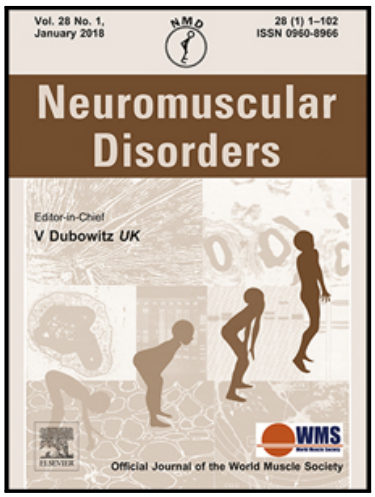

Respiratory and upper limb function as outcome measures in ambulant and non-ambulant subjects with Duchenne muscular dystrophy: a prospective multicentre study

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Highlights

- This study contributes to the natural history of DMD, linking the ambulant and non-ambulant phases
- Respiratory measurements, upper limb function, pinch and grip force are reported
- A composite score combining respiratory outcomes, upper limb function and strength is explored

Respiratory and upper limb function as outcome measures in ambulant and non-ambulant subjects with Duchenne muscular dystrophy: a prospective multicentre study.

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Abstract

The field of translational research in Duchenne muscular dystrophy (DMD) has been transformed in the last decade by a number of therapeutic targets, mostly studied in ambulant patients. A paucity of studies focus on measures that capture the non-ambulant stage of the disease, and the transition between the ambulant and non-ambulant phase.

In this prospective natural history study, we report the results of a comprehensive assessment of respiratory, upper limb function and upper limb muscle strength in a group of 89 DMD boys followed in 3 European countries, 81 receiving corticosteroids, spanning a wide age range (5-18 years) and functional abilities, from ambulant (n=60) to non-ambulant (n=29).

Respiratory decline could be detected in the early ambulatory phase using Peak Expiratory Flow percentage predicted (PEF%), despite glucocorticoid use (mean annual decline: 4.08, 95%CI [-7.44,-0.72], p=0.02 in ambulant; 4.81, 95%CI [-6.79,-2.82], p<0.001 in non-ambulant). FVC% captured disease progression in non-ambulant DMD subjects, with an annual loss of 5.47% (95%CI [-6.48,-4.45], p<0.001). Upper limb function measured with the Performance of Upper Limb (PUL 1.2) showed an annual loss of 4.13 points (95%CI [-4.79,3.47], p<0.001) in the non-ambulant cohort. Measures of upper limb strength (MyoGrip and MyoPinch) showed a continuous decline independent of the ambulatory status, when reported as percentage predicted (grip force -5.51%, 95%CI [-6.54,-4.48], p<0.001 in ambulant and a slower decline -2.86%; 95%CI -3.29,-2.43, p<0.001, in non-ambulant; pinch force: -2.66%, 95%CI [-3.82,-1.51], p<0.001 in ambulant and -2.23%, 95%CI [-2.92,-1.53], p<0.001 in non-ambulant).

Furthermore, we also explored the novel concept of a composite endpoint by combining respiratory, upper limb function and force domains: we were able to identify clear clinical progression in patients in whom an isolated measurement of only one of these domains failed to appreciate the yearly change. Our study contributes to the field of natural history of DMD, linking the ambulant and non-ambulant phases of the disease, and suggests that composite scores should be explored further.

Introduction

Duchenne muscular dystrophy (DMD) is a rare, X-linked neuromuscular disorder with an estimated incidence of approximately 1 in 3.500 to 1 in 5.000 live male births. [1-3] DMD is

caused by mutations in the dystrophin gene (*DMD*) that lead to an absence or near-absence of dystrophin, a protein essential for muscle cell integrity.[4] The profound deficiency of dystrophin seen in DMD results in progressive muscle degeneration and loss of function, culminating in premature death, typically by age 30.[5] DMD usually presents in early childhood with motor difficulties including delayed motor milestones, frequent falls, easy fatigability, as well as calf muscle hypertrophy. Mobility continues to decline over the course of the disease, with loss of ambulation by the early teen years and subsequent deterioration in upper extremity function to the point that patients are unable to perform even the most basic self-care tasks. Scoliosis can develop due to weakness of trunk muscles and often requires surgical correction to maintain respiratory capacity.[6] Absence of dystrophin in the heart leads to cardiomyopathy, and respiratory muscle decline ultimately results in dependence on assisted ventilation.[7] Death is usually caused by cardiac and/or respiratory failure and related complications. [8, 9] There is currently no cure for DMD. DMD patients require a variety of interventions including medication, physiotherapy, nutritional and psychosocial support, and orthopaedic, respiratory, and cardiac care. [10-12] The current mainstay of DMD therapy consists of oral glucocorticoids, which improve muscle strength and delay loss of ambulation, development of cardiomyopathy and the need for ventilatory support.[13] Deflazacort recently received Food and Drug Administration USA (FDA) approval.[14] Furthermore, new therapies to increase dystrophin production in small genetic subsets of DMD have recently become available: ataluren (conditional approval in EU); [15] eteplirsen (accelerated approval in US). [16] Finally, a number of adeno-associated virus (AAV) mediated micro-dystrophin gene transfer clinical trials are on-going. However, clinical trials target mostly ambulant patients and

this is reflected in the choice of the primary outcomes (e.g. six-minute walking test, NorthStar ambulatory assessment, 4 stair climb) excluding the participation of patients who are about to lose or already lost ambulation. Among the few exceptions, the trials evaluating idebenone (NCT03603288, NCT01027884) [17-19], pamrevlumab (NCT02606136), allogenic cardiospheres (NCT03406780), and AVV-mediated gene therapy IGNITE-DMD (NCT03368742) included non-ambulant patients.

The increased survival and prolonged functional capacity of DMD subjects are important incentives to target clinical trials also towards non-ambulant DMD patients; in addition capturing the transition phase between ambulant and non-ambulant patients with meaningful outcome measures is important, as the eventual loss of ambulation is a likely event even for the ambulant DMD patients receiving experimental therapies. Therefore, the success of those trials depends on the establishment of outcome measures that are reliable and sensitive to change in disease progression across the loss of ambulation. During the non-ambulant phase of the disease, natural history studies on upper limb and respiratory function are critical to provide relevant information, as highlighted in recent literature regarding pulmonary endpoints in DMD. [9, 20, 21] Furthermore, in response to the demand for monitoring motor function in the older subjects, the Performance of the Upper Limb (PUL), a functional scale dedicated to evaluating upper extremities in DMD has been developed,[22] (Mayhew et al. under review) necessitating further evaluation in other cohorts of patients.[23-26]

The aim of this prospective, longitudinal, multicentre natural history study was to provide novel information on outcome measures that can support drug development in DMD subjects

irrespective of their ambulatory status. Respiratory function, upper limb function and strength measurements were included in the assessments. Furthermore, with a focus on the non-ambulant cohort, the concept of a composite endpoint was explored by integrating the components of respiratory function, upper limb function and strength, with the goal to capture disease progression with a multi-component approach.

Methods

DMD subjects with a confirmed molecular and clinical diagnosis were recruited as part of a prospective, longitudinal, multicentre study across 5 centres in Europe (London and Newcastle, UK; Paris, France; Leiden and Nijmegen, The Netherlands), which aimed to assess the natural history of ambulant and non-ambulant patients with DMD. The key inclusion criteria for ambulant subjects were the following: age above 5 years old with a diagnosis of DMD documented by genetic testing (if a muscle biopsy was available, it had to contain less than 10% of revertant fibres) able to walk independently for at least 75 meters in 6 minutes at recruitment; receiving the standards of care for DMD as recommended by the DMD Care Considerations Working Group;[27] having a percentage predicted forced vital capacity (FVC) above 30%. The key inclusion criteria for non-ambulant subjects were as follows: age between 5 and 18 years, loss of the ability to walk 10 meters without support; being capable of sitting upright in a wheelchair. Any subject with severe intellectual impairment that prevented cooperation during examination, symptomatic cardiac failure or anticipated surgery within 2 years from recruitment was excluded from the study. Subjects were assessed 6-monthly

according to a shared protocol including among other measurements the Performance of Upper Limb (PUL version 1.2)[22, 24] and respiratory function (absolute and percentage predicted values for FVC and peak expiratory flow (PEF)) and strength measurement with the MyoGrip and MyoPinch dynamometers.[28] For strength measurements, the dominant side was assessed. All patients were given between two and five trials and the maximal value was recorded. For each muscle function tested, if the difference between the first two measurements was lower than 10% of the greater, the greater was accepted. If not, a subsequent measurement was made until two trials ranged within 10% (see Servais et al. 2013, for more details). The subjects were vigorously encouraged to produce their maximal voluntary effort. Grip and pinch maximal strength were expressed in kg and percentage of predicted values for age using predictive equations computed on a control population (internal database of the Institute of Myology, Paris and UCL Great Ormond Street Institute of Child Health & Great Ormond Street Hospital Trust, London). Part of the data have been published for grip strength.[29] The respiratory function tests were performed by qualified respiratory physiologists or specialist neuromuscular physiotherapists according to the recommendations of the American Thoracic Society and the European Respiratory Society (ATS/ERS).[30] Each parameter was expressed as an absolute value and corresponding percentage predicted value. The absolute value was selected as the largest from 3 consecutives attempts at each visit. The percentage predicted value was determined using the relevant reference equations (best effort/predicted x100).[31, 32] For non-ambulant patients, ulnar length or arm span was used to derive height. Arm span was determined for each individual with arms extended laterally with palms facing forward, kept at shoulder height, from the tip of the middle (longest) finger

of one side to the other recorded to the nearest 0.1 cm with a flexible, non-stretch tape. For the weakest patients with joint contractures, measurements were performed by adding the following segment lengths: right hand, forearm, arm, trunk width, left arm, forearm, hand. General demographics were also collected including date of birth, type of mutation and steroid treatment and regime. Steroid regime was defined as daily when taken every day, and intermittent when taken with different intervals (alternate days; periods of 10 days taking the medication followed by 10 days not taking it). Training by the same lead physiotherapist was provided to all clinical evaluators to ensure standardization of assessment procedures and scoring.

Ethics review boards at participating institutions approved the study protocol, consent and assent documents. Informed consent/assent was obtained for each participant prior to conducting the study. This study is registered with the Clinical Trial Gov website with the number: NCT02780492.

Statistical methods

Characteristics of the sample are presented as mean (SD), or frequency (percentage) unless otherwise stated. Considering ambulant and non-ambulant boys separately and using all available outcome data, we estimated the annual change for each of the outcomes using mixed effects regression models, accounting for the longitudinal data. All models were adjusted for steroid regimen and age at entry to the study. Boys who lost ambulation during the study were included in both sets of analyses and their age at the first visit after losing ambulation was used

as baseline age in the non-ambulant models. Results are presented as mean annual change with 95% confidence intervals. In addition, as the outcomes are measured on different scales, we calculated standardised annual changes, using an internal standardisation for the ambulant cohort and for the non-ambulant cohort. The standardised annual change is a re-scaling of the annual change, it corresponds to the average annual change relative to the variability of the change between boys.

For the non-ambulant cohort we defined a composite score involving 3 outcomes, representing 3 different clinical aspects of function. For PUL total score we defined an annual decline in score during year 1 (between visit 1 and visit 3) of >=4 points, and an annual improvement in score of >=4 points as clinically meaningful. This was also based on previously reported data.[33] Similarly, for FVC % we defined an annual change of more than 5% as meaningful [21] and for MyoGrip measurement of force, annual changes greater than 3% were used. [34] Boys who experienced a deterioration for a particular function based on the criteria above were assigned a score of -1. Vice-versa, where improvement was observed a score of +1 was assigned. Where no change was observed the boy was assumed to be stable for the function and given a score of zero. We calculated the composite score for each of the non-ambulant boys by summing these 3 scores and where data was not complete for year 1 for a particular boy we used the first available complete data in subsequent years. All analyses were conducted in Stata v15 [35] and a P value < 0.05 was considered statistically significant.

Results

In this prospective and on-going study a total of 89 boys were included with confirmed clinical and genetic diagnosis of DMD (Table 1). All mutations were predicted to lead to a DMD phenotype, the majority being out of frame *DMD* gene deletions (see Table 1-appendix). At recruitment 60 boys were ambulant with a mean age of 7.9 years (ranges: 5- 13.6 years). The majority of the ambulant DMD boys (40/60) were on daily glucocorticoids, 17 on intermittent and 3 had not started glucocorticoids yet. The remaining 29 boys were non-ambulant with a mean age of 14.2 (ranges: 8.4, 18 years), of which 24 were on glucocorticoids (15 on a daily regimen) and 5 had stopped glucocorticoid therapy after loss of independent ambulation. Boys were assessed every 6 months. The majority of subjects (n=75, 84%) had at least 3 visits, so 1 year follow-up (table1). Eleven subjects lost independent ambulation during follow-up.

In relation to respiratory function (Figure 1A), a significant decline in FVC % predicted was observed in the non-ambulant population consisting of 5.47% (95%CI [-6.48, -4.45], p<0.001) annual decline, whilst no significant change was observed in the FVC % of ambulant boys (1.92, 95%CI [-0.30, 4.14], p=0.09). A decline in PEF% predicted was observed as early as in the ambulant stage of the disease and further into the non-ambulant stage with a PEF % annual deterioration of 4.08 % (95% CI [-7.44, -0.72], p=0.02) and 4.81 % (95% CI [-6.79, -2.82], p<0.001) respectively (Table 2). Upper limb function assessed by the PUL remained stable in the ambulant population, while in the non-ambulant patients an annual total loss of 4.13 PUL points (95% CI [-4.79, -3.47], p<0.001) and a loss of 0.97 point at the shoulder level (95%CI [-

1.25, -0.69], p<0.001) was observed (Table 2, Figure 1B). When expressed in absolute values (kg), the strength for both grip and pinch shows a significant improvement in ambulant patients, followed by a decrease in non-ambulant patients. However, when expressed in percentage of predicted values, measurements of upper limb force revealed a steady decline across age and ambulatory status with an annual loss of grip force of 5.51% predicted (95% CI [-6.54, -4.48], p<0.001) and 2.86% predicted (95% CI [-3.29, -2.43], p<0.001) in the ambulant and non-ambulant DMD respectively, and a decline in pinch force of 2.66% (95% CI [-3.82, -1.51], p<0.001) in ambulant and 2.23% (95% CI [-2.92, -1.53], p<0.001) in non-ambulant subjects (Table 2, Figure 1C).Table 1 reports the annual changes in ambulant and non-ambulant subjects adjusted for age and glucocorticoids use at baseline. In the supplemental material (appendix-table 2), we report standardised annual change from ambulant and non-ambulant subjects.

Furthermore, focusing on the non-ambulant cohort alone, we explored the concept of a composite score by combining the FCV% (change of >=5%), PUL total score (change of >=4 points) and the MyoGrip measurement of force (change of >=3%). Out of 28 non-ambulant subjects who had all the assessments done, 21 showed a global decline on the composite score, which might not have been captured by assessing one parameter alone (i.e. 12 subjects declined in respect to the PUL, 13 in respect to FVC% and 14 in respect to grip force). Only one boy showed a decline in all the three domains: respiratory function, upper limb function and force. Four subjects (12, 14, 24 and 26) showed decline in one domain and improvement in another resulting in a composite score that was indicative of stable disease. Two subjects remained stable across all the three domains.

Discussion

In our multicentre, prospective, longitudinal natural history study, respiratory function, upper limb function and strength were assessed in a cohort of 89 ambulant and non-ambulant DMD subjects treated according to the international standards of care and evaluated with a standardised protocol by trained physiotherapists. The progression of the disease was highlighted across the ambulatory stages of the disease with a particular focus on those outcome measures that are independent from ambulation. We observed an annual loss FVC % predicted of 5.47 (95% CI -6.48, -4.45, p<0.001) in the non-ambulant subjects (n=29), which is also in line with what has been previously reported in the literature, [17, 36-38] while this measure did not decline in the ambulant population (n=60). In accordance with recent observations [9, 21] we observed a deterioration in PEF% predicted in young ambulant children with an annual decline of 4.08 PEF % (95% CI-7.44, -0.72, p=0.02), reflecting that maximal expiratory muscle pressure required to perform this assessment is impaired already in this young DMD population. In the non-ambulatory cohort the annual decline of PEF % predicted was 4.81% (95% CI -6.79, -2.82, p<0.001), hence a very similar annual decline as in the ambulant patients. Our data are in keeping with the only other natural history study that specifically explored this outcome in which a similar rate of yearly decline (5%) was reported.[37] Percentage predicted PEF was the primary endpoint in clinical trials testing idebenone [39]. The phase 3 clinical trial reported higher rates of annual decline in the placebo group (8.84% per year) compared with Mayer et al. and our findings.[37] However, in contrast with the patients

in the natural history studies including ours, the ones in the clinical trial were not treated with glucocorticoids. [39]. Newer evidence from the CINRG natural history study indicates that steroids are capable of delaying the onset of respiratory force decline but do not alter the slope of decline once this has started. [21] Our data are consistent with the observations from the CINRG natural history study, confirming the similar course of disease in DMD between our European and the US studies.

Upper limb function evaluated by the PUL (v1.2) appears to be a more sensitive measure of disease progression in the non-ambulant cohorts than in the ambulant, principally because of a ceiling effect in the ambulant group. The total score showed an annual decline of 4.13 points (95%CI -4.79, -3.47, p< 0.001), and a loss of performance at shoulder level of 0.97 scores (95% CI-1.25, -0.69, p< 0.001) in the non-ambulant population. In the ambulant population, the shoulder sub-domain detected a mild decline over the course of the year, which however was not statistically significant, but would be in line with the proximal to distal progression of the disease. These PUL findings corroborate the results observed in DMD populations followed at other European sites (Mayhew et al. under review)[25].

When measuring distal upper limb strength with the MyoGrip and MyoPinch [28] a steady decline of the percentage predicted grip and pinch force were observed. Grip force showed a more rapid annual decline in ambulant (-5.51%, 95% CI [-6.54, -4.48], p<0.001) and a slower decline in non-ambulant boys (-2.86% [95% CI -3.29, -2.43] p<0.001) when compared to pinch force (-2.66 %, 95% CI [-3.82, -1.51], p<0.001 in ambulant and -2.23 %, 95% CI [-2.92, -1.53], p<0.001 in non-ambulant boys). Similar trajectories have been observed in previous studies

using the same dynamometers. [34, 40] Importantly, these results demonstrate that DMD children never reach a normal force capacity when compared to healthy children. DMD children peak at about 60% of normal total grip force predicted. Results also emphasize how the distal upper extremities loose strength during the early stages of the disease. Muscle strength expressed as percentage predicted seems to be a consistent clinical outcome measure, which, together with the respiratory function measures, bridges the early ambulatory stage of DMD towards the later non-ambulatory phase. Non-ambulant subjects lose less strength probably because they have less total strength to lose. However, when looking at relative declines (with respect to their remaining strength), they lose more than ambulant patients.

Finally, with the aim to capture progression in multiple domains of DMD in the non-ambulant phase, we explored the concept of a composite endpoint using a descriptive analytical approach. With a focus on the non-ambulant cohort (n=28), the following parameters were selected on the basis of the observed annual decline, which is also in line with the literature: FVC% (5% change), PUL total score (4 points change) and grip strength (3% change).[33, 34, 37] Table 3 highlights the high individual variability in disease progression when individual domains are considered: with the exception of one boy (subject 23) who showed decline in the respiratory, upper limb function and force domains, all other subjects showed a more heterogeneous picture. However, using our exploratory composite score, decline was detected in 21/28 subjects (75%) over the course of one year. It must be highlighted that 4 subjects showed decline in one domain and improvement in another (subjects 12, 14, 24, 26) resulting in a composite score that is indicative of stable disease.

We are aware that care should be used when considering a composite score, as the different subdomains are likely to progress with different slopes and have different linearity, and in this context more work will be required to assess the validity of our proposed exploratory measure in the future. We defined the thresholds for improvement or decline for each subdomains in the composite score at the mean annual decline considered clinically significant for that parameter, but we have not defined the relationship of linearity for each of these outcome measures. In addition, the minimally clinically important difference (MCID) for these domains have yet to be established: in our exploratory composite endpoint we therefore have weighted all 3 domains equally, however DMD boys may experience that a decline in one domain is more impactful than in the others. Furthermore, given the small number of patients included and the exploratory nature of this analysis, these results need to be interpreted with caution. This exploratory composite score is also meant to spark debate on the multiple dimension of disease progression that is not captured by the current assessment tools, and that it could be potentially complemented with other progression disease biomarkers such MRI, which has previously been shown to progress in close correlation with force/function measurements. [40, 41]

An example of a composite score is the one used in a phase 3 study of the safety and efficacy of laronidase in the storage disorder Mucopolysaccharidosis I (MPS I) (NCT00146770) comparing placebo with treatment arm. In this study, a composite endpoint was used that summed up clinically significant changes across five efficacy variables (percent predicted normal FVC, 6-minute walk test distance, shoulder flexion range of motion, apnoea-hypopnoea index, and visual acuity), providing a global response to the treatment. A similar approach could

potentially be explored also in DMD, given the heterogeneity of this disease and its multi-systemic manifestations across the population. Of course, such concept requires further evaluation in larger cohorts of patients, and assessment of the contribution of the changes across the entire range of the scales for each of the sub-domains.

In summary, our prospective study, for the first time, combines outcomes of respiratory, upper limb function and precise upper limb force dynamometry, across ambulatory and non-ambulatory DMD subjects. On the other hand and lending support to the validity of our observations, the rate of decline of PEF% predicted and FVC % predicted in non-ambulant patients as well as in the late ambulant phase are in the same range as those independently observed in the CINRG natural history study, but extended further to capture younger DMD boys. [21] Our study has however limitations. The small number of subjects included in our study, can limit some sub-group analyses and generalization of results. Secondly, our cohort of patients does not reflect the full genotype spectrum of DMD, and it is known that genotype can influence functional capacities. [25, 42-45] . Finally, patients in our cohorts were on different glucocorticoid regimens, the potential impact of which could not be compared due to the small numbers.

We demonstrate that progressive motor and respiratory function decline are features of DMD in the ambulant and non-ambulant phase of the disease, albeit at different rates for the different parameters studied. These observations should allow for clinical study designs, which

aim at slowing decline or even improving function in DMD across the ambulant and non-ambulant phases of the disease.

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Authors contributions:

VR contributed to protocol development, set up of the database, patients' recruitment, data collection, data curation, data analysis, wrote the first draft of the manuscript and contributed to its revision.

VS contributed to patients' recruitment, data curation and manuscript revision.

DR contributed to the data curation, data analysis and revision of the manuscript.

VD contributed to protocol development, patients' recruitment, data curation and manuscript revision

JD contributed to data collection and data curation.

AM contributed to protocol development, data collection, data curation and manuscript revision.

ME to protocol development and manuscript revision

JB contributed to data collection and data curation

MG contributed to data collection and manuscript revision

MVdH contributed to data collection and manuscript revision

VJJGM contributed to patient recruitment and manuscript revision

IJMdG contributed to patient recruitment and manuscript revision

EHN contributed to patient recruitment and manuscript revision

LS contributed to patient recruitment and manuscript revision

VS contributed to patient recruitment and manuscript revision

TV contributed to protocol development and manuscript revision

JYH contributed to protocol development, data analysis, data curation and manuscript revision

FM obtained the funding, contributed to protocol development, patient recruitment and manuscript revision.

Competing interest's disclosure

VR was a Solid Biosciences employee at the time of writing this manuscript.

AM has participated in SAB meetings for Summit, PTC and Biogen and performs Consultancy work (training physiotherapists) for: Roche, Pfizer, PTC, Summit, Sarepta, Santhera, Italfarmaco, Amicus, Biogen and Avexis.

ME is managing director of Atom International Limited.

MvdH performs Consultancy work (training physiotherapists) for: Roche, PTC, Sarepta, Santhera, Italfarmaco, Amicus and MNK

EHN reports grants from Spieren voor Spieren, Duchenne Parent Project, ZonMW and AFM, and was local PI in studies conducted by BioMarin, GSK, Lilly, Santhera, Italfarmaco, Roche, and WAVE Life Sciences outside the submitted work. He also reports consultancies for BioMarin, Summit and WAVE. All reimbursements were received by the LUMC. No personal financial benefits were received.

IdG has participated in consultancy and educational meetings of PTC and Sarepta. Radboudumc is participating in clinical trials from Italfarmaco, Santhera and Roche (IdG PI).

JJGMV has been involved in Duchenne trials that are sponsored by Biomarin/Prosensa, GSK, Santhera or Lilly, in two European FP7 project on Duchenne muscular dystrophy (BIOIMAGE NMD, #602485, and SCOPE DMD, #60157), and a natural history study supported by AFM. All reimbursements were received by the LUMC, JJGMV had no personal financial benefit.

LS is part of the SAB of Sarepta, Santhera, of the steering commitee of Roche. LS has consultancy running for Roche and Biophytis. LS is PI in Sarepta studies, Wave, Santhera and Givinostat.

FM is a member of the Rare Disease Scientific Advisory Group for Pfizer, and has participated to SAB meetings for PTC, Sarepta, Wave Therapeutics and Summit. UCL and Great Ormond Street Hospital are recipient of grants from Pfizer, Italfarmaco, Wave, Sarepta and Summit regarding clinical trials (Muntoni PI).

TV is an SAB member of Constant Pharma and Metriopharm; he served as consultant for: Audentes, BioLeaders, Biophytis, Capricor, DebioPharm, Fibrogen, Italfarmaco, Lysogene, Santhera, Sarepta, Servier, Solid, and Summit. He acted as a PI for Prosensa, GSK, Sarepta, PTC, Santhera.

JYH is a co-inventor of the MyoGrip and MyoPinch devices.

All the other authors have no conflicts to declare.

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Figure 1

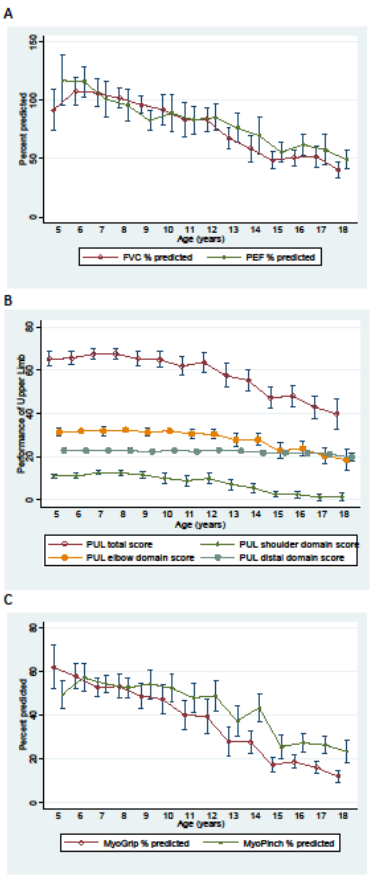

Figure 1. Mean and 95%CI in ambulant and non-ambulant DMD boys for (A) respiratory function (FVC% and PEF%) in relation to age (B) Performance of the upper limb functional scale: total score and sub-domains in relation to age (C) MyoGrip and MyoPinch in % of predicted values for age. Note: maximum PUL 1.2 total score = 74, maximum shoulder domain score = 16; maximum elbow domain score = 34; maximum distal domain score 24.

Table 1. General characteristics of subjects at their initial assessment

FVC= force vital capacity; PEF= peak expiratory force; PUL= performance of the upper limb

	Ambulant (N=60)	**Non-ambulant (N=29)**
Age at recruitment, mean (range)	7.9 (5, 13.6)	14.2 (8.4, 18)
Steroids: Daily	40 (66.7%)	15 (51.7%)
Intermittent	17 (28.3%)	9 (31.0%)
Not started	3 (5.0%)	
Stopped		5 (17.3%)
Number of visits median (range)	4.5 (1, 9)	6 (2, 10)
Duration of follow up median (range)	2.0 (0, 4.9)	3.0 (0.5, 4.5)
Initial FVC absolute value mean (range) (l)	1.40 (0.71, 2.46)	2.02 (0.80, 4.21)
Initial FVC % predicted mean (range)	92.17 (54, 140)	62.10 (28, 108)
Initial PEF absolute value mean (range) (l/min)	2.46 (0.8, 5.23)	3.19 (1.29, 5.13)
Initial PEF % predicted mean (range)	100.95 (34, 149)	65.56 (22.12, 107)
Initial PUL total score mean (range)	65.2 (32, 74)	50.4 (10, 74)
Initial PUL shoulder level sub-score mean (range)	11.1 (0, 16)	3.6 (0, 16)
Initial Myogrip absolute value mean (range) (Kg)	6.65 (2.89, 14.62)	6.50 (0.53, 13.75)
Initial Myopgrip % predicted mean (range)	55.13 (23.47, 105.60)	21.11 (1.18, 36.58)
Initial Myopinch absolute value mean (range) (Kg)	2.31 (0.73, 6.24)	2.03 (0.28, 3.91)
Initial Myopinch % predicted mean (range)	55.69 (23.85, 115.50)	29.39 (3.50, 51.29)

Table 2. Estimated annual changes from baseline for respiratory and upper limb measurements

FVC= force vital capacity; PEF= peak expiratory force; PUL= performance of the upper limb

	AMBULANT (n=60) **Mean change (95% CI)** **p- value**	**NON-AMBULANT (n=29)** **Mean change (95% CI)** **p-value**
FVC absolute value (l)	0.14 (0.11, 0.17) <0.001	-0.06 (-0.10, -0.02) <0.01
FVC % predicted	1.92 (-0.30, 4.14) 0.09	-5.47 (-6.48, -4.45) <0.001
PEF absolute value (l/min)	0.45 (0.34, 0.56) <0.001	0.24 (0.10, 0.37) <0.001
PEF % predicted	-4.08 (-7.44, -0.72) 0.02	-4.81 (-6.79, -2.82) <0.001
PUL total score	0.36 (-0.62, 1.34) 0.48	-4.13 (-4.79, -3.47) <0.001
PUL shoulder level sub-score	-0.13 (-0.62, 0.36) 0.61	-0.97 (-1.25, -0.69) <0.001
Myogrip absolute value (Kg)	0.23 (0.08, 0.38) <0.01	-0.39 (-0.50, -0.29) <0.001
Myopgrip % predicted	-5.51 (-6.54, -4.48) <0.001	-2.86 (-3.29, -2.43) <0.001
Myopinch absolute value (Kg)	0.09 (0.04, 0.14) <0.01	-0.08 (-0.13, -0.03) <0.01
Myopinch % predicted	-2.66 (-3.82, -1.51) <0.001	-2.23 (-2.92, -1.53) <0.001

Table 3. Composite endpoint in non-ambulant DMD integrating PUL total score , FVC% predicted and MyoGrip.

A clinically meaningful change for the PUL total score = 4 points, for the FVC% = 5% and for the MyoGrip = 3%. A decline was captured as -1, unchanged measurements as 0 and improvement as 1.

Subject	PUL total	FVC%	MyoGrip%
1	-1	-1	-1
2	-1	-1	0
3	-1	-1	0
4	-1	-1	0
5	-1	0	-1
6	-1	0	-1
7	-1	0	0
8	-1	0	0
9	-1	0	0
10	-1	1	-1
11	-1	1	0
12	-1	1	0
13	0	-1	-1
14	0	-1	-1
15	0	-1	-1
16	0	-1	-1
17	0	-1	0
18	0	-1	0
19	0	-1	0
20	0	0	-1
21	0	0	-1
22	0	0	0
23	0	0	0
24	0	1	0
25	1	-1	-1
26	1	-1	-1
27	1	0	-1
28	1	0	-1