Key Words list: PFT, sleep study, DMD, Duchenne, Respiratory function, respiratory decline, nocturnal hypoventilation, Sleep disordered breathing.

Abbreviations list:

AHI: apnea-hypopnea index

BMI: body mass index

B-NH: borderline nocturnal hypoventilation

CnAHI: central apnea hypopnea index

CS: corticosteroids

FVC: forced vital capacity

FVC%: forced vital capacity % predicted

GLI: Global Lung Initiative

LVFS: left ventricular fractional shortening

LOA: loss of ambulation

NH: nocturnal hypoventilation

NMV: Nocturnal mechanical ventilation

OAHI: obstructive apnea-hypopnea index

OSA: obstructive sleep apnea

PFT: pulmonary function test

PPV/NPV: positive predictive value/ negative predictive value

SS: sleep study

TST: total sleep time

ABSTRACT

Background: The decline of respiratory function in Duchenne muscular dystrophy (DMD) is associated with sleep disordered breathing (SDB) and alteration of nocturnal gas exchange, first manifesting as nocturnal hypoventilation (NH). However, the correlation between pulmonary function measured by spirometry (PFT) and the onset of SDB with or without NH is unclear.

Aim: To identify the prevalence and features of SDB and to investigate the relationship between lung function determined by forced vital capacity (FVC) and sleep abnormalities in a large paediatric DMD population.

Methods: Retrospective, single-center cohort study. FVC% predicted (FVC%) was calculated using predicted equations from the Global Lung Function Initiative. NH was defined by transcutaneous (tc) CO2 >50 mmHg for >25% of total sleep time (TST); borderline NH by a mean tcCO2 between 45-50mmHg or tcCO2>50mmHg for ≤25% of TST; Clinically meaningful obstructive sleep apnea (OSA) by obstructive Apnea–Hypopnea Index >5. The sensitivity, specificity, positive and negative predictive value of FVC<50% to indicate presence of nocturnal hypoventilation were calculated.

Results: 134 patients underwent 284 sleep studies and 1222 PFT. Mean (SD) age at first and last sleep study was 12.9 (2.7) and 14.3 (2.6) years. Borderline NH (n=31) was detected in both ambulant and early-non ambulant subjects, while 100% of NH cases (n=14) were non-ambulant. NH was detected in 4/14 patients despite an FVC>50%. Seventeen/26 patients with OSA presented with concomitant NH or borderline NH. FVC<50% was associated with NH indicating a sensitivity and specificity of 73% and 86%, respectively. Positive and negative predictive value were 32% and 97% respectively. PFT showed a non-linear, sudden FVC% decline in 18% of cases.

Conclusions: FVC% <50 was associated with NH in close to a third of patients. CO2 elevation can be associated with obstructive/pseudo-obstructive events and was also observed in early non-ambulant cases or in the presence of FVC>50%. These results are relevant for the clinical management of SDB.

INTRODUCTION

Duchenne muscular dystrophy (DMD) is an X-linked recessive neuromuscular disorder affecting around 20 (95%CI 16.6-23.6) per 100.000 live male births.[1] DMD is characterized by progressive weakening of skeletal, respiratory, and cardiac muscles leading to loss of ambulation (LOA) by a median age of 10-12 years. [2-5] Along with cardiac failure and arrythmias, respiratory impairment is the leading cause of morbidity and mortality in DMD. [6, 7] The standard of care recommendations on provision of respiratory intervention with non-invasive nocturnal mechanical ventilation (NMV) have contributed to prolong survival over the past decades. [3, 8-12] However, the optimal timing for NMV introduction remains uncertain and usually driven by expert consensus, as there is limited published evidence available for this recommendation. [13, 14]

Respiratory function is routinely measured with pulmonary function tests (PFT), [13, 15] with several parameters providing information regarding inspiratory and/or expiratory function. [16, 17] Forced vital capacity (FVC), measured by spirometry, quantifies dynamic lung volumes and is considered the most informative test to guide therapeutic interventions.[18] Whilst FVC < 1 L has been associated with decreased 3-year survival and 4.1-fold increase in mortality risk, [19, 20] and values <0.68 L with a higher risk of awake hypoventilation, [21] the relationship between pulmonary function and the risk of nocturnal hypoventilation is yet to be clarified. Current guidelines include FVC percentage predicted (FVC%) below 50 as an indication for NMV, particularly in late non-ambulatory stages.[13] In clinical practice though, irrespective of FVC% values , NMV is often not considered until sleep study reveal an abnormal result,[18] which in DMD can be either nocturnal hypoventilation (NH, the first manifestation of chronic respiratory insufficiency) or other sleep disordered breathing (SDB), or in presence of other clinical indications. [22-24]

In the present study we aimed to identify the prevalence and features of SDB in a large pediatric DMD population, and to investigate the relationship between FVC/FVC% and sleep abnormalities.

1. METHODS

1.1. Study Design

This is a retrospective cohort study conducted on pediatric patients (<18years) affected by DMD. We included all subjects with a consistent phenotype, a pathologic *DMD* variant expected to lead to a DMD phenotype, and/or absence of dystrophin on muscle biopsy at immunohistochemistry (IHC). All patients performed at least one cardiorespiratory SS at Great Ormond Street Hospital (London) between 2010-2020. We ultimately considered patients with SS performed before NMV initiation and within 6 months from the first available PFT (Figure 1), a timeframe which was considered reasonable in view of the expected annual decline in FVC%.

1.2. Standard Protocol Approvals, Registrations, and Patient Consents

This study was registered as clinical audit (Ref number 2871) in agreement with Great Ormond Street Hospital trust guidelines and approved by the Institutional Review Board. The ethics board determined that participant consent was not required for a registered audit.

1.3. Data Collection

PFTs are routinely collected on DMD patients attending clinics (every 6-12 months, according to the standard of care); raw data are prospectively and automatically stored in a secured database. This dataset was used to obtain the following variables: age at achievement of specific FVC% thresholds and peak of FVC L/%.

Raw data extracted from sleep studies are prospectively stored in a separated secured database. Importantly, the definitions for respiratory events used for scoring purposes and summarized in Table 1 were retrospectively applied based on both raw parameters (e.g., AHI, CO2 levels etc.) and sleep study reports. From clinical records, we retrieved information at the time of every sleep study including age, weight, height, BMI, ambulation status, corticosteroid (CS) treatment, cardiac function and treatment, scoliosis (severity defined as mild <20°, moderate 20-44°, severe ≥45°, when Cobb Angle was available), and spinal fusion. Information regarding genotype, age at LOA, age at NMV initiation/indication for NMV, presence of behavioral problems or learning difficulties and age at CS initiation were also collected. PFTs parameters were imported from the first dataset.

Pulmonary Function Test

Spirometry was performed according to the Pediatric ARTP Standards by specialist pediatric physiologists.[25] FVC% predicted was calculated using predicted equations from the Global Lung Function Initiative (GLI), including those observations performed prior to the implementation of GLI in our centre.[26] Tests were conducted in the sitting position, over 10-30 minutes, allowing for patient recovery. We considered the longitudinal trajectory of the following measures: FVC (L) and FVC%. Where possible (namely, when we had multiple longitudinal measurements), we calculated the age at FVC% predicted < 60/50/40 (representing progressive thresholds of restrictive respiratory impairment commonly considered in clinical practice), the peak value of FVC (L) and FVC% (which are expected to occur between 10-14 years and 7-9 years, respectively) and age when achieved. [27]

Sleep study

The presence and severity of sleep disordered breathing was assessed via a cardiorespiratory sleep study coupled with transcutaneous (tc) capnography. Sleep studies are not routinely performed in all DMD boys and indications for sleep study may include the presence of symptoms suggestive for hypoventilation/obstruction, pre-surgical screening, occurrence of severe chest infection, low FVC% values, or baseline screening after losing ambulation. However, information regarding the indication for sleep study was not available for every single patient. Further details regarding the device we used are provided in eMethods in the Supplement. We applied criteria to define respiratory events as indicated in Table 1. Specifically, hypoventilation was defined as carbon dioxide >6.7 kPa (>50 mmHg) for >25% of the recorded night as per American Association of Sleep Medicine (AASM) rules.[28, 29] The OAHI threshold of 5 (instead of 1) was used to define clinically significant OSA, as per AASM and European Respiratory society (ERS) guidelines indicating that when OAHI is above 5, OSA is unlikely to resolve spontaneously and the child is at risk for morbidity (i.e. needing closer follow-up, or initiation of treatment in presence of symptoms/other comorbidities). [30] We used the International Classification of Sleep Disorders (ICSD-3) criteria to define central apnea (namely, CnAHI≥5 and CnAHI >50% of the total AHI). To define isolated hypoxemia, we selected an adapted threshold that has been considered clinically meaningful in previously published pediatric populations

[31]. As there is no consensus regarding the definition of borderline-hypoventilation, we applied the criteria indicated in Table 1.

Definition of LOA groups

To correlate age at LOA with the achievement of specific FVC% thresholds, we categorized patients according to the age at LOA ≤ 9.3 years (Group A), 9.3<LOA<12.0 (Group B), LOA ≥12 years (Group C), and ambulant patients at last follow-up (FU) (Group D). The cut-offs of 9.3 and 12 years represent the 1st and 3rd quartile, respectively, of the estimated age at LOA in this cohort, calculated with Kaplan Meier method. The division in 3 groups rather than 4 was chosen to be more representative of "classic" DMD phenotype vs extremes.

1.4. Statistical analysis

Descriptive statistics are presented for all measures, mean and standard deviation were used for normally distributed data and median, IQR and range for skewed data. Categorical data were summarized as frequency and percentage. Individual FVC trajectories were plotted as line charts. Kruskal Wallis was used to compare median values between groups (e.g., LOA groups, NIV indication). Kaplan-Meier method was used to estimate median (IQR or 95% CI) time to event for pre-specified FVC% thresholds. Log-rank tests and Cox regression analysis were used to explore the relationship between LOA groups and time at the achievement of specific FVC% thresholds (i.e., <60/50/40). Multivariable Cox regression analysis was used to adjust for the effect of CS-treatment for those patients who had not achieved such "milestones", we used their age at last PFT as a censored measure.

To establish the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the FVC%<50 threshold to detect NH we considered the first available sleep study for each patient. To describe the prevalence of DMD-related complications (e.g., scoliosis, cardiomyopathy etc.) and concomitant treatments at the time of an abnormal sleep study, we selected the first study in which NH, B-NH, OSA, or hypoxemia were detected for each patient.

We used Stata, R and SPSS software for statistical analysis and a P value < 0.05 was considered significant for all analyses.

1.5. Data Availability

Aggregated data not provided in the article because of space limitations may be shared at the request of any qualified investigator for purposes of replicating procedures and results.

2. RESULTS

2.1. Patients' selection process

Sixteen out of 176 screened patients (9%) were excluded because they could not perform reliable spirometry. Additional 24 patients were excluded because they were either already on NMV at the time of first SS or because spirometry was not conducted within 6 months as per inclusion criteria. Of these 24, 12 subjects had been started on NMV at a median age of 14.4 years.

2.2. Patients' characteristics

This study cohort comprises 134 DMD patients <18 years. 284 SS satisfied inclusion criteria (60 patients had 1 SS, 29 two SS, 24 three SS, and 21 more than four SS). The median (IQR) age at first and last sleep study was 13 years (11-15) and 15 years (13-16) respectively. A total of 1222 PFTs across a median period of 5.9 years (range 0-12.4) per subject were examined and 1217/1222 were considered reliable for analysis. The mean (SD) time between SS and its coupled PFT was 2.4 (1.3) months. Population characteristics are summarized in Table 2.

Estimated median age at LOA in CS-naïve (n=35) was 9.6 years (95% CI 9.1-10.1; IQR 8.8-10.6), in CS-treated patients (> 6 months) (n=98) the median age at LOA was 10.8 years (95% CI 10.4-11.2; IQR 9.7-12.7). One hundred and eighteen (88%) subjects lost ambulation before their last SS, 123 (92%) before their last PFT. At last SS, 27 (20%) subjects were CS-naïve, 27 (20%) had been previously treated with CS ("past"), 79 (59%) were still under CS-treatment.

Thirty-four patients (25%) were established on NMV after last SS, at a median age of 14.9 years. A detailed description of patients' characteristics at the time of NMV establishment according to NMV are listed in eAppendix 1 and eTable1 in the Supplement.

2.3. Progression of respiratory function

As previously described, the progression of FVC% in DMD follows a biphasic trajectory, with acquisition of a peak value in the first decade of life followed by a decline. Patients losing ambulation earlier displayed lower FVC L and % values at peak and achieved FVC L peak at a younger age (eFigure 1).

FVC% decline towards clinically meaningful thresholds was then assessed. Overall, the estimated median (IQR) age when FVC% predicted fell below 60%, 50% and 40% was 15.1 (13.5-16.9), 16.2 (14.7-17.7) and 17.4 years (15.8-18.9), respectively. For each of the FVC% thresholds there was a significant relationship between age at LOA and median age at reaching the threshold (Figure 2 A-C; P <0.001). For FVC%<50 the median (IQR) age for group A, B, and C was 14.4 (13-15.2), 15.9 (14.8-17.5), and 17.7 years (17.5-18.1), respectively. After adjusting for CS treatment, differences between LOA groups remained unchanged.

The overall estimated median (IQR) time from LOA to FVC<50% was 5.8 years (4.2-6.7), with no significant differences between LOA groups (P= 1.66) (Figure 2D).

A subset of patients demonstrated a more rapid decline.

We observed that 23/134 subjects presented an unexpected drop of respiratory function (defined by a decline of >10% in FVC L *and* >20% of FVC% between two assessments occurring within a year). The drop occurred within a median of 8 months (IQR 6.5- 11.7 months) between subsequent assessments. Additional 9 subjects had a decline in FVC% > 10 /year. No significant change in height/ height measurement method accounted for such FVC% variation. For the former 23 patients, the median (IQR) age at FVC drop was 14.6 years (13-16.2), occurring at a median (IQR) of 5.5 years (3.2-7.2) after LOA. Median (IQR) FVC% before and after drop were 46.6% (30.8-58.9) and 31.4% (22.2-40.4). Eleven /23 individuals presented with cardiomyopathy at the age of drop, but ten had a normal cardiac fractional shortening (data not available in 2). The was no prevalence of a specific genotype in patients presenting with sudden drop of FVC L/% (Figure 3A, B). LOA did not occur earlier in these 32 individuals compared to those who declined linearly.

2.4. Characterization of sleep disordered breathing

2.4.1. Overnight gas exchange - Hypoventilation

Fourteen patients (15/284 SS) were diagnosed with NH, eleven from their first available sleep study. All subjects were non ambulant, with a median (range) age at LOA of 9.4 years (6.6-11.7). The median (IQR) age at NH was 14.9 years old (14.2-16.3), with all but two outliers being in a late-non

ambulatory stage (from 4.5 to 7.8 years after LOA). Median (IQR) FVC L and FVC % were 1.4 L (0.9-1.8) and 39% (26.9-63.9), respectively. Nine/14 subjects had severe scoliosis, and 2 moderate scoliosis (Cobb Angle 40 and 42°, respectively). Six/14 subjects were CS-naïve, 6 were past users, and only 2 were currently CS-treated. Ten out of 14 subjects had an underlying cardiomyopathy with a median (IQR) LVFS% of 23.5% (20.7-24.8). The median BMI at first study was 20.2 (range 6.6-37.9). Notably, none of the patients had a history of recurrent chest infections. Seven out of 14 subjects additionally had OSA (OAHI>5) with 3/7 with OAHI>10.

There were two outliers aged 11.2 and 11.5 years respectively who presented with NH in an early-non ambulant stage, in the absence of scoliosis and cardiac impairment. The first had OSA-associated NH, the second isolated NH with only REM-related central events.

Information regarding the presence/absence of a sudden decline in FVC was available in 10/14 individuals. If we exclude the two outliers, 5/8 subjects who developed NH presented a sudden decline of respiratory function before last sleep study.

2.4.2. Overnight gas exchange - Borderline nocturnal hypoventilation (B-NH)

A total of 31 patients (43 SS) met the criteria for borderline nocturnal hypoventilation (B-NH), at a median (IQR) age of 14.2 (12.4-15.8) years. In non-ambulant subjects, B-NH was detected at a median (IQR) of 4.2 (2-5.4) years after LOA. Seven patients with B-NH were still ambulant. In 26/43, B-NH was associated with significant respiratory events, 5 studies had OAHI>10, 3 CnAHI>5. Median (IQR) FVC L and FVC % were 1.7 L (1.2-2.3) and 59% (38.6-84.8), respectively.

Fifteen/31 patients showed no or only mild scoliosis, 4 had a moderate spinal curvature, 7 had severe spinal curvature, and 5 had previously received spinal fusion. Fifteen/31 patients were currently on CS, 9 were past users, and 6 were CS-naïve (including one 4.2-year-old boy who had not yet started CS). Ten out of 30 subjects (data not available in 1) had an underlying cardiomyopathy, with a median (IQR) LVFS% of 27.5% (22.8-29), and 13/24 patients were on cardiac medication (either ace-inhibitors or ace-inhibitors + beta blockers). The median (IQR) BMI at first study was 23.1 (16.6-27.1).

2.4.3. Overnight gas exchange - Hypoxemia

Ten patients (11 SS) had nocturnal hypoxemia as per criteria indicated in Table 1. Two had clusters of hypoxemia associated with obstructive events and B-NH. Two had prolonged hypoxemia associated with obstructive events (moderate and severe) without significant CO2 retention. None of the patients with hypoxemia had concomitant nocturnal hypoventilation (according to AASM criteria). Patients with hypoxemia without OSA (n=6) had median age 15.2 years (range 13.5-17.1), median FVC L 1.5 L (range 1.1-2.3), and median FVC% 49.4% (range 36.7-59). Three out of six patients had cardiomyopathy, 5 were on CS-treatment (1 past user), 4 had no or mild scoliosis and 2 had moderate scoliosis. The median BMI was 23.3 (range 22.7-27.8).

2.4.4. Respiratory events - OSA

A total of 26 patients (34 SS) were diagnosed with OSA based on the detection of OAHI>5, at a median (IQR) age of 14.1 years (11.6-15.2). In 2 patients OSA was diagnosed at 4.2 and 4.6 years old, respectively. In non-ambulant patients, OSA was detected at a median (IQR) of 3.5 (1.7-5.7) years after LOA. Five patients were still ambulant, and 10 were in an early non-ambulatory phase. Only 4 individuals reported daytime symptoms (information was available in 20). OSA was associated with B-NH and NH in 12 and 4 patients, respectively.

Median (IQR) FVC L and FVC % were 1.6L (1.3-2.0) and 58.3% (42.8-74.3), respectively. Fifteen / 26 patients showed no or only mild scoliosis, 2 had moderate scoliosis, 5 had severe spinal curvature, and 3 had previously received spinal fusion. Fifteen/26 patients were currently on CS, 4 were past users, and 7 were CS-naïve (including one 4.2-year-old boy who had not started CS yet). Ten out of 26 subjects had an underlying cardiomyopathy with a median (IQR) LVFS% of 24.5% (22.3-25.8), and 13/22 patients were on cardiac medication. The median (IQR) BMI at first abnormal sleep study was 23.6 (17.7-27.5).

2.5. Relationship between SDB and FVC%

Seventy-nine sleep studies out of 284 were recorded when patients showed an FVC% <50. In 66% of these, there was no significant alteration of gas exchange, in 20% there was B-NH, and in 14% there was NH.

To establish the sensitivity and specificity of the FVC%<50 threshold in capturing NH we only considered the first available sleep study for each patient. Of the 132 first assessments, 107 were normal, 14 borderline (B-NH) and 11 abnormal (NH). If we consider normal/borderline vs abnormal (NH) the sensitivity of FVC<50% in picking up NH was 73% (8/11), specificity 86% (104/121), the positive predictive value (PPV) 32% (8/25), and negative predictive value (NPV) of 97 % (104/107) (eTable 2). If we consider the accuracy of FVC<50% in detecting not only NH but also B-NH, PPV and NPV would be 44% and 87%, respectively.

3. DISCUSSION

The decline of respiratory function in DMD leads to the progressive alteration of nocturnal gas exchanges. Nocturnal hypoventilation, which can be effectively treated with NMV, usually represents the first manifestation. In DMD, NH results from rapid shallow breathing in supine position during sleep and the decrease in the ventilatory response to hypercapnia.[32] This initially affects rapid eye movement (REM) sleep, when the decline in central ventilatory drive is associated with diaphragm dependence and upper airway hypotonia.[33] Importantly, not only NH may precede daytime hypercapnia by several years, but it contributes to its occurrence by desensitizing the response of central chemoreceptors to CO2-induced pH changes. [34]

Current guidelines include FVC%< 50 as an indication for NMV. This is based on experts' opinion, considering such threshold as red flag of an increased risk of CO2 retention during sleep, [13, 18] and also indirectly suggested by one study indicating that the odds of hypoventilation may increase by 20% for every 10% predicted reduction in FVC%. [35] However, there is surprisingly limited published evidence on the correlation between PFT and the onset of NH and other SDB in DMD.

In the current study, we observed that almost one in two patients scoring FVC%<50 presents some degree of CO2 retention during sleep (i.e., ~30% had NH and ~ 20% borderline NH), with the odds of

detecting an abnormal exam based on FVC<50% increasing by 12% when we expanded the criteria of NH to include borderline CO2 elevation. Both specificity and NPV for NH at first sleep study were above 85%, meaning that it is unlikely to find NH if a patient has an FVC value >50%. Indeed, around nine out of ten DMD boys presenting with FVC>50% had a normal overnight gas exchange but it is of note that 28% of patients with NH had FVC>50%. While these findings support the common practice prompting the initiation of NMV based on alteration of SS rather than FVC% alone,[36] the key question that remains open is when to start screening patients for sleep abnormalities and which clinical parameters mandate a closer follow-up and a more pro-active approach.

While the detection of NH was mostly explainable by the functional status of these patients, most being late non-ambulant often with associated scoliosis and cardiomyopathy, we also observed exceptions. As an example, two boys presented with NH already at 11 years in the absence of significant comorbidities (only in one case NH was associated with significant obstructive events and FVC >60%). This is in keeping with a previous study suggesting that a specific sub-group of younger DMD patients might be at higher risk of early NH, for reasons that still need to be elucidated. [35]

It is important to remark that the spectrum of sleep abnormalities in pediatric DMD is not fully deciphered. The current definition of NH itself by the American Academy of Sleep Medicine has limitations, being based on NH associated to OSA in healthy children. A recent experts consensus has therefore expanded the possible criteria to initiate NMV in DMD.[13] We observed the presence of B-NH in around 10% patients referred to sleep studies for the first time, also in a milder stage of the disease (e.g., early-non ambulant, mild scoliosis, no cardiomyopathy). The most relevant factors contributing to the progression from B-NH to NH such as scoliosis[27], as well as the timing of progression itself, need to be identified and further analyzed in depth.

The trajectory of respiratory deterioration rather than single observations could be important. In DMD FVC (L) increases when patients are ambulant, plateaus during early non-ambulatory stages and subsequently declines, with a degree of variability among subjects. [7, 27] When considered as % of predicted, FVC peaks at a median age of 6-9 years, followed by a mean annual decline of 4-6.1%. [17, 27, 37-40] There is increasing evidence that prolonged ambulation and CS-treatment are associated with a

higher peak of FVC (L), FVC% (and Peak expiratory flow- PEF) and with an older age at which the threshold of FVC (L) < 1 L is achieved, although the rate of annual decline is thought to remain unchanged. [3, 37, 41]

In keeping with what previously reported, we confirmed a strong association between the age at loss of ambulation, the peak value of both FVC L and % (the former occurring around 2 years after LOA), and the time at achieving significant FVC% thresholds.

Other aspects that should be considered are the phenotypic spectrum of the DMD population and different trajectories of clinical progression also observed regarding motor function.[42] While the age at LOA, [41] absolute value of FVC (L) at peak,[40] and different genotypes [37, 43, 44] have been variably associated with different rates of FVC% deterioration, their interplay on the subsequent decline is not fully understood. Additionally, it is likely that further and yet not well characterized factors contribute to these events. Indeed, while the rate of FVC% decline after LOA is often considered to be linear,[27] this might not be always the case, as demonstrated by the fact that nearly one fifth of our cohort displayed a sudden drop of respiratory function years after having lost ambulation. The determinants of such drop and whether patients presenting a sudden respiratory deterioration are the ones at higher risk of experiencing early NH need to be clarified in a prospective manner, with important consequences on the provision of a more tailored care to patients.

Despite NH being relevant as indicator for NMV establishment, the most common form of sleep disordered breathing in the ambulatory and early non-ambulatory stage of DMD is OSA.[35, 45] Current guidelines indicate that reported symptoms (e.g., daytime sleepiness, reduced performance, morning headache) should warrant the execution of SS, when obstruction is suspected. [18] However, similar to what observed with nocturnal hypercapnia,[46] patients with DMD very seldom report daytime symptoms in the presence of OSA (only 20% in this cohort). [47]

Interestingly, we detected OSA later than previously reported. [45] Possible explanations include the overall older age at first SS, the use of different criteria to define OSA and the fact that the events scored as obstructive in DMD might differ from the general population. This is particularly true in the second decade when, due to diaphragmatic dysfunction, pseudo central/diaphragmatic sleep disordered

breathing is potentially diagnosed as obstructive. [32, 48] During REM sleep, the combination of reduction of airflow identified as apnea or hypopnea and the presence of out of phase residual movements of abdomen and chest are scored as obstruction at cardiorespiratory polygraphy (as per AASM criteria) but are in fact, particularly in children with intrinsic muscle weakness, the expression of an ineffective breath due to weakened diaphragm (eFigure 2).

It needs to be considered that in our center we use CRSS (i.e., level 3 sleep study) rather than full polysomnography for the diagnosis of pediatric SDB. This can have led to the underestimation of the severity of sleep apnea as respiratory events would be scored based on oxygen desaturations only and the ones leading to cortical arousals would not be scored.

In the context of OSA, CS-induced weight gain is understandably a major concern based on risk profiles observed in the adult population. Nevertheless, we found no significant correlation between OAHI and BMI, possibly reflecting a higher prevalence of diaphragmatic SDB in this population compared to younger cohorts. Further research to clarify the morbidity of obstructive SDB is warranted considering that "true" OSA is a well-established contributing factor to cardiovascular risk (including arrythmias, a potential cause of sudden death), at least in adults. [49] This is particularly relevant if we considered that reduced heart rate (HR) variability and higher resting average HR[50] are common findings in DMD, although their genesis remains unclear.

There were several limitations in our study. First, although both SS and PFT results were prospectively recorded, additional data was retrospectively collected from case note review. Second, and most importantly for the main aim of the study, NH was detected only in a relatively small proportion of SS and this was a major shortcoming in creating a strong prognostic model. Moreover, NH was already present from the first available SS in 11/14 patients; as such, we might have overestimated the age at onset of NH. Third, there is no consensus regarding the definition of B-NH and also the definition of NH in the pediatric population is debated[14], and our results should be interpreted accordingly. Lastly, sleep events which are commonly reported as OSA following criteria created for healthy population might in fact represent diaphragmatic weakness.

4. Conclusions

Both in clinical setting and in translational research, there is an urgent need to identify disease-specific outcome measures to assess the progression of respiratory function in DMD. This is relevant since FVC alone may fail to identify those patients who experience signs of respiratory failure or those who are at higher risk of progression towards nocturnal hypoventilation. Our data shows that both lower FVC% values and severe scoliosis are associated with NH, but the FVC threshold of 50% is not accurate enough in predicting NH.

Current guidelines suggest that NMV usually becomes necessary in late-non ambulatory stage. However, in light of the early detection of borderline tcCO2 elevation and the high incidence of OSA/"diaphragmatic SDB" also in ambulant/early-non ambulant patients, along with the limited reliability of patients-reported symptoms in DMD, we advocate the necessity for early screening, in particular within 3 years after LOA even in individuals with FVC%> 50%. Large, prospective studies are needed to identify those parameters who mandates a closer follow-up also in a less-advanced stage of the disease.

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TABLES

Table 1. Sleep study criteria

Definition of SDB	Criteria
Alteration of gas exchange	
Nocturnal Hypoventilation (NH)	tcCO2 >50 mmHg for >25% of total sleep time (TST) [28]
Borderline nocturnal hypoventilation (B-NH)	Mean tcCO2 levels 45-50 mmHg *and/or* tcCO2> 50 mmHg≤25% of TST (namely, not meeting NH criteria)
Hypoxemia	Mean SpO_2 <94% *and/or* SpO_2 <90% for ≥2% of TST
Respiratory Events	
Clinically meaningful Obstructive sleep apnea (OSA)	OAHI > 5 (Severe if OAHI>10)[30]
Central apneas	CnAHI≥5 and CnAHI >50% of the total AHI

Table legend: Criteria to define gas exchange abnormalities and respiratory events. With the exception of NH and B-NH, such criteria were not mutually exclusive. *Abbreviations: AHI, Apnea-hypopnea index. Cn, central; CO, carbon oxide; OAHI, obstructive Apnea-hypopnea index; OSA, obstructive sleep apnea; SpO2, oxygen saturation; TST, total sleep time.*

Table 2. Population Characteristics

	Patients (n 134)
Pulmonary function test - PFT (n 1222)	
Age at first PFT, years	
Mean (SD)	9.7 (3.5)
Age at last PFT, years	
Mean (SD)	15.2 (2.6)
Cardiorespiratory sleep studies - SS (n 284)	
Age at first SS, years	
Mean (SD)	12.9 (2.7); range 4.2-17.2
Age at last SS, years	
Mean (SD)	14.3 (2.6); range 6.3-17.8
Sleep study results, n (%)	
CO2 retention:	
Normal	224 (79)
NH	15 (5)
B-NH	43 (15)
n/a	2 (1)
Hypoxemia	11 (4)
OAHI>5	34 (12)
PFT results at the time of first SS	
FVC L, mean (SD)	1.88 (0.6); range 0.59-3.3
FVC %, mean (SD)	65 (20); range 17-111
PFT results at the time of last SS	
FVC L, mean (SD)	1.87 (0.8); range 0.59-3.9
FVC %, mean (SD)	53 (20); range 17-90
Ambulatory status at first SS, n (%)	
Ambulatory	31 (23%)
Non-ambulatory	103 (77%)
Ambulatory status at last SS, n (%)	
Ambulatory	16 (12%)
Non-ambulatory	118 (88%)
Estimated age at LOA*, years	
Median (IQR)	10.4 (9.3-12)
Corticosteroid use, n (%)	
CS-naïve	35 (26%)
CS-treated	98 (74%)
Genotype, n (%)	
Deletions	83 (62)
Duplications	19 (14)
Point mutations	20 (15)
Frameshift	8 (6)
Promoter region	1 (1)
Undefined	3 (2)

*Overall age at LOA was estimated using the Kaplan Meier method. Mean (SD) time between sleep study and related spirometry was 2.4 (1.5) months. *Abbreviations: CS, corticosteroids; FVC, forced vital capacity; LOA, loss of ambulation; B-NH, borderline nocturnal hypoventilation, PFT pulmonary function test; oAHI, obstructive apnea-hypopnea index; SS, sleep study.*

FIGURES LEGEND

FIGURE 1. **Patients' selection**. We included patients with at least one cardiorespiratory sleep study performed before NMV initiation (off-NMV) and a pulmonary function test available within 6 months from sleep study. Abbreviations: GOSH, Great Ormond Street Hospital; NMV, nocturnal ventilation; PFT, pulmonary function test; pts, patients.

FIGURE 2. **Age at meeting specific FVC% thresholds in patients categorized according to the age at loss of ambulation.** Kaplan-Meier curves representing time to FVC <60% (A), <50% (B), <40% (C) and time from loss of ambulation to FVC<50% (D) according to the age at LOA. Numbers at risk are expressed as absolute (%).

FIGURE 3. **Rapid FVC decline**. Individual trajectories of FVC% (A) and FVC L (B) of the 32 patients identified as presenting a sudden rapid decline of respiratory function. FVC and FVC% are depicted with reference to time from loss of ambulation (LOA). To help visualize the different slope of progression compared to "average" DMD, we added a dotted line in Panel A representing what would be like the putative 6.1% annual decline previously reported in the literature.

SUPPLEMENT

E-FIGURE 1. Association between age at loss of ambulation and peak FVC/FVC%. The peak of FVC L and % predicted was available in 93 and 53 subjects, respectively. Despite the small sample size of each group, there was a statistically significant difference between the different LOA groups in terms of peak of FVC L (P<0.001), age at peak FVC L (P<0.001), peak FVC % (P 0.02), but not age at peak FVC% (P 0.23).

Box plots representing the median and IQR values of FVC L peak (A), age at FVC L peak achievement (B), FVC% peak (C), age at FVC% peak achievement (D) according to age at loss of ambulation. The median (range) age at last spirometry of patients belonging to LOA group A, B, C and D was 13.8 (9-18.9), 16.4 (10.3-22), 16.9 (14.1-18.8) and 13.3 (6.4-17.3) years, respectively. There were 17 subjects in Group A, 46 in Group B, 21 in Group C and 9 in Group D with available FVC peak. There were 10 subjects in Group A, 26 in group B, 9 in Group C, 8 in group D with available FVC% peak. Abbreviations: FVC: forced vital capacity.

E-FIGURE 2. Difference between OSA and Diaphragmatic SDB in DMD.

Of the 34 studies in which an OAHI>5 was scored, eight (7 pts) showed clear diaphragmatic SDB, 16 (14 pts) showed OSA, 6 showed mostly OSA with a bit of diaphragmatic SDB, and 2 showed a mixed picture of OSA, central and diaphragmatic SDB.

Panel A. DMD patient with OSA. Significant respiratory effort in both thoracic and abdominal bands (orange circles) with paradox and significantly reduced nasal flow =OSA (arrow). Associated desaturation with each respiratory event and overall increase in CO2. In between event, recovery breaths with even large efforts (asterisk).

Panel B. DMD patient with pseudo-OSA/diaphragmatic SDB. Minimal respiratory effort during the event with “recovery” breath representing normal effort – associated reduced nasal flow (arrow) as not enough effort to generate flow – associated desaturation and overall increase in CO2. This is more in keeping with respiratory muscle fatigue with both effort bands (in particular thoracic) showing minimal effort during these events. This has to be scored as OSA following AASM guidelines as there

is movement during the event so cannot be scored as a pure central event and there is paradox during the hypopnoeas. However, the pattern of breathing is clearly different.