overweight, so weight was not a confounding factor in this work.

IL-6, VCAM-1 and ICAM-1 are all thought to play a role in atherosclerosis and their elevation may predispose these patients to future cardiovascular morbidity. Furthermore, ICAM-1 mediates influenza virus penetration into lung epithelial cells [298] and is also one of the

receptors for rhinovirus [297]. We speculate that increased ICAM-1 expression could make NMD children more vulnerable to viral infections and pulmonary exacerbations.

In this study, there was no correlation between hypoventilation and airway inflammation measured using EBC. However, we found that EBC is a difficult technique in NMD because of problems with the use of the mouthpiece, reducing the power of the study.

The study is novel and investigates potential systemic consequences of SDB in NMD children. It is exploratory, and the lack of healthy controls is an acknowledged weakness. The comparison between the serum levels of cytokines in this cohort of patients with an ODI ≥ or < 3 and the levels in healthy non-obese children used as controls in previous studies are presented in Table 4.

	NMD children ODI<3	**NMD children ODI≥3**	**Healthy controls**	**OSA children**	**Reference**
IL-10, pg/ml	0.27 (±0.16)	0.39 (±0.27)	2.10 (±0.28)	2.62 (±0.39)	[245]
IL-6, pg/ml	1.50 (±2.91)	1.33 (±0.52)	1.10 (±0.18)	1.66 (±0.23)	[245]
IL-1β, pg/ml	1.87(±4.76)	6.64 (±13.16)	0.42 (±0.27)	0.36 (±0.16)	[245]
TNF-α, pg/ml	1.62 (±0.48)	1.80 (±0.47)	126.200 (±9400)	125.800 (±8300)	[245]
IL-17, pg/ml	NA	NA			
IL-23, pg/ml	1.07 (±0.83)	2.20 (±1.74)	12.29 (±0.73)	14.58 (±0.75)	[245]
hsCRP, mg/l	0.57 (±0.47)	12.84 (±20.60)	0.41 (±0.48)	1.90 (±0.44)	[245]
ICAM-1, ng/ml	343.12 (±120.22)	473.21 (±241.75)	369.8 (±116.4)	421.7 (±115.6)	[307]
VCAM-1, ng/ml	362.81 (±142.58)	411.01 (±93.80)	NA	NA	

Table 4. Mean (±SD) serum cytokines levels in NMD patients from this cohort (stratified according to oxygen desaturation index, ODI) and paediatric healthy controls and OSA children from published studies

IL-10: Interleukin 10, **IL-6:** Interleukin 6, **IL1-β:** Interleukin 1 beta, **TNF-α:** Tumour Necrosis Factor alpha, **IL-17:** Interleukin 17, **IL-23:** Interleukin 23, **hsCRP:** high sensitivity C Reactive Protein, **ICAM-1:** Intercellular Adhesion Molecule 1, **VCAM-1:** Vascular Cellular Adhesion Molecule 1

6. CONCLUSIONS AND FUTURE STUDIES

NMD children with SDB unrelated to OSA have elevated levels of pro-inflammatory cytokines and adhesion molecules, which may predispose to systemic co-morbidities. In our population, those with an elevated ODI had raised IL-6 levels, and those with elevated CO_2 had elevated cell adhesion molecules VCAM-1 and ICAM-1.

Intermittent hypoxia was associated with increased levels of IL-6 in keeping with previous finding in OSA-related hypoxia. The circulating isoforms of VCAM-1 and ICAM-1, derived from the cleavage of the transmembrane isoform, were associated with overnight hypercapnia. The concept that systemic high levels of CO_2 could also stimulate a pro-inflammatory environment is new, and is particularly relevant in NMD. It is not known whether CO_2 alone is directly causally related to the increased mediators of inflammation or also the mechanisms behind CO_2 retention such as respiratory muscle weakness or atelectasis play a role. These patients invariably suffer from overnight hypoventilation at some point in the course of the disease. IL-6, as previously described, plays an important role in the enhancement of the pro-inflammatory cascade. It is also an important mediator of the vascular pro-atherogenic processes. VCAM-1 and ICAM-1 are also recognised mediators of atherogenesis. The membrane-bound isoform of ICAM-1 is as receptor for virus and other infective agents. Taken together, this suggests that any long-surviving children with NMD suffering from uncorrected SDB may be more prone to viral infections or future cardiovascular morbidity if they are not effectively treated.

The results presented in this chapter need confirmations with a more robust study design, with larger numbers of NMD children and an age-matched healthy population. Moreover, stratification by the underlying genetic condition should be performed, as, for instance, DMD boys progress towards more severe overnight hypoventilation than patients with SMA type 2

and 3 at the same age. Another relevant issue is the evaluation of the causative role that inflammation and its mediators have in the long-term complications of the long-surviving NMD children. The assessment of co-morbidities and inflammatory levels in teenage-young adults and of the presence/severity of SDB and/or NIV requirement at younger age may help answer this question. The long-term progression of the inflammatory state and its association with cardiovascular co-morbidities may be important, for example, in long-surviving patients with DMD. Long-term observations in NMD children could clarify whether there is a susceptibility to infections mediated by ICAM-1 and whether NIV has a role in reducing the systemic inflammatory state and the risk of multi-organ co-morbidities. We could not assess inflammation pre- and post- NIV establishment to see whether treatment of the SDB results in reduced inflammation, and this is another important aspect for future study. NIV itself can be associated with rhinitis and upper airway inflammation [308]. Randomised controlled trials are needed to determine whether early treatment of SDB with NIV can reduce inflammation and thereby affect future morbidity, and whether other anti-inflammatory strategies would be beneficial.

Chapter 5

CONCLUSIONS

The neuromuscular field is facing exciting times as novel therapeutic opportunities are becoming available, particularly for DMD and SMA. New disease phenotypes are consequently emerging. The extent of this remarkable progress on patients' motor function can be more readily estimated if we have a portfolio of validated assessment tools and high-quality natural history data. However, the progression and the specific features of respiratory function in most diseases remain only partially known. Firstly, long-term respiratory natural history data are scanty for most NMD. Secondly, the existing tools used to assess respiratory function and the severity of SDB are not always ideal. The main reasons are either that they not targeted for young non-cooperative children, or that they are validated only for otherwise healthy children. Furthermore, forced manoeuvres may be impossible, or underestimate underlying pulmonary function, if the child is weak. Thirdly, the progressive overnight ventilatory impairment leading to SDB not only causes morbidity in its own right but may also initiate a cascade of events leading to other, systemic complications. New long-term complications such as renal stones and osteopenia secondary to immobility are already emerging [309-311], and more can be anticipated. Similarly, as patients with cystic fibrosis live longer, the importance of early recognition and treatment of complications such as diabetes and bone disease has emerged. The projects included in this thesis were aimed to develop these three nascent issues.

Statement of principal findings

- **Objective 1 (Chapter 2) - To identify the natural history of decline in respiratory function in the commonest paediatric neuromuscular disorder (DMD) and the role of treatment and co-morbidities.**

The effect on respiratory function of either deflazacort or prednisolone administered either daily or intermittently was unknown.

The hypothesis of this work was that different corticosteroids administered by different dosing regimens would have a differential impact on the respiratory decline in DMD and on the achievement of respiratory endpoints when compared to no treatment. The main aim of the project was to identify the impact of corticosteroids on respiratory function, i.e. deflazacort and prednisolone, administered either intermittently or daily vs no treatment. The secondary aim was to describe the long-term respiratory progression and the age at which important respiratory thresholds were reached in a large UK cohort of paediatric DMD followed in a single tertiary care centre.

Two-hundred-ninety-three DMD boys were followed for a mean of 5.6 years during a 10-year period. Information on corticosteroid therapy was missing for 23 patients. Irrespective of the regimen, corticosteroid-treated boys (n=248) reached clinically meaningful respiratory thresholds (FVC % pred. <50% and NIV requirement) later than steroid naïve patients (n=22) as previously reported [106]. The earlier onset of respiratory impairment in corticosteroid-naïve boys was associated with attaining a lower peak FVC % rather than a faster annual rate of decline. Corticosteroids, particularly if administered daily, had a positive effect on lung function (higher peak FVC %) before the onset of decline. The respiratory progression in boys treated with corticosteroids was then subdivided according to the chosen steroid and the regimen of administration. Irrespective of the regimen, deflazacort was significantly less

effective in maintaining FVC > 50% than prednisolone ($p<0.05$). The project achieved its main aim. The time to achieve long-term respiratory milestones in treated and untreated patients was able to be determined even when further breaking down the cohort into subgroups. However, the different sizes of each subgroup could have affected the exact estimation of the yearly rate of respiratory progression. The number of steroid-naïve patients was small, making estimates of untreated natural history less reliable. The other main weakness is the lack of randomisation to steroid therapy, and the different regimes.

- **Objective 2 (Chapter 3) - To define the reliability of standard and novel outcome measures for the assessment of respiratory and sleep abnormalities in paediatric neuromuscular disorders**

Three separate yet related projects were included in Chapter 3.

Project 1. "Non-invasive assessment of respiratory pattern in Spinal Muscular Atrophy type 1 by Structured Light Plethysmography"

The hypothesis of this work was that the breathing pattern of infant and children with SMA type 1, captured via SLP, would reflect their clinical status. The main aim was to determine whether SLP would capture the classical respiratory features of young children with SMA type 1. The secondary aim was to correlate the grade of abnormality of breathing pattern captured by SLP and the severity of peripheral motor dysfunction by standardized clinical scales.

SLP was used to capture chest and abdominal movements during quiet breathing in 19 infants and young children with SMA type 1 (median age 2.3 years). SLP traces identified the selective components of chest and abdomen to breathing. The breathing pattern captured by SLP in the study population was then compared to published reference values for age-matched children. The breathing pattern of children with SMA type 1 enrolled in the study was characterised by rapid shallow breathing, a predominantly abdominal contribution to breathing and chest-

abdomen asynchrony. The extent of the thoraco-abdominal asynchrony, measured by the SLP phase angle, negatively correlated with patients' motor function assessed via CHOP-INTEND. The first project has only partially achieved the aim of defining the reliability of SLP in children with SMA type 1. The small sample size is small, whether there is a differential response of respiratory and peripheral muscles to Nusinersen therapy could not be determined, and there was no control population.

The two other projects described in Chapter 3 assessed the role of oximetry and capnography in otherwise healthy children and NMD children, in order to improve efficiency and cost-effectiveness of screening NMD patients for SDB.

Project 2. "The McGill score as a screening test for obstructive sleep disordered breathing in children with co-morbidities"[312]

The hypothesis of this study was that the positive and negative predictive values of the McGill score in detecting OSA would be worse in children with associated comorbidities such as NMD compared with otherwise healthy children. The main aim was to determine the relationship between the McGill score and CR Polygraphy in the diagnosis of OSA in children referred to a tertiary care paediatric sleep centre.

The McGill score is currently used to grade the severity of changes in overnight oximetry. In 50 children with neurological conditions including NMD, only 70% (for OSA defined as oAHI $\geq$ 1 at CR Poly) and 60% (for OSA defined as oAHI $\geq$ 5 at CR Poly) of those with abnormal McGill scores had OSA. A high number of false negatives caused this low PPV in NMD. As expected, the McGill score had a good PPV and NPV in otherwise healthy children. Thus the starting hypothesis was confirmed.

Project 3. "Reducing the need for CO_2 monitoring in the investigation of paediatric sleep disordered breathing"[313]

The hypothesis of this work was that overnight CO_2 data did not change management in otherwise healthy children who are being investigated for obstructive sleep apnoea (OSA) but was important in those with pre-existing medical conditions or co-morbidities, when added to CR Polygraphy data. The main aim was to determine how often the addition of overnight CO_2 monitoring changed the clinical management of patients when added to CR Polygraphy.

The addition of capnography to CR Polygraphy did not change management in 130 otherwise healthy children but it changed treatment in 12 out of 112 (11%) NMD children. All 12 patients had critical levels of CO_2 requiring the establishment of NIV, again confirming the original hypothesis.

- **Objective 3 (Chapter 4) - To investigate whether the presence and severity of sleep disordered breathing in paediatric neuromuscular disorders is associated with a pro-inflammatory airway and systemic state that could potentially play a role in the development of co-morbidities**

Children with NMD are at risk of SDB, including nocturnal hypoventilation and OSA. The hypothesis of this project was that SDB in NMD would be associated with systemic and airway inflammation. The main aim was to relate systemic (serum) and airway (EBC) levels of pro-inflammatory cytokines and adhesion molecules to the severity of SDB to determine whether there is any evidence of systemic and airway inflammation in children with NMD with SDB and to determine whether any systemic and airway levels of inflammation present relate to the severity of SDB.

In 23 NMD children, in line with previous findings in OSA, the presence of chronic intermittent hypoxia, defined as an oxygen desaturation index per hour of sleep above 3 (ODI>3/h sleep), was associated with increased levels of IL-6. Hypercapnia, defined as tcCO2>6.7kPa (50 mmHg) for >2% sleep, was associated with an increase of circulating isoforms of the adhesion

molecules VCAM-1 and ICAM-1. This project was exploratory, it has provided pilot data for future larger and more detailed studies to confirm or otherwise the findings. Overall, it has achieve the objective of showing preliminary evidence that there is a link between inflammation and SDB in NMD.

In summary, the projects included in this thesis add novel perspectives to the current management of NMD. They expanded the knowledge of the effect of standard of care therapies on respiratory function in DMD, provided new insights on the correlation between breathing pattern and motor function in SMA type 1, contributed to the most efficient use of sleep assessments including the need for CO_2 monitoring in NMD and, lastly, identified potential new pathways linking SDB and systemic co-morbidities in NMD.

Strengths and weaknesses

The studies were all performed in tertiary care centres with considerable expertise in NMD, either in Italy (Paediatric Neurology and Muscle Disease Unit, IRCCS Istituto Giannina Gaslini, Genoa) or in the UK (Dubowitz Neuromuscular Centre, UCL Great Ormond Street Institute of Child Health and Department of Pediatric Respiratory Medicine, Royal Brompton Hospital, London).

The strength of the first project is that high-quality lung function data was obtained by highly skilled operators using the same equipment and protocols over the whole study period. Additionally, the analysis included a broad cohort of~300 DMD boys followed for over 5 years by the same tertiary care centre (Dubowitz Neuromuscular Centre, UCL Great Ormond Street Institute of Child Health). The retrospective design meant some children switched between corticosteroid treatment and dose regimens during the study. Those who switched therapeutic regimes were stratified in subgroups according to the steroid and regimen they have been using

for > 60% time of the time of observation throughout the study. The corticosteroid treatment was not randomly assigned.

The sample size of the two projects aiming to evaluate the McGill score and CO_2 levels in otherwise healthy and in NMD were large enough to ensure reliable comparisons. The use of standardised equipment and protocols for sleep assessment is a strength.

The main limitations are the design of the two prospective studies included in Chapter 3 and Chapter 4. The former evaluated the breathing pattern of SMA type 1 captured by SLP and the latter assessed the relationship between SDB and inflammation. They both had a relatively small sample size (respectively 19 and 23 children) and more importantly, they both lacked an age-matched control population. In both cases, the results obtained were compared to published values for aged-matched populations. Hence the data are preliminary, to inform the design of future studies.

Finally, the comparison of oximetry with CR Poly rather than PSG in the diagnosis of OSA, likely caused an underestimation of McGill's PPV and NPV in NMD but also in the otherwise healthy children. Nonetheless, the conclusion, that CO_2 monitoring is essential in NMD, is not affected.

Strengths and weaknesses in relation to other studies

The studies presented in this thesis are relatively novel and there are only a few previous manuscripts in the field.

The effect of different corticosteroid regimens was known only for motor function in DMD; the effects on respiratory progression had not previously been studied. We did not observe any difference in long-term respiratory function between daily and intermittent regimens, while deflazacort was associated with less stability in lung function compared with presnisolone. The studies on motor function showed slightly different results. DMD patients treated daily were

reported to lose ambulation 2.5 years later than those treated intermittently [193] and deflazacort was more effective in delaying the loss of ambulation and motor skills [194, 196]. The studies on motor function were smaller (114)[195, 196], or followed patients over a two-year follow-up [194]. The only ongoing randomised controlled trial, the "FOR DMD" *(ClinicalTrials.gov Identifier NCT01603407)* will address the question whether the intermittent (i.e. 10 days on/10 days off treatment) or daily regimen is more effective over five years [314]. However, as the age at enrolment is 4 to 7 years, DMD boys could still be too young to reach clinically meaningful respiratory endpoints.

The results obtained in the SLP assessment of the breathing pattern in SMA type 1 during wakefulness are similar to those obtained in recent cross-sectional studies that utilised OEP [29], respiratory inductance plethysmography [230] and FOT [216]. However, OEP is not easily feasible in clinical settings and FOT was performed in older SMA type 1 patients than those in the study presented in this thesis. Only seven children with SMA type 1 underwent respiratory inductance plethysmography, and further larger studies need to confirm whether it is be a feasible and reliable tool.

The McGill score had an excellent PPV in otherwise healthy children as in previous studies, capturing nearly 100% of children with OSA [76]. The use of CR Poly in the project presented in the diagnosis of OSA instead of full PSG (above) could in part explain why the score in otherwise healthy children had lower a PPV than previously reported [315].

Finally, little was known about whether inflammation is present in NMD patients with SDB not related to OSA. While the role of IL-6 has been extensively studied both in adults and in children with OSA [243, 263, 264, 316] and its role in atherogenesis has been described [267], the finding linking the adhesion molecules such as ICAM-1 and VCAM-1 to abnormally high levels of CO2 has never been reported. Both adhesion molecules are considered downstream

mediators of tissue damage in children and adults of OSA particularly if obese [294, 295]. Patients' body weight did not play a role in the patients analysed in the study presented as their BMI was within WHO ranges of normality.

Meaning of the studies

The projects presented have described new tools for clinicians looking after the neurological, respiratory and sleep aspects of NMD children. Firstly, mostly for the commonest paediatric NMD such as DMD it is important clinicians know what to expect from the respiratory natural history, for instance what is the expected age at significant milestones. Secondly, paediatric neurologists need to be aware, when prescribing corticosteroids that the compounds currently available administered at different regimens might differentially affect respiratory and peripheral motor function. The results of the first project also suggested that corticosteroids, with their beneficial role in delaying the respiratory impairment, should not be stopped, as often happens, when boys lose ambulation, but again, a further randomised controlled trial is needed to confirm this, especially considering the potentially deleterious effect of corticosteroids on bone mineral density. Finally, these data can help the design of future clinical trials. It provides respiratory trajectories for steroid-treated DMD disease and can help accurate power calculations, especially in studies adding additional treatments to steroid therapy.

With the advent of new therapeutic options for SMA, the need for new respiratory outcome measures has emerged. This is particularly true for children at the severe end of the spectrum, (SMA type 1). They are those who mostly derived benefit from the new treatments from a motor and respiratory perspective, but also those whose age and clinical severity prevent the performance of standardised lung function tests. The results of the study presented have clinical implications. Children with SMA type 1 had improved motor function after treatment with Nusinersen and gene therapy. Those results were associated with different extents of

improvement of breathing pattern characterised by the reduction of paradoxical breathing [317]. Furthermore, treated children had almost invariably a reduced or delayed requirement for ventilatory support [124, 129] presumably because of improved respiratory muscle function. Early changes in the breathing pattern, such as the reduction of paradoxical breathing expressed by the phase angle, may be useful in the follow-up of these children, but this needs to be determined in future work. The phase angle was shown to be strongly correlated with motor function and, can be regularly assessed by SLP in clinical settings. New tools are also needed for the identification and follow-up of SDB in NMD. Oximetry alone is still frequently used as first line overnight assessment in NMD, because it is simple to perform at home. However, not only it is not suitable for detecting obstructive events due to the high rate of false positive but also it would miss the diagnosis of hypoventilation if not coupled with capnography. Previous work showed that 13 of 29 NMD children with nocturnal hypoventilation had normal oximetry [73]. The novel results in this thesis are that CR Poly alone is not sufficient for the management of SDB in children with NMD if CO_2 monitoring is not performed.

Finally, many more of NMD children are reaching adulthood than in the past, but they almost invariably develop SDB needing long term ventilatory support. IL-6 was associated with high number of overnight desaturations. IL-6 is known to be involved in pro-atherogenic processes and in the maintenance of the inflammatory cascade [267]. VCAM-1 and ICAM-1 were associated with high levels of CO_2. These adhesion molecules have multiple roles. Both are expressed on endothelial cells during inflammation and facilitate the adhesion of leukocytes, which is also the first step towards the formation of atherogenic plaque [293, 295]. They were detected in alveolar epithelial cells made hypoxic and are thought to be mediators of hypoxic lung damage [290]. Hypoxia-induced vascular remodelling is a multi-factor process leading to progressive vasoconstriction and chronic pulmonary hypertension [318, 319]. ICAM-1 is also

a receptor for influenza virus and rhinovirus. Influenza virus triggers the expression of ICAM-1 on the cell surface. The binding of rhinovirus to ICAM-1 triggers its endocytosis and its translocation into the cytosol [297, 298]. Children with NMD suffering with SDB might therefore be more prone to viral infections and cardiovascular morbidity in the long-term if they are not effectively treated. These results, although may have important clinical implications, derived from a small cross-sectional study and therefore need to be investigated further.

Unanswered questions and future research

DMD standards of care have affirmed that ambulatory status (ambulant, early non-ambulant, late non-ambulant) affects respiratory risk. More work is needed to better characterise the respiratory and sleep features in DMD and their progression within each of these three phases of the disease. The UK North Star network and other international multicentre networks such as AFM (EU) and CNRG (US) have made available a large pool of data for the description of natural history of DMD. They have helped the identification of trajectories of survival, motor function and the identification of gene modifiers through the availability of very large cohorts with high-quality data. So far, the North Star network includes over 1000 DMD patients, both children and adults followed across UK centres.

To test the hypothesis that patients' age, motor status and *DMD* mutations in addition to steroid treatment would differentially affect respiratory function, a prospective multicentre observational study should be set-up in a large population of paediatric and adult DMD patients within the North Star network. This study should entail the six-monthly recording of daytime clinical symptoms of hypoventilation, FVC and PEF absolute and % pred. according to GLI data [19], tests of expiratory and inspiratory muscle function such as PCF and SNIP and yearly overnight gas exchange data and. Patients should be grouped according to their age range and

their ambulation status. Patients' genetic background, height and weight, severity of scoliosis and corticosteroid treatment can be used as covariates for analyses of progression. The results of this work will provide estimates of the risk of developing SDB and NIV requirement according to patients' motor status and daytime respiratory function. These results will also help the design of future clinical trials by providing the expected rate of daytime and overnight respiratory progression according to the age/motor function at patients' enrolment.

As previously mentioned, in children with SMA type 1 the improvement of breathing pattern, expressed as reduction of paradoxical breathing, could represent a more sensitive and potentially early sign of respiratory response to the new treatments [317]. The further step will be to test the hypothesis that the breathing pattern captured by SLP and motor function would respond differently to Nusinersen and other treatments such as gene therapy in children with SMA type 1. A study enrolling children with SMA type 1 who were not yet treated with any of the novel therapies can address this question. Subjects (ideally n >30 to detect differences in 10° phase angle with a CI 95%, https://www.openepi.com/SampleSize/SSCohort) will undergo SLP recording of tidal breathing and a motor function assessment via CHOP-INTEND at baseline, defined as before the first dose of either Nusinersen or the one-off infusion of gene therapy or the oral Risdiplam and after one month and one year from the first dose. For those receiving Nusinersen, the first four doses administered during the first month represent the loading dose. The same time points will be maintained for children receiving other therapies. At the three time points, for each child, the requirement of ventilatory support, the respiratory rate, the contribution of chest and abdomen to the breath and the degree of paradoxical breathing and the rate of change will be correlated with motor function.

Additionally, despite the fact that different NMDs such as SMA type 1, CMD and some CMYO children all have early onset hypotonia, the extent of involvement of respiratory muscles and

ventilatory requirements are different and not fully elucidated. A study with first a cross-sectional component and a second longitudinal component will test the hypothesis that SLP could capture individual differences in the awake and sleep breathing pattern in children affected by specific NMDs and healthy controls. To ensure a comparison with 95% CI of detecting meaningful ($p<0.05$) differences between cases and controls, at least 36 children for each diagnostic group (SMA type 1, LAMA2-related CMD, early onset CMYO) and at least 10 age-matched controls should be enrolled (https://www.openepi.com/SampleSize/SSCC). Patients should have early onset (before 6 months of age) clinical signs of hypotonia. All children enrolled will undergo a five-minute recording of SLP during awake tidal breathing and sleep followed by motor function scoring with CHOP-INTEND at baseline and after 6 months to evaluate the presence of changes in respiratory pattern within the first year of age when children with hypotonia are more vulnerable to respiratory co-morbidities. The respiratory variables at baseline will be compared between diagnostic groups and with healthy controls. Motor and respiratory function will be correlated. The data gathered at 6 months will be compared with those at baseline for each diagnostic group in order to assess whether SLP detects small variations in the disease progression, which are useful in clinical follow-up.

Finally, if the results on inflammation linked to desaturations and hypercapnia in NMD were confirmed, the pathways downstream IL-6, VCAM-1 and ICAM-1 may be targets for future treatments. For example, strategies targeting the IL-6 receptor were effective in restoring the respiratory function in a murine model of DMD (mdx mouse) [320]. However, whether there is any link between SDB and inflammation in NMD needs to be confirmed by robustly designed studies. These studies need to take into account several aspects. Firstly, duplicate blood (and EBC) samples should be collected from the same patient at different time points to assess between-occasion repeatability. For example, levels of cytokines such as IL-1β and TNF-α exhibit a circadian rhythm. Secondly, a standardised protocol of assessment including

daytime respiratory and motor function should be carried out in children with NMD to analyse whether the disease status and other underlying conditions such as respiratory muscle weakness or intrinsic lung diseases affect patients' inflammatory state. Thirdly, the collection of serum and EBC cytokines across different NMD diagnostic groups will clarify whether there are intrinsic disease-related differences in the expression of particular cytokines. Cytokine levels should also be compared with age-matched healthy controls and otherwise healthy controls with OSA in order to estimate differences in the degree of inflammation in each disease.

Once good baseline data have been collected, and any abnormalities in cytokines are present in specific diagnostic categories of NMD children with SDB, randomised controlled trials are needed to test whether there is a causative relationship between SDB and systemic co-morbidities. In light of the association between adhesion molecules and hypercapnia found in the study presented and the well-identified causative role of ICAM-1 and VCAM-1 on atherogenesis, the first hypothesis to test would be whether NMD children with hypoventilation had increased atherogenic risk.

In the context of OSA, plasma exosomal microRNAs play an important role in endothelial dysfunction in both children and in adults. Exosomes are endosomal-derived vesicles that are secreted in several biological fluids and contribute to cell-to-cell communication [321]. Several physiological functions of exosomes have been identified in vitro when different types of microRNAs change their abundance inside the vesicles. Exosomes also exhibit pro-angiogenesis, pro-coagulant and pro- or anti-inflammatory effects as well as having effects on vascular tone and the arterial wall, most likely related to the ability of exosomes to transport and transfer of proteins between cells, and as well as microRNAs, and other mediators. The mechanism by which microRNAs are received and processed by target cells is nevertheless undefined [322, 323].

Furthermore, in association with the analysis of circulating exosomes and microRNA, the assessment of arterial peripheral flow can provide mechanistic insight on the association found between high CO_2 and increased serum levels of ICAM-1 and VCAM-1. Endothelium modulates vasodilatation in response to increases in blood flow-associated shear stress mediated by the release of nitric oxygen [324]. This phenomenon has been described as endothelium-dependent flow-mediated vasodilatation (FMD). The non-invasive assessment of FMD uses high-resolution ultrasound to measure dilatation of the artery in response to hyperaemic shear stress induced by blood pressure cuff-induced occlusion of the radial and ulnar arteries, and then release of the cuff. The presence of vascular dysfunction is reflected by an impaired FMD response in either the magnitude of vessel dilatation or the time taken to reach maximal dilation post cuff deflation. This vascular hyperemic response has been used as a surrogate marker for endothelial function in adults and children in order to evaluate the association of obstructive SDB and the risk for hypertention and atherosclerosis. Children with obesity-associated OSA [325] but also lean children with even only mild obstructive SDB had a significant increase of the time to FMD during hyperaemic stress when compared to controls [326]. In non-obese children with OSA, this abnormal vascular response to ischemia-reperfusion was reversed after adenotonsillectomy [327].

In order to test the association between abnormally high levels of CO_2 and atherogenic risk in NMD, the expression of microRNA stimulating ICAM-1 and VCAM-1 (miRNA-222, miRNA-223, miRNA-1185) [328-330] and the response of arterial flow to shear stress will be compared in children with hypoventilation, children with lesser degrees of hypercapnia, children with OSA and controls. At least 40 NMD patients in two diagnostic groups and 10 age-matched healthy controls (https://www.openepi.com/SampleSize/SSCC) would be required. Participants will undergo full PSG and spirometry at baseline. The number of respiratory infections in the previous six months will be recorded. Systemic markers will be

collected in serum and urine and upper airway markers from EBC and nasal brushing on the night before the sleep study and also in the morning on waking. Arterial ultrasound and measurement of FMD will be performed at baseline in the morning. The systemic and airway levels of ICAM-1 and VCAM-1, the circulating levels of the microRNAs known to be associated to the expression of ICAM-1 and VCAM-1 (mi-RNA 222, miRNA-223 and miRNA-1185) and the blood flow velocity will be compared in a. patients with hypercapnia ($tcPaCO_2 \geq 50$ mmHg > 2% total sleep time) without obstructive events (oAHI < 1); b. patients with hypoventilation ($tcPaCO_2 \geq 50$ mmHg > 25% recording [65]) without obstructive events (oAHI < 1); c. patients with OSA (oAHI ≥ 1) and d. healthy controls.

Metabolomic studies in the four subgroups will also potentially allow the profiling of exosomes secreted at different degrees of CO_2 retention. Another method of measuring inflammation is the e-nose that allows the profiling of airway inflammation. Human-exhaled breath contains over 3000 volatile organic compounds (VOCs) in gas phase. These VOCs are produced during metabolic as well as during disease processes in the airways and elsewhere in the body [331]. It has been shown that VOCs analysis may potentially be used as non-invasive diagnostic test for lung inflammatory diseases such as asthma [332], cystic fibrosis [333], COPD [334] and cancer. The introduction of e-noses in the biomedical setting, and analysis of the exhaled breath has become a feasible option, due to the ability to perform real-time profiling of "breathprints" as derived from the composite nano-sensors arrays of e-noses. Commercially available handheld electronic nose connected to a modified spacer with a reverse valve system allowing tidal inspiration through a face mask, inspiratory VOC filter and tidal expiration into the spacer. Breath was collected over five minutes in some reports [333]. As opposed to EBC collection for cytokines arrays, e-nose technology seems to be suitable even for NMD patients as it does not require a tight seal around a mouthpiece or any forced breathing manoeuvres. This is clearly a potentially very promising and powerful tool.

In conclusion, the projects presented in this thesis have answered at least partly most of the research questions propounded at the commencement of the work. These results highlighted ways in which the clinical management of NMD children can be improved in the future. The long-term respiratory progression of DMD was not established until the work reported here. There has been a trend to stop steroids in non-ambulant DMD, but the work reported here suggests this may not be appropriate, and a new clinical trial is needed to test whether the risks of continuing steroids in non-ambulant boys are outweighed by the benefits. Baseline data of the breathing pattern of infants and young children with SMA type 1 captured by new non-invasive techniques is of interest in view of the new therapeutic options available. Additionally, there is the need to customise the existing sleep assessments for the NMD population. Currently, many of these patients undergo standardised tests that do not take into account their unique combinations of central involvement, upper airway obstruction and hypoventilation. This thesis has clinical implications, but also opens new research questions in the field that should be further explored, such as the exploration of the long/term implications of the potential association between inflammation and non-obstructive SDB in NMD.

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