




RESEARCH ARTICLE

Upper limb disease evolution in exon 53 skipping eligible patients with Duchenne muscular dystrophy

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Abstract

Objective: To understand the natural disease upper limb progression over 3 years of ambulatory and non-ambulatory patients with Duchenne muscular dystrophy (DMD) using functional assessments and quantitative magnetic resonance imaging (MRI) and to exploratively identify prognostic factors. **Methods:** Forty boys with DMD (22 non-ambulatory and 18 ambulatory) with deletions in dystrophin that make them eligible for exon 53-skipping therapy were included. Clinical assessments, including Brooke score, motor function measure (MFM), hand grip and key pinch strength, and upper limb distal coordination and endurance (MoviPlate), were performed every 6 months and quantitative MRI of fat fraction (FF) and lean muscle cross sectional area (flexor and extensor muscles) were performed yearly. **Results:** In the whole population, there were strong nonlinear correlations between outcome measures. In non-ambulatory patients, annual changes over the course of 3 years were detected with high sensitivity standard response mean ($|SRM| \geq 0.8$) for quantitative MRI-based FF, hand grip and key pinch, and MFM. Boys who presented with a $FF < 20\%$ and a grip strength $> 27\%$ were able to bring a glass to their mouth and retained this ability in the following 3 years. Ambulatory patients with grip strength $> 35\%$ of predicted value and $FF < 10\%$ retained ambulation 3 years later. **Interpretation:** We demonstrate that continuous decline in upper limb strength, function, and MRI measured muscle structure can be reliably measured in ambulatory and non-ambulatory boys with DMD with high SRM and strong correlations between outcomes. Our results suggest that a combination of grip strength and FF can be used to predict important motor milestones.

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked neuromuscular disease caused by mutations in the dystrophin gene that affects approximately 1 in every 5000 boys.¹ Progressive muscle weakness starts before the age of 5 years and leads to death around the end of the third decade.² Treatment with glucocorticoids and advances in multidisciplinary care programs have delayed the loss of ambulation and considerably increased the life expectancy.^{3,4}

Over the last 7 years, several clinical trials have been conducted in non-ambulatory patients with DMD (NCT02814019, NCT03406780, and NCT03603288).⁵ No drug has yet demonstrated sufficient efficacy in a double-blind placebo-controlled study to result in a market approval, though five drugs have been FDA-approved on the basis of retrospective comparison or surrogate biomarker. So far, important aspects for quality of life, like upper limb function and lung capacity were used as primary endpoints. Understanding upper limb involvement and weakness progression and the validation of outcomes sensitive to change and the ability to predict natural course of loss of various functional abilities are of primary importance in evaluating drug efficacy in patients at risk of losing ambulation or who are already non-ambulatory.

We previously showed that quantitative magnetic resonance imaging (MRI) and precise strength measures could reliably measure disease progression over a 1-year period.^{6,7} The objective of the present study was to investigate upper limb evolution over a longer time frame using strength and function assessments, and quantitative MRI. In addition, we initiated an explorative approach about identifying predictive values of these measures on important clinical milestones such as loss of ambulation or loss of hand-to-mouth ability in boys with DMD.

Material and Methods

Participants and study design

The 7-year natural history study included patients with DMD eligible for exon-skipping therapy. Subjects were evaluated in two investigator centers (GOSH in London and I-Motion in Paris). Patients were recruited directly from these two centers. In order to increase our sample, we also called on Belgian, Romanian, Polish, and German networks. Initially, ambulatory and non-ambulatory patients were included, and glucocorticoid status was not an inclusion criterion. Ambulation was defined as being able to walk 10 meters without any kind of assistance. In August 2015, in anticipation of future clinical trials, an

amendment to the initial study protocol (Amendment #3 in France, Amendment #1 in the United Kingdom) specified that henceforward only non-ambulatory patients treated with glucocorticoids could be included. Throughout the whole study duration imaging and clinical raters were blinded to use of glucocorticoids. The study was approved by the local ERB (CPP Ile de France VI, ID RCB-2010-A01138-31) and registered on clinicaltrials.gov (NCT01385917). All patients or legal guardians signed an informed consent. The study was strictly monitored according to established standard operating procedures (SOPs), ICH/GCPs, and current legislation for clinical trial regulation. The study details have been reported in more detail elsewhere.⁶

Strength and functional assessments

Every 6 months patients underwent functional assessments with the MFM 32 (<https://mfm-nmd.org/?lang=en>),⁸ grip strength assessments with the MyoGrip and key pinch strength assessments with the MyoPinch (Ateliers Laumonier, Nesles-La-Vallée, France), and upper limb distal coordination and endurance assessments with the MoviPlate (Valotec, Villejuif, France).⁹ The physiotherapists conducting the assessments were trained and certified for the MFM and the MyoTools (MyoGrip, MyoPinch, and Moviplate). They followed strict SOPs as previously described.^{6,10,11} Grip and pinch strengths are reported either in absolute values (kg) or in percentage of predicted normal values for age (%pred).

MRI

All quantitative MRI data were acquired annually on either a 3-T (France, Paris) or a 1.5-T (United Kingdom, London) clinical system (Siemens Healthineers). The reproducibility of the examinations performed on the 1.5T and 3T scanners in London and Paris was assessed on four subjects, two healthy volunteers and two patients with an autoimmune disease. The inter-site variability, as estimated by the mean standard deviation between sites, was 1% for lean cross-sectional areas (ICSA), 1.2% for Fat fraction (FF) and 1.2 msec for water T2 (unpublished data from the BIOIMAGE-NMD project, FP7 funded translational research program) for data processed by the same operator. These data indicate a low impact of the environment, i.e., scanner, magnetic field, imaging sequences, technologists, and on the results obtained in the two participating centers. Patients were placed in the supine head-first position, and dominant and nondominant forearms were scanned in two subsequent sessions on the same day. Quantitative water-fat imaging was performed using a 3D gradient echo (3-point Dixon) sequence with three echo times (TE₁, TE₂,

and TE₃ values of 2.75, 3.95, and 5.15 msec, respectively) with the volume centered on the thickest part of the forearm. Total MRI acquisition time was approximately 20 min per forearm. Adequate repositioning of the image volume at follow-up exams was performed using the images of the preceding year. Regions of interest were drawn manually, by the same person, in the flexor and the extensor muscle group of each forearm. FF values (expressed in absolute %) and ICSA (expressed in mm²) and defined as the lean muscle cross-sectional area corresponding to the muscle fraction containing the contractile apparatus were computed as previously reported in both the flexors and the extensors.⁶

Statistical analyses

Patients were classified according to their ambulation status at each visit. Descriptive statistics were computed at baseline for both groups of patients as mean and standard deviation (SD). Standard response means (SRMs) were computed to quantify the effect size of the changes between visits and were calculated as the mean change score divided by the SD of change scores. An SRM ≥ 0.8 in absolute value was considered to reflect a high responsiveness to change. Differences between dominant and nondominant sides and between visits were tested using a Wilcoxon signed-rank test.

Differences between groups were tested using a Mann–Whitney test. Spearman's rho correlation coefficients were computed to explore correlations between variables. We also performed a Mann–Whitney test in both ambulatory and non-ambulatory populations to test the effect of glucocorticoids compared to treatment-naïve patients over time on the various outcome measures. All analyses were performed using IBM SPSS v.22.0 statistical software. A $p < 0.05$ was considered as statistically significant and a Bonferroni correction was applied in case of multiple tests. SRM and changes from baseline are presented for the first three years of the seven years of follow-up; loss of subjects to follow-up precluded longer term analysis.

Data availability

Anonymized data can be made available to qualified investigators on request. All data requests will be reviewed by the study executive committee.

Results

Population description at baseline

Forty DMD patients presenting with mutations theoretically treatable by correction of skipping of dystrophin

exon 53 were included in the study. Mean duration of follow-up was 3.5 ± 1.8 years yielding 288 visits. The number of patients pursuing the study decreased with time (Fig. 1). Reasons for drop-out varied. For example, five ambulatory patients entered therapeutic trials and six others became non-ambulatory during the study. Four patients died during the 7 years of the study, at ages ranging from 14 to 19 years. The causes of death were respiratory complications ($n = 2$) and cardiac arrest ($n = 2$). At time of death, one patient was on glucocorticoids, one patient stopped 10 years before and two patients never initiated the treatment. These latter required nocturnal noninvasive ventilation until death. Characteristics of patients at baseline are presented in Table 1.

With the exception of the MoviPlate scores, all the variables were significantly different between ambulatory and non-ambulatory patients (all p values < 0.001). In individual patients, bilateral measurements were highly correlated ($\rho > 0.89$, all p values < 0.001). Strength measures, FF, and ICSA (flexors and extensors) were not statistically different between sides. MoviPlate scores were significantly better on the dominant side ($p < 0.001$).

Sensitivity to change

In addition to differences in relationships with time (e.g., exponential decrease for grip strength and sigmoidal increase for FF), individual trajectories of all outcome measures were heterogeneous (Fig. 2). Significant reductions relative to baseline were observed at all visits for grip and pinch strength in non-ambulatory patients and at 12, 24, and 36 months in the overall population. Total MFM value was significantly decreased with high SRM values relative to baseline starting from 24 months in both non-ambulatory and overall patient populations. FF increase in the flexor muscle group was significant (with high SRMs) from 12 months onwards in the non-ambulatory population and from 24 months in the overall patient population. The ICSA (flexors and extensors) values tended to decrease with time but not always significantly (Table 2).

Patients treated with glucocorticoids were not evenly distributed over the various age groups. The large majority of those treated with glucocorticoids were young, ambulatory patients. Thus, these results present a critical bias and a limited interpretation. Non-ambulatory patients on steroids at baseline were stronger than the treatment-naïve population but yearly changes were not statistically different. As only three treatment-naïve ambulatory patients were included, we found no significant differences, neither at baseline nor in follow-up (Table S1).

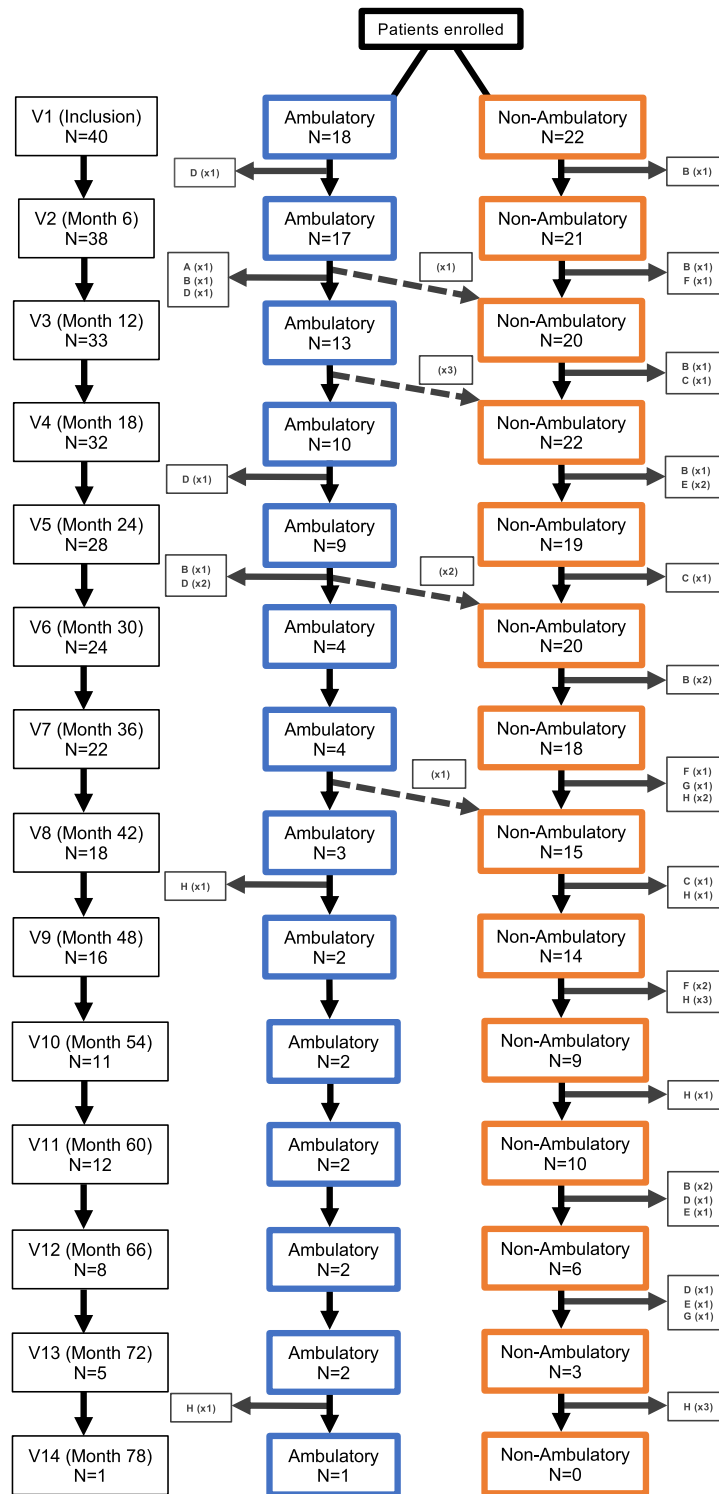


Figure 1. CONSORT diagram. Reasons for premature withdrawal included: (A) loss of follow-up, (B) withdrawal, (C) physician decision to withdraw, (D) enrollment into other clinical trials, (E) death, (F) missed visit, (G) last visit missed, (H) protocol completed, (--->) loss of ambulation.

Table 1. Baseline characteristics.

	A	NA	All
<i>N</i>	18	22	40
Age (years)	9.1 (2.5)	13.8 (2.5)	11.7 (3.4)
Weight (kg)	32.7 (13.5)	45.6 (15.7)	39.6 (15.9)
Height (cm)	125.8 (11.2)	144.8 (11.9)	136.1 (15.0)
BMI (kg/m ²)	19.3 (4.0)	20.6 (6.9)	20.0 (5.6)
Number of patients with right dominant side (%)	16 (88.9%)	21 (95.5%)	37 (92.5%)
Age of onset of first symptoms (years)	2.6 (1.3)	3.3 (1.6)	3.0 (1.5)
Age at diagnosis (years)	4.0 (2.5)	5.1 (2.1)	4.6 (2.3)
Age at loss of ability to walk (years)	NA	9.2 (2.0)	NA
Number of patients on glucocorticoids (%)	15 (83.3%)	14 (63.6%)	29 (72.5%)
Brooke score	1.3 (0.5)	4.0 (1.4)	2.8 (1.7)
Walton score	3.9 (1.5)	8.9 (0.8)	6.7 (2.7)
Grip strength (kg) – dominant side	6.9 (3.0)	3.3 (2.4)	4.9 (3.2)
Grip strength (%pred) – dominant side	49.3 (16.3)	13.4 (11.0)	29.5 (22.6)
Grip strength (kg) – nondominant side	6.9 (2.8)	3.3 (2.7)	4.9 (3.3)
Grip strength (%pred) – nondominant side	49.8 (17.4)	13.1 (11.2)	29.6 (23.6)
Pinch strength (kg) – dominant side	2.2 (0.7)	1.1 (0.6)	1.6 (0.8)
Pinch strength (%pred) – dominant side	51.0 (13.8)	18.0 (10.3)	32.9 (20.4)
Pinch strength (kg) – nondominant side	2.1 (0.6)	1.1 (0.7)	1.6 (0.8)
Pinch strength (%pred) – nondominant side	49.4 (14.0)	17.6 (11.2)	31.9 (20.3)
MoviPlate score (#) – dominant side	47.0 (15.3)	42.9 (11.0)	44.7 (13.1)
MoviPlate score (#) – nondominant side	42.6 (13.4)	42.5 (11.0)	42.6 (12.0)
MFM total score (%)	77.6 (11.8)	34.1 (12.2)	53.7 (24.9)
MFM D1 (%)	56.7 (22.9)	1.3 (2.6)	26.2 (31.8)
MFM D2 (%)	64.1 (7.0)	48.2 (23.4)	68.8 (29.2)
MFM D3 (%)	88.1 (9.7)	70.58 (15.1)	78.6 (15.5)
FF extensors (%) – dominant side	9.7 (5.6)	32.1 (18.6)	23.1 (18.4)
FF extensors (%) – nondominant side	9.0 (5.6)	35.2 (17.2)	24.4 (18.8)
FF flexors (%) – dominant side	11.3 (8.5)	39.5 (19.6)	27.8 (21.1)
FF flexors (%) – nondominant side	11.2 (7.5)	39.8 (16.7)	28.6(19.7)
ICSA extensors (mm ²) – dominant side	417.6 (119.4)	310.4 (79.1)	348.5 (106.9)
ICSA extensors (mm ²) – nondominant side	420.1 (123.2)	325.8 (103.3)	361.6 (118.6)
ICSA flexors (mm ²) – dominant side	650.9 (206.5)	444.3 (169.9)	515.3 (205.7)
ICSA flexors (mm ²) – nondominant side	641.1 (165.6)	414.5 (148.1)	500.5 (188.8)

Results are presented as means \pm SD, except for number of patients with right dominant side and number of patients on glucocorticoids where results are presented in percentage. Abbreviations: A, ambulatory; NA, non-ambulatory; BMI, body mass index; MFM, motor function measure; FF, fat fraction; ICSA, lean muscle cross-sectional area.

Correlations between outcome measures

Strong significant correlations were observed between total MFM and relative grip and pinch strength scores. Equally strong correlations were found between FF (flexors and extensors) and grip and pinch relative values and between ICSA and grip and pinch absolute values. The strongest correlations were observed between FF and total MFM. Table 3 summarizes the correlation analyses. Relationships between variables were generally not linear (Fig. 3).

Exploratory predictive approach

In order to identify factors predictive of clinically significant milestones, we assessed the correlation between

different variables: the changes in grip strength at 6 or 12 months or flexor FF with the probability of losing ambulation or with the loss of 3 points on the MFM or 1 point on the Brooke score within 3 years. Some weakly significant correlations were detected, but the high inter-patient variability precluded the identification of a strong predictive factor using a single variable (data not shown).

We then investigated whether the combination of strength and MRI-based data were predictive of clinically significant milestones and abilities, such as the loss of ambulation or the loss of the ability to drink a glass of water independently (equivalent to Brooke score 3). The combination of low flexor FF and high grip strength was a 3-year positive prognosis factor for maintaining ambulation and for the ability to raise a glass to the mouth independently. As indicated in plots of grip strength (%pred)

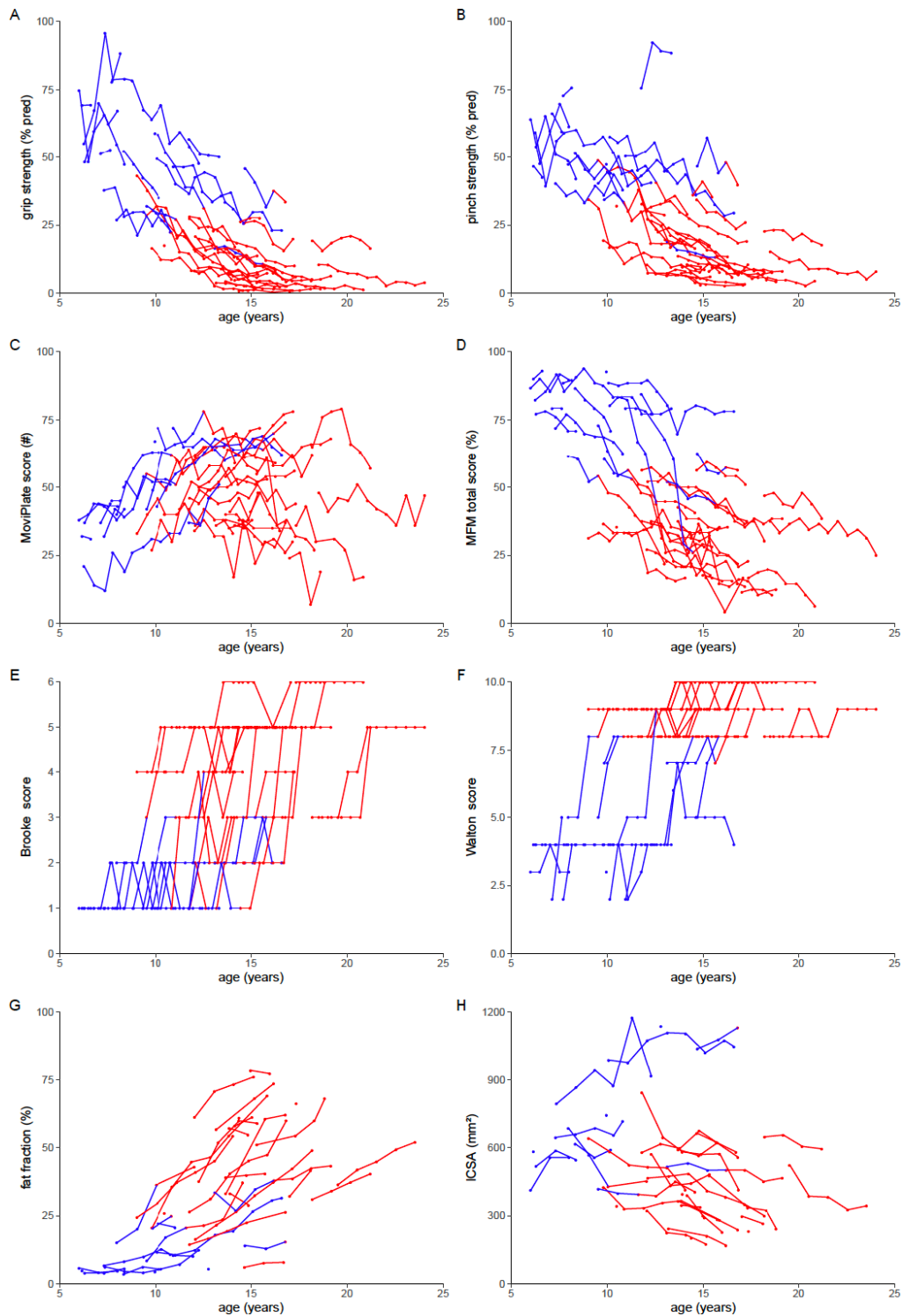


Figure 2. Longitudinal evolution of clinical outcome measures. Outcomes (A) grip strength as percentage of predicted value, (B) pinch strength as percentage of predicted value, (C) MovPlate score, (D) MFM total score expressed as percentage, (E) Brooke score (scale from 1 to 6), (F) Walton score, (G) FF expressed as percentage, (H) ICSA expressed as square millimeter of the flexors as a function of age in years for non-ambulatory (red) and ambulatory (blue) subjects. Each line connects data points for an individual subject.

Table 2. Standardized response means and differences from baseline (Δ) for the various outcome measures.

		6 months			12 months			24 months			36 months		
		A	NA	All	A	NA	All	A	NA	All	A	NA	All
		(n = 13)	(n = 20)	(n = 33)	(n = 13)	(n = 20)	(n = 33)	(n = 9)	(n = 19)	(n = 28)	(n = 4)	(n = 18)	(n = 22)
Brooke score	Δ	0.12	0.24	0.18	0.31	0.30	0.30	0.56	0.90	0.79	0.50	1.11	1.00
	SRM	0.24	0.44	0.36	0.49	0.37	0.42	1.06	0.90	0.90	0.87	1.15	1.08
Walton score	Δ	0.82	0.05	0.40	1.00	0.20	0.52	0.67	0.90	0.82	1.25	1.17	1.18
	SRM	0.77	0.10	0.45	0.61	0.38	0.45	0.60	0.72	0.69	0.50	0.66	0.64
Grip strength (kg)	Δ	0.28	-0.15	0.05	0.45	-0.26	0.02	0.78	-0.55	-0.13	2.73	-1.12	-0.42
	SRM	0.31	-0.28	0.06	0.24	-0.50	0.01	0.43	-0.57	-0.09	1.79	-0.90	-0.21
Grip strength (% pred)	Δ	-0.96	-1.40	-1.21	-1.36	-3.11	-2.42	-6.09	-6.83	-6.59	-2.81	-10.72	-9.28
	SRM	-0.11	-0.72	-0.20	-0.09	-0.96	-0.25	-0.48	-1.04	-0.75	-0.27	-1.09	-0.91
Pinch strength (kg)	Δ	0.14	-0.08	0.02	0.14	-0.13	-0.02	0.13	-0.14	-0.05	0.62	-0.34	-0.17
	SRM	0.30	-0.58	0.06	0.39	-0.56	-0.08	0.53	-0.52	-0.18	5.30	-0.99	-0.34
Pinch strength (%pred)	Δ	-0.32	-1.78	-1.13	-1.91	-3.56	-2.91	-4.83	-5.55	-5.32	-0.31	-10.06	-8.29
	SRM	-0.04	-0.86	-0.18	-0.36	-0.77	-0.59	-1.01	-1.27	-1.20	-0.10	-1.35	-1.06
MoviPlate score (#)	Δ	2.71	3.50	3.14	3.46	1.30	2.15	5.67	4.16	4.64	17.33	1.83	4.05
	SRM	0.38	0.80	0.54	0.47	0.15	0.26	0.59	0.35	0.42	1.87	0.14	0.30
MFM total score (%)	Δ	-0.93	-1.73	-1.36	-3.57	-2.16	-2.73	-5.37	-7.19	-6.61	-14.34	-14.18	-14.21
	SRM	-0.22	-0.38	-0.31	-0.51	-0.50	-0.50	-0.62	-1.18	-0.96	-0.58	-1.09	-0.95
MFM D1 (%)	Δ	-3.64	0.01	-1.67	-0.27	-7.89	-3.36	-6.21	-8.54	-6.96	-9.68	-19.89	-11.54
	SRM	-0.41	0.00	-0.26	-0.53	-0.23	-0.34	-0.49	-0.51	-0.50	-0.74	-0.57	-0.61
MFM D2 (%)	Δ	-0.21	-4.93	-2.76	-1.29	-5.78	-3.96	-4.63	-10.14	-8.37	-20.13	-20.90	-20.76
	SRM	-0.05	-0.56	-0.38	-0.41	-0.58	-0.49	-0.63	-1.19	-0.99	-0.61	-1.10	-0.98
MFM D3 (%)	Δ	2.60	0.47	1.45	0.72	-2.02	-0.90	-0.52	-4.02	-2.90	5.98	-11.11	-8.00
	SRM	0.33	0.05	0.17	0.09	-0.23	-0.11	-0.05	-0.37	-0.27	0.56	-0.99	-0.63
FF (%) extensors	Δ	—	—	—	0.09	2.95	1.83	1.72	10.70	8.25	3.85	15.36	13.83
	SRM	—	—	—	0.04	0.39	0.30	0.32	1.12	0.87	0.79	1.60	1.40
FF (%) flexors	Δ	—	—	—	0.49	4.42	2.82	1.25	11.66	8.82	6.15	15.59	14.33
	SRM	—	—	—	0.14	0.81	0.54	0.68	1.38	0.98	0.91	2.21	1.66
ICSA (mm ²) extensors	Δ	—	—	—	6.43	-28.77	-14.37	31.88	-35.66	-16.36	34.70	-51.05	-39.62
	SRM	—	—	—	0.15	-1.17	-0.39	0.53	-0.65	-0.26	0.51	-1.05	-0.70
ICSA (mm ²) flexors	Δ	—	—	—	8.06	-58.15	-32.24	42.93	-75.50	-43.20	64.82	-86.05	-65.93
	SRM	—	—	—	0.11	-0.91	-0.43	0.67	-0.78	-0.42	0.83	-1.00	-0.67

Significant negative changes are highlighted in gray. $|SRM| \geq 0.8$ are in bold (when in the expected direction). In case of bilateral measures, only the dominant side is shown. Abbreviations: A, ambulatory; NA, non-ambulatory; MFM, motor function measure; FF, fat fraction; ICSA, lean muscle cross-sectional area.

versus flexor FF (% of baseline), patients with a FF of greater than 10% and a grip strength less than 35% at any point of the study had either lost ambulation or would lose it in the following 3 years (Fig. 4A–C). In contrast, patients with a flexor FF less than 10% and a grip strength greater than 35% at any point were ambulatory and did not lose ambulation within the next 3 years. The combination of grip strength and FF was also predictive of the Brooke score: boys who presented at any point with a FF less than 20% and a grip strength greater than 27% retained the ability to independently bring a glass of water to their mouth for the following 3 years (Fig. 4D–F).

Discussion

This study of DMD patients followed at least 3 years showed that high precision dynamometry and muscle

MRI can reliably be used over time to assess upper limb performance evolution in ambulatory as well as non-ambulatory boys who have mutations theoretically eligible for exon 53 skipping. The SRMs are indicative of the suitability of these techniques for use as outcome measures in a 1-year clinical trial.

Measurements of motor function, strength, or daily activities such as the performance of upper limb (PUL),^{12,13} the Brooke score,¹⁴ the 9-Hole Peg test,¹⁴ MyoGrip/MyoPinch,⁹ ActiMyo,¹⁵ and the MFM⁸ as well as questionnaires like the Egen Klassifikation^{14,16,17} have been used to evaluate changes over time in non-ambulatory DMD patients. Different blood and urine biomarkers responsive to treatment have also been proposed, but none are currently qualified as surrogate endpoints or as fully qualified biomarkers.¹⁸ Quantitative MRI-based evaluation of the progression of muscle fat

Table 3. Correlations between variables.

	Brooke	Walton	Grip strength (kg)	Grip strength (%)	Pinch strength (kg)	Pinch strength (%)	MoviPlate (#)	MFM total score (%)	FF flexors (%)	FF extensors (%)	ICSA flexors (mm ²)	ICSA extensors (mm ²)
Walton	0.906											
Grip strength (kg)	-0.691	-0.713										
Grip strength (%)	-0.772	-0.771	0.909									
Pinch strength (kg)	-0.747	-0.735	0.903	0.898								
Pinch strength (%)	-0.793	-0.773	0.855	0.950	0.960							
MoviPlate (#)	-0.430	-0.388	0.552	0.399	0.580	0.450						
MFM total score (%)	-0.826	-0.842	0.751	0.860	0.836	0.877	0.444					
FF flexors (%)	0.888	0.870	-0.776	-0.883	-0.849	-0.892	-0.305	-0.921				
FF extensors (%)	0.868	0.829	-0.656	-0.799	-0.773	-0.831	-0.210	-0.975	0.967			
ICSA flexors (mm ²)	-0.613	-0.677	0.865	0.800	0.820	0.781	0.396	0.742	-0.757	-0.646		
ICSA extensors (mm ²)	-0.609	-0.620	0.716	0.800	0.746	0.773	0.176	0.721	-0.772	-0.745	0.841	

Values are Spearman rho correlation coefficients. Significant correlations are highlighted in gray. When bilateral, only the dominant side was considered. Abbreviations: MFM, motor function measure; FF, fat fraction; ICSA, lean muscle cross-sectional area.

replacement is a well-established outcome in natural history studies in ambulatory DMD patients¹⁹ and is also suitable for upper limb skeletal muscle evaluation, as previously shown in non-ambulatory DMD patients.^{6,7,20} Clinical trial readiness in this population requires a better understanding of the long-term robustness and sensitivity with which various measures are able to detect a change over a given period of time.

The population in this study is comparable to those in other prospective natural history studies conducted in non-ambulatory patients with DMD. In all but two studies that included older patients,^{10,12} the overall mean ages at baseline were similar to that of this cohort.^{14,21,22} Although only one prospective study has included exclusively glucocorticoid-treated patients,²¹ it is also common to include patients who have discontinued treatment once becoming wheelchair-bound or even treatment-naïve patients.^{7,10,12,14,22} In terms of functional status, the mean MFM total score in our study was 34%, which corresponds to the MFM score of a previous study that included only glucocorticoid-treated patients.²¹ Other studies included patients with a Brooke score ranging from 1 to 5;^{10,14} the mean Brooke score of ambulatory patients in our cohort was 1.3 and that of non-ambulatory subjects was 4.0.

Our population included only patients theoretically treatable with agents that alter splicing of dystrophin exon 53, who present with a more severe phenotype than other patients with DMD.²³ This study was initially planned to pave the way for a clinical trial in this population.²⁴ Given the approval of golodirsen, an antisense oligonucleotide that induces skipping of exon 53,^{25,26} and the development of other therapies with similar modes of action, the present set of data offers the opportunity to benchmark treated patients with natural history of patients with the same genotype.

Although data were collected over a period of 78 months in some patients, we limited the SRM analysis to the first 36 months to avoid bias related to different follow-up durations. This study constitutes, to our knowledge, the longest prospective natural history study which included non-ambulatory patients at baseline. In previous studies, only ambulatory patients had been followed for this length of time.^{2,3,27} In non-ambulatory subjects, cross sectional studies^{4,16,28,29} and prospective studies with 12-month,^{6,7,10} 18-month,²¹ and 24-month^{12–14,22} follow-up periods have been described.

We showed that in non-ambulatory patients, changes were detected annually over the course of 3 years with

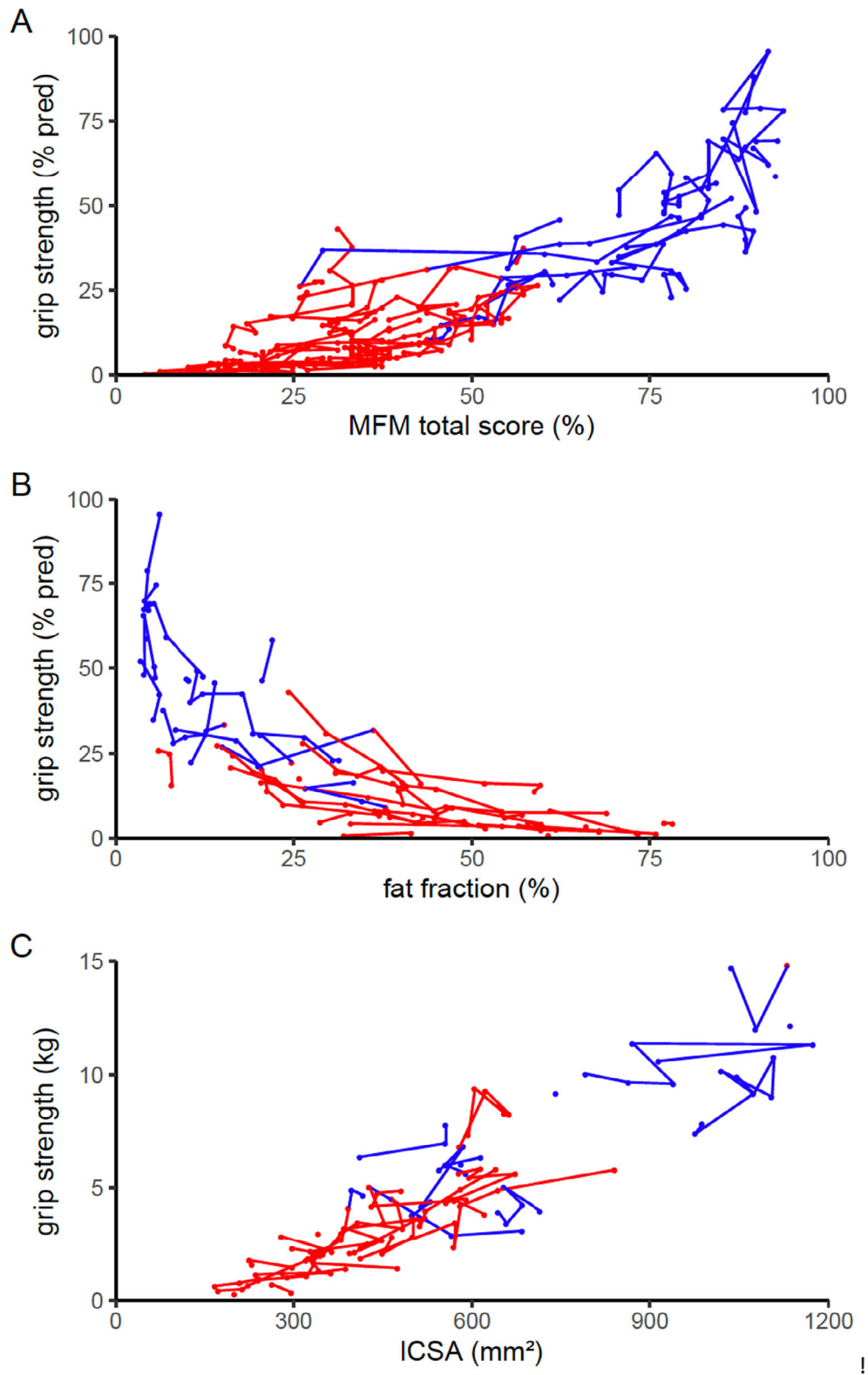


Figure 3. Relationship between grip strength and MFM total score, FF, and ICSA. Correlations of (A) percentage of predicted grip strength to MFM total score, (B) percentage of predicted grip strength to FF of the flexors, (C) grip strength absolute value (expressed in kg) to ICSA of the flexors (expressed in mm²). Each line connects data points for individual non-ambulatory (red) or ambulatory (blue) subject.

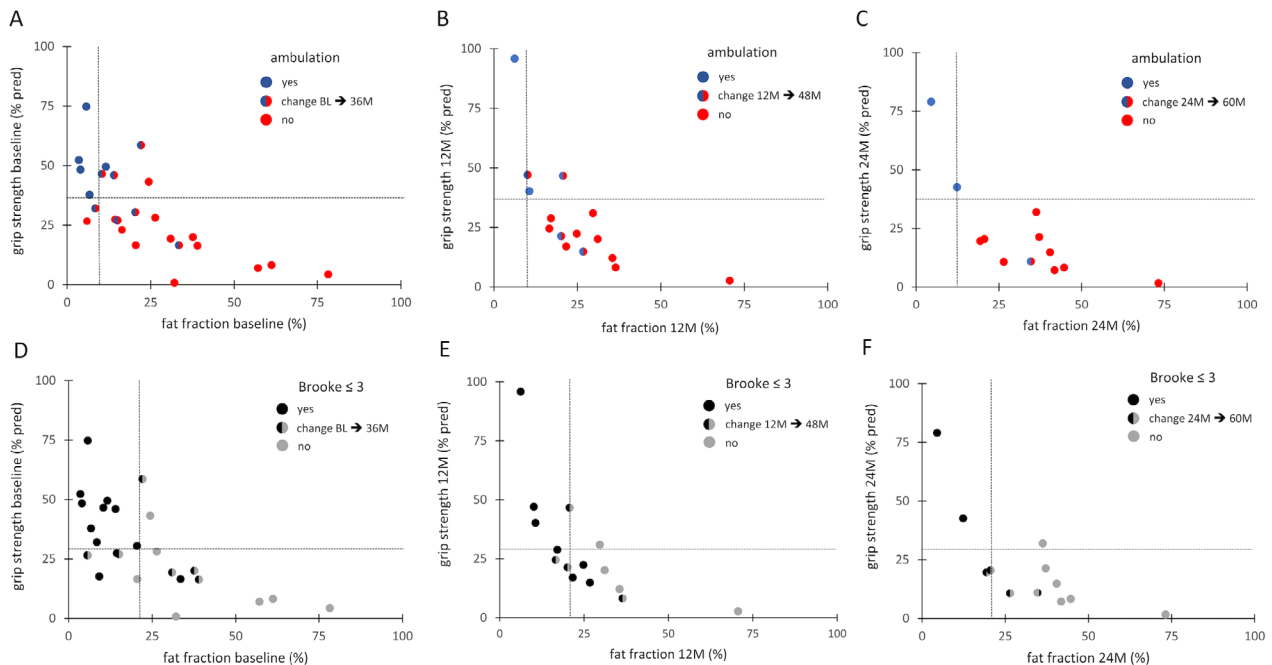


Figure 4. Correlation between flexor FF and grip strength with clinical parameters. Grip strength (%pred) versus FF in the flexors (% of baseline) for (A) subjects who were ambulatory (blue), non-ambulatory (red), or changed between baseline and 3 years (blue/red) ($n = 25$), (B) subjects who were ambulatory (blue), non-ambulatory (red), or changed between 12 months and 4 years (blue/red) ($n = 15$ from the cohort baseline 3 years), (C) subjects who were ambulatory (blue), non-ambulatory (red), or changed between 2 and 5 years (blue/red) ($n = 12$ from the cohort 1–4 years), (D) subjects who had Brooke score ≤ 3 (black) or >3 (gray) or who changed (black/gray) between baseline and year 3 ($n = 25$), (E) subjects who had Brooke score ≤ 3 (black) or >3 (gray) or who changed (black/gray) between 12 months and year 4 ($n = 15$ from the cohort baseline 3 years), and (F) subjects who had Brooke score ≤ 3 (black) or >3 (gray) or who changed (black/gray) between year 2 and year 5 ($n = 12$ from the cohort 1–4 years).

high sensitivity ($|SRM| \geq 0.8$) for grip and pinch strength, function (MFM, Brooke), and composition of the forearm muscles (quantitative MRI-based FF), illustrating the ability of these measures to quantify disease progression in individual patients accurately over 1 year and beyond. As in our previous study conducted in a smaller independent cohort,¹⁰ we found a stronger correlation ($\rho = 0.86$) between hand grip strength and function (MFM total score) than in another study.²⁸ We reproduced the strong correlations described between quantitative MRI-based FF (both flexor and extensor) and function and strength measures previously demonstrated in cohorts of DMD^{6,30} and spinal muscular atrophy³¹ patients. The results of our study also confirm that when considering the strength data in absolute terms (i.e., in kg), the disease progression can be partially masked by the effect of the growth, especially in boys still in the ambulatory phase of the condition. This decreases the sensitivity to change of such data, making it necessary to express the strength relative to baseline values as recently suggested.³²

As a group, the DMD patients in our cohort presented with a clear disease progression; at an individual level, however, we observed a large heterogeneity in disease

evolution on a clinical basis and from an imaging perspective. The difficulty of prediction of FF evolution at an individual level has already been highlighted based on studies of the lower limbs of ambulatory patients, although recent logistic modeling has shown promising improvements.^{33,34} In our study, we observed a similar heterogeneity in the FF evolution in the forearm muscle groups with annual increases ranging from zero to 20%–25% depending on the individual; this range is similar to that previously reported for lower limb muscles.²⁰

Although our data indicate that FF (and ICSA), grip strength (expressed in %pred), and the MFM score are sensitive and reliable for use in the follow-up of the natural evolution of the disease, correlating yearly changes with clinically meaningful endpoints remains challenging in view of this heterogeneity of trajectories. While the loss of ambulation is certainly the most commonly used milestone in DMD,^{33,34} there is a need for other meaningful upper limb milestones for non-ambulatory patients. In this regard, we investigated how the combined value of flexor FF and grip strength can predict the Brooke score evolution. This exploratory approach spotlighted the predictive nature of the combination of FF and grip strength

of the 3-year preservation of ambulation in ambulatory patients and the maintenance of the ability to carry a glass of water to the mouth in non-ambulatory patients. Increased understanding of these relationships based on larger and independent cohorts will enable stratification of patients for future clinical trials.⁶

At the time of preparing this natural history study, available and clinically relevant outcome measures were chosen that were sensitive to change in both ambulatory and non-ambulatory patient groups.^{8,9,35,36} On 1 January 2021, there are only three trials registered in clinicaltrials.gov that are currently recruiting non-ambulatory patients (NCT03354039, NCT04371666, and NCT04004065), whereas five exclude non-ambulatory patients. Non-ambulatory subjects are likely excluded because these patients are not expected to respond as well as ambulatory subjects to treatment and because of decreased available muscle mass and increased retractions. As described in spinal muscular atrophy, disease duration is a constant predictive factor of treatment efficacy.³⁷ It has been thought that as non-ambulatory patients constitute a more fragile population with higher comorbidity, changes in clinical condition would be more difficult to quantify than in ambulatory subjects. This study clearly demonstrates, however, that SRM of clinical and MRI endpoints in non-ambulatory patients are comparable to those considered as acceptable in ambulatory patients.³⁸

This study has some limitations. First, the inclusion only of patients theoretically treatable with agents that promote exon 53 skipping may be perceived as a limitation, since this genotype results in the most severe symptoms, which could lead to an overestimation of the SRM.²³ Nevertheless, this restriction ensured a more homogeneous population, and this population is specifically targeted by clinical trials²⁵ (NCT02500381). Second, this study focused only on upper limbs; we did not evaluate global natural history such as cardiac and respiratory evolution. Furthermore, we had to limit the number of functional evaluation scales to reduce patient burden and to avoid fatigability induced by redundant measures. At the time the study was initiated, the PUL was not available.^{13,39} This scale partially overlaps with the distal motor function domain of the MFM. Third, only two sites were involved in patient analysis; however, patients were included from different sites and countries that followed diverse guidelines of standard of care management. In fact, not all patients were on glucocorticoids at baseline and treatment initiation time, type, and posology varied, which may have influenced the disease course.⁴⁰ At the time of study initiation, glucocorticoids use in non-ambulatory patients with DMD in France was very limited. Several studies have since then demonstrated the potential benefit of

glucocorticoids including in the non-ambulatory²⁹ and in the overall population,⁴¹ and more and more non-ambulatory patients are nowadays on glucocorticoids. Our study was not designed or powered to demonstrate glucocorticoids effect.

Conclusions

This study revealed a continuous decline as well as a strong correlation between upper limb muscle FF, function, and strength over a 3-year period in non-ambulatory and ambulatory DMD patients. Patients with DMD, even with a single genotype, constitute a heterogeneous population and some patients may present with an unpredictable clinical or MRI evolution. In addition, this study permitted an initiatory approach to predict patient evolution over a 3-year period by using combined clinical and MRI evaluations. The added value of these longitudinal and correlative data of clinically relevant outcome measures will enable the scientific community and industry partners to better stratify patient populations for future clinical trials of the upper limb in non-ambulatory patients with DMD.

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Author Contributions

All the authors contributed to the study conception and design, analyzed and interpreted the data, commented on previous versions of the manuscript, and read and approved the final manuscript.

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Conflict of Interest

CL has no direct disclosures to declare; she is an MFM, ActiMyo, and ATOM trainer. HR, AMS, TG, MA, VC, VD, IL, and JLL report no disclosures relevant to the manuscript. EG is president of BIOSSEC and was hired by Généthon for the statistical analyses. FM has no direct disclosures to declare; he consults for Pfizer, Sarepta, Santhera, and Dyne Therapeutics. JYH is a coinventor of the MyoGrip, MyoPinch, and MoviPlate. PGC receives support from the European Community and the *Association Française contre les Myopathies*. LS is a coinventor of the MoviPlate; he consults for Pfizer, Sarepta, Santhera, Catabasis, RegenexBio, Affinia, Biophytis, and Fibrogen.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Mean and standard deviation (SD) in both ambulatory and non-ambulatory populations to test the effect of glucocorticoids on the various outcome measures. The variables at baseline were not statistically different between glucocorticoid users and nonusers ($p > 0.0038$ with Bonferroni correction), except for MFM D2 and total score in non-ambulatory patients. Their changes over time were not different. Note that the populations are not balanced in age and ambulatory status as explained in the main text.