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TITLE:

Measurements of Motor Function and Other Clinical Outcome Parameters in Ambulant Children with Duchenne Muscular Dystrophy

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SUMMARY:

The aim of this study is to present the most reliable clinical outcome measures and their correlations with quantitative muscle MRI in ambulant patients with Duchenne muscular dystrophy.

ABSTRACT:

While the number of new treatment options tested in patients with Duchenne muscular dystrophy (DMD) is increasing, there is still no defining of the most reliable assessments regarding therapeutic efficacy. We present clinical and radiological outcome measures used in ambulatory patients participating in our trial "Treatment with L-citrulline and metformin in Duchenne muscular dystrophy". The motor function measure is a validated test in patients with neuromuscular disorders that consists of 32 items and assesses all three dimensions of motor performance including standing and transfer (D1 subscore), axial and proximal motor function (D2 subscore), and distal motor function (D3 subscore). The test shows high intra- and inter-rater variability but only when strictly following guidelines of the materials, examination steps, and calculation of scores. The 6-minute walk test, timed 10-meter walk/run test, and supine-up time are commonly used timed functional tests that also sufficiently monitor changes in muscle function; however, they strongly depend on patient collaboration. Quantitative MRI is an objective and sensitive biomarker to detect subclinical changes, though the examination costs may be a reason for its limited use. In this study, a high correlation between all clinical assessments and quantitative MRI scans was found. The combinational use of these methods provides a better understanding about disease progression; however, longitudinal studies are needed to validate their reliability.

INTRODUCTION:

Outcome measures that reliably reflect treatment response are an essential requirement of successful clinical trials. Due to the rapid development of new therapeutic strategies, stronger effort has been made to define reproducible as well as sensitive methods that monitor clinical outcomes.

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder and the most common type of muscular dystrophy in children. It is characterized by severe involvement of predominantly the skeletal and heart muscle and a progressive disease course, with loss of ambulation around 8-12 years old and premature death mainly before 30 years old¹. Validated tests such as motor function measure and timed function tests are widely accepted as clinical tools for monitoring disease progression, as they assess many aspects of daily life functions. Furthermore, in ambulatory cases, they seem to be more sensitive than quantitative muscle strength measures, which cannot be appropriately performed in weak and non-cooperative patients²⁻³.

The motor function measure (MFM) assesses functions of the neck, trunk, arm, and leg muscles and abilities such as standing, transferring, and walking. It can even be performed in patients who have lost ambulation, as it reflects three dimensions of motor performance⁴. The MFM (validated for patients aged 6-60 years with DMD) was evaluated based on the MFM User's Manual⁵. It includes 32 items and is divided into three subdomains: D1 (assessment of standing and transfer), D2 (assessment of axial and proximal motor function), and D3 (assessment of distal motor function). All items are scored on a 4-point scale (0-3). The test is validated in neuromuscular disorders and can sufficiently monitor changes in muscle function and predict loss of ambulation. Moreover, it agrees with clinical changes perceived by the treating physicians and

patients with DMD⁶⁻⁷. Timed function tests are also commonly used as outcome measures, though they are mainly performed in ambulatory patients. Among these, the 6-minute walk test (6MWT) has received special attention since it shows the highest test-retest reliability, predicts clinical decline and loss of ambulation, and correlates more accurately with muscle function measures compared to quantitative muscle strength measures⁸⁻⁹. The test measures the maximal walking distance of a patient in 6 minutes¹⁰. It is guided by two trained professionals, a “follower” who walks 1-2 meters behind the patient, and an “evaluator” who records the time. Other timed function tests have lower test-retest reliability and do not reflect endurance, an important marker of ambulatory function⁸⁻¹¹. These tests include the timed 10-meter run/walk test (10MWT), which measures the best performance of walking/running for a 10-meter distance, and the supine-up time, which measures the ability to stand up from supine position². The use of the motor function measure and timed function tests as primary and secondary endpoints in clinical trials is justified; however, a major limitation is that none are independent of patient collaboration and the skills of the evaluator.

Quantitative MRI (QMRI) is an objective method to visualize well-described morphological abnormalities of the musculature including edema, muscle degeneration, and increased content of adipose and connective tissue¹². The use of MRI as a diagnostic tool in neuromuscular disorders has already been established, but its role in monitoring disease progression and treatment response is still limited to clinical trials. T2-relaxation time is known to be increased in muscle dystrophies due to muscle damage, edema, fatty replacement, and inflammation, and further information about muscle fat content can be extracted through calculation of the mean fat fraction (FF). QMRI has been shown to be a promising biomarker, as measurements have correlated with clinical outcome and disease progression, while a mean fat fraction of 50% predicted loss of ambulation¹³⁻¹⁴. Moreover, QMRI has been able to detect subclinical changes in patients with stable or even improved outcome measures¹⁵⁻¹⁶. QMRI data of extensors muscles has also shown to be meaningful regarding its correlation with clinical outcomes¹⁷. QMRI is a non-invasive and sensitive method; nevertheless, its cost and the possibility of reduced compliance in younger children may limit its use.

The reliability of functional tests and QMRI has been previously shown in Becker’s muscular dystrophy¹⁸. The aim of this cross-sectional study was to highlight sensitive clinical and radiological outcome measures in ambulant children with DMD, as the need for standardized and disease-specific assessments is increasing in the era of clinical trials in neuromuscular disorders.

PROTOCOL:

Before recruitment, the study was approved by the local ethics committee [Ethics Committee of the two Basel Cantons (EKBB 63/13)] and the Swiss Drug Agency (Swissmedic 2013DR3151) and registered under ClinicalTrials.gov (NCT01995032).

1. Clinical Assessment of Muscle Function

1.1. Motor function measure (MFM)

1.1.1. Have the patient perform each of the following tasks and score them as shown in **Table 1**.

Note: The MFM contains 32 independent items that must be performed in the given order to avoid unnecessarily repositioning and exhaustion of the patient⁵. See **Table 1** for a detailed description of each task and scoring.

1.1.1.1. Ask the patient to lie down on his back. Ask him to hold his head in midline position and turn it from one side to the other. In this and all subsequent steps, score the patient on a scale of 0 to 3 (see **Table 1**) based on performance of the task.

1.1.1.2. Ask the patient to lie down on his back with the head in midline position. Ask him to raise the head and maintain the position.

1.1.1.3. Ask the patient to lie down on his back. Ask him to bring one knee to the chest.

1.1.1.4. Ask the patient to lie down on his back with one leg flexed both at the hip and knee at approximately 90°. Ask him to place the lower leg parallel to the mat with the foot in plantar flexion. Ask him to perform a maximal dorsiflexion of the foot.

1.1.1.5. Ask the patient to lie down on his back. Ask him to place one upper limb beside his body and to bring the hand to the opposite shoulder.

1.1.1.6. Ask the patient to lie down on his back with the lower limbs half-flexed and the feet resting on the mat slightly apart. Ask him to maintain this position and raise the pelvis.

1.1.1.7. Ask the patient to lie down on his back. Ask him to turn over onto the stomach and free both upper limbs.

1.1.1.8. Ask the patient to lie down on his back. Ask him to sit up.

1.1.1.9. Ask the patient to sit on the mat. Ask him to maintain the seated position and keep the hands in contact in front of the trunk.

1.1.1.10. Ask the patient to sit on the mat and place a tennis ball in front of him. Ask him to touch the ball and sit back again.

Note: The tennis ball should be at a distance so that the patient must lean his trunk forward about 30° away from the starting position in order to touch it.

1.1.1.11. Ask the patient to sit on the mat with the lower limbs in front of him. Ask him to stand up.

1.1.1.12. Ask the patient to stand in front of the chair. Ask him to sit down on the chair.

1.1.1.13. Ask the patient to sit on the chair. Ask him to maintain the seated position as straight as possible.

1.1.1.14. Ask the patient to sit on the chair with the head in complete flexion. Ask him to raise the head and maintain this position.

1.1.1.15. Ask the patient to sit on the chair in front of a table with the forearms (except for the elbows) on the table. Ask him to place both hands on top of the head.

1.1.1.16. Ask the patient to sit on the chair in front of a table with the forearms on the table. Place a pencil on the table and ask him to touch the pencil.

Note: The pencil should be placed at a distance equal to the length of the patient's upper limb.

1.1.1.17. Ask the patient to sit on the chair in front of a table with the forearms on the table. Ask him to pick up the coins next to his hand and to hold them in the same hand.

Note: All coins should be placed next to the patient's hand and picked up successively with one hand.

1.1.1.18. Ask the patient to sit on the chair in front of a table with the forearms on the table. Place a CD glued to a piece of cardboard on the table. Ask him to place one finger in the center of the CD and to trace around the edge of the disc with the finger.

1.1.1.19. Ask the patient to sit on the chair in front of a table with the forearms on the table. Hold a pencil and a paper on the table. Ask him to pick up the pencil and draw inside the frame.

1.1.1.20. Ask the patient to sit on the chair in front of a table with the forearms on the table. Put a sheet of paper in his hands and ask him to tear the paper at least 4 cm.

1.1.1.21. Ask the patient to sit on the chair in front of a table with the forearms on the table. Place a tennis ball next to his hand. Ask him to pick up the ball, raise it, and turn the hand.

1.1.1.22. Ask the patient to sit on the chair in front of a table with the forearms on the table. Place a diagram with pictures on the table. Ask him to place the finger at the center of the diagram on the word "start", then place the finger on the drawings.

1.1.1.23. Ask the patient to sit on the chair with the arms next to his body and the table at a distance equivalent to the length of his forearm. Ask him to place both hands on the table.

1.1.1.24. Ask the patient to sit on the chair with both feet on the ground. Ask him to stand up.

1.1.1.25. Ask the patient to stand with the upper limbs resting on a piece of equipment for support. Ask him to release the support and stand straight.

1.1.1.26. Ask the patient to stand with the upper limbs resting on a piece of equipment for support. Ask him to release the support and raise one foot.

1.1.1.27. Ask the patient to stand without support. Ask him to touch the floor with one hand and stand up again.

1.1.1.28. Ask the patient to stand and walk 10 steps on his heels.

1.1.1.29. Ask the patient to stand without support. Draw a straight line (about 6 m long and 2 cm wide) on the floor, and ask him to walk on the line.

1.1.1.30. Ask the patient to stand without support. Ask him to run.

1.1.1.31. Ask the patient to stand on one foot without support with the other foot off the ground. Ask him to hop in place.

1.1.1.32. Ask the patient to stand without support. Ask him to squat and stand up again.

1.1.2. Calculate the scores.

1.1.2.1. Add the scores of all 32 items, divide the sum by 96, and multiply it by 100 to calculate the final score.

1.1.2.2. To calculate scores of the subdomains, add the score of all items in that domain and divide it by the maximum score for the domain, then multiply it by 100.

Note: All scores must be calculated as percentages.

1.2. 6-minute walk test (6MWT)

1.2.1 Task performance

1.2.1.1. Let the patient rest for 10 min prior to testing. Demonstrate the walking process.

1.2.1.2. Ask the patient to stand at the starting line on the right side of the 0 cone. Give the instructions, "Ready, set, go".

1.2.1.3. When saying "go", let the patient start walking around the cones without crossing the middle and, if possible, without slowing down or stopping.

1.2.1.4. At 6 min, stop the timer and let the patient stop walking. Count down the final seconds of the test and mark the point at which the patient stopped.

1.2.2. Calculation of the scores

1.2.2.1. Record each timepoint at which the patient passes a cone.

1.2.2.2. Calculate the total distance by adding a and b , where a is defined as the distance of the final lap (between the last cone rounded until the finishing point at 6 min), and b is defined as the distance in meters prior to the last cone (distance at the time of the last cone rounded).

1.3. Timed 10-meter walk/run test (10MWT)

1.3.1 Task performance

1.3.1.1. Ask the patient to stand at the starting line. Stand at the 12-meter mark and give the instructions, "Ready, set, go".

1.3.1.2. When saying "go", let the patient start walking/running.

1.3.1.3. Measure the time and observe the quality of the walk/run. Stop the timer when the second foot of the patient passes the finish line at 10 m.

1.3.1.4. Repeat the test three times, and use the fastest performance to calculate the score.

1.3.2 Calculation of the scores

1.3.2.1. Score the patient on a 6-point scale (1-6) based on the quality of the walk/run during the fastest trial. Score 1 if he is not able to walk by himself, and score 2 if he is not able to walk by himself but is able to walk when supported by a knee-ankle-foot orthosis or another person.

1.3.2.2. Score 3 if he is not able to increase the walking speed and his gait remains highly adapted and lordotic. Score 4 if he is able to increase the walking speed but not able to run while the gait remains moderately adapted.

1.3.2.3. Score 5 if he is almost running but cannot raise his feet from the ground. Score 6 if he is able to run and raise both feet from the ground.

1.4. Supine-up time

1.4.1. Task performance

1.4.1.1. Ask the patient to lie down on the examination table in supine position.

Note: In the case where a mat is necessary, be sure that it is fixed and not slippery.

1.4.1.2. Give the instructions, “Ready, set, go”. When saying “go”, let the patient start standing up as fast as he can.

1.4.1.3. Measure the time and observe the quality of the task. Stop the timer when the patient has assumed an upright position with arms by his side. Provide a chair after the patient has attempted to stand from the floor for 30 seconds.

1.4.1.4. Repeat the test three times, and use the fastest performance to calculate the score.

1.4.2. Calculation of the scores

1.4.2.1. Score the patient on a 6-point scale (1-6). Score 1 if he is not able to stand up from supine position. Score 2 if he is able to stand up from supine position when using a furniture for support.

1.4.2.2. Score 3 if he turns over in supine position and needs both hands “climbing up” on the legs to reach the standing position. Score 4 if he turns over in supine position and needs one hand on the leg to reach the standing position.

1.4.2.3. Score 5 if he turns to the side and uses one or both hands on the ground but not on the leg to reach the standing position. Score 6 if he is able to stand up without turning over or using the hands on the legs.

2. Quantitative Muscle MRI

2.1. Perform axial MRI of the thighs including all muscles (flexors, extensor, and adductors) on a 3 Tesla scanner using a 36-channel peripheral angio and spine coil. Perform localizers and slice positioning as previously described^{11,17}.

2.2. Use a three-dimensional (3D) gradient echo sequence with two different echo times for in-phase and opposed-phase imaging [30 slices, repetition time (TR) = 20 ms, echo time 1 (TE1) = 2.45 ms, echo time (TE2) = 3.68 ms, flip angle = 15, acquisition time = 2 min 49 s] and a multi-contrast spin echo with 14 echo times to quantify the transverse relaxation times. Use a field of view of 400 x 400 mm and 384 x 384 matrix to achieve 1 mm in-plane resolution and 3 mm slice thickness.

2.3. Manually draw regions of interest (ROI) on the MR images containing the whole muscle area of flexors, extensors, and adductors of each leg.

2.4. Use the two-point Dixon method and generate relative fat content maps using the pixelwise fat fraction, given $f/(f + w)$, where f = fat images, w = water images¹⁷.

2.5. Calculate the T2-relaxation time and mean fat fraction for each muscle group.

REPRESENTATIVE RESULTS:

Baseline data of 47 ambulatory male patients (aged 6.5 to 10.8 years) with DMD were analyzed. All patients participated previously in the “Treatment with L-citrulline and metformin in Duchenne muscular dystrophy” study. Patients were enrolled from the University Children's Hospital Basel and from the patient registries of Switzerland, Germany, and Austria. Except for one patient, who refused to take part in the scanning, MRI of all thigh muscles was performed^{13,17}. MRI examinations were blinded to clinical status and motor function tests.

Statistical analysis was performed using the R Core Team (2017). The Pearson product-moment correlation (r) was used to estimate associations, and the Spearman rank correlation coefficient (rs) was used to perform the analysis. A significance level of 0.05 was chosen.

Clinical examination was performed in all 47 patients aged 6.5-10.8 years [mean 8.2, standard deviation (SD) 1.1], according to the protocol. **Table 1** shows the detailed description and the scoring system of the MFM, **Figure 1A** illustrates the examination steps of all 32 items, and **Figure 1B** shows the 6MWT in a selected patient with DMD. QMRI of the thigh muscles was performed in all patients except one, who refused the examination. T2 measurements of one patient had to be excluded from the analysis due to movement artifacts.

The median MFM total score was 78.1% [interquartile range (IQR) 75.0-83.3], while the median value of the D1 subscore reached 56.4% (IQR 48.7-66.7), median of the D2 subscore 97.2% (IQR 94.4-96.6) and median of the D3 subscore 90.5% (83.3-95.2). The mean distance of the 6MWT was 359 m (SD 76.4). The mean time was 6.7 seconds (SD 1.8) for the 10MWT and 10.2 seconds (SD 6.4) for the supine-up test. There were no correlations between clinical assessments and height, weight, and BMI of the patients. The total MFM, D1 subscore, and 6MWT did not correlate with age; however, the 10MWT and the supine-up time showed a positive correlation with the age of the patients. All clinical tests were significantly intercorrelated: the MFM total score and its D1 subscore, the 6MWT and 10MWT, were highly correlated ($p < 0.001$) with each other.

During investigation of the magnetic images, the mean fat fraction and global T2 time showed a strong intercorrelation with each other and negative correlation with the D1 subscore of the MFM and 6MWT ($p < 0.001$). There was also a highly positive correlation between the QMRI data, 10MWT, and supine-up time ($p < 0.001$). The extensor muscles of the thigh showed the strongest correlation with the functional tests, though the adductor muscles were more severely affected than the flexors and extensors. Both the T2 relaxation time and mean fat fraction correlated with the ages of patients. **Figure 2** shows a representative example of the correlation of baseline QMRI data with motor function tests in two patients with DMD.

The detailed description of all baseline values and their correlations can be found in our previous publication¹⁷.

FIGURE AND TABLE LEGENDS:

Table 1: Detailed description of all 32 items of the MFM, including the definition of the starting position, specific task, and scoring system. Red = D1, blue = D2, and yellow = D3 subdomains.

Figure 1: Illustration of the MFM and 6MWT in an 8-year-old patient with DMD. (A) All 32 items of the MFM are represented; numbers in red box = D1, blue = D2, and yellow = D3 subscores. The first rows represent the starting positions and second rows represent the tasks to perform (arrow). It should be noted that no equipment was required to support the patient in item 25. (B) The starting position of the 6MWT is illustrated on the left side, while the right-sided image shows a patient performing the test on a 30 m corridor under supervision of a physiotherapist.

Figure 2: Representative correlation of the baseline QMRI data and clinical assessments in two patients with DMD. Patient 1, with more severe clinical involvement assessed by the MFM (in %), 6MWT (in meters), 10MWT (in seconds), and supine-up time (in seconds), showed prominent fatty degeneration (FF in %) of the thigh muscles, particularly of the adductors (arrow). Patient 2, with better clinical performance, showed less pronounced fatty degeneration of the adductors (arrow). For comparison, clinical assessments (median MFM in %, mean 6MWT in meters, mean 10MWT and supine-up time in seconds) and QMRI data (mean FF in %) of all 47 patients (mean age in years) at baseline are represented in the table.

DISCUSSION:

Several promising outcome measures have been used in clinical trials in patients with Duchenne muscular dystrophy (DMD). The MFM is a validated and reproducible functional test that involves a detailed examination of crucial motor functions in 32 steps⁴, while the 6MWT can provide useful information about the patient's endurance.

All currently validated tests have limitations due to inter- and intra-rater variabilities and all require cooperation of the patient and expertise of the examiner. To reduce limitations, it is crucial that the evaluator adheres to the protocol and recommended examination materials. Particularly when performing the MFM, the specific definitions of certain positions must be considered. Furthermore, the starting positions followed by single steps of each item must be strictly followed and clearly presented. Any factors that may interfere with test performance should be avoided, such as wearing uncomfortable clothes or using slippery examination materials. Likewise, the patients should not be allowed to use any orthotic devices while performing these tests. When completing the 6MWT, it is necessary to give the patient enough time to rest before the test.

The MFM has many advantages that qualify it as a useful tool in clinical trials. Its application is not limited to patients in adulthood, giving researchers the unique opportunity to follow children from the age of 6 and demonstrate clinical changes and therapy response throughout many years. The test is suitable for both ambulatory and non-ambulatory patients, showing a potential superiority to other tests such as the North Star Ambulatory Assessment^{2,8-11}. Besides, the MFM is less dependent on a patient's compliance compared to tests of motor strength such as manual muscle testing. Timed function tests provide information about a patient's endurance and can predict disease progression. In particular, the 6MWT has been described as a reproducible outcome measure; however, it shows an age-dependency due to the different stages of motor development. Independently of age, a rapid clinical decline may be shown in patients performing

6MWT at distances of less than 350 m at inclusion, so that results of the 6MWT may be used as prognostic parameters¹¹.

However, there is still a need to describe broader functions not assessed by the commonly used clinical tests. Limitations in daily life activity and reduced quality of life are not captured routinely, and some effort has already been made to assess these aspects using electronic devices and questionnaires¹⁹. In addition, the more sensitive evaluation of retained functions of the upper limbs in non-ambulatory patients has gained increasing interest^{20,21}. Quantitative MRI has also become important in clinical trials when assessing involvement of the musculature. Fat replacement can be measured using the mean fat fraction, while the T2 relaxation time provides information about the presence of edema and inflammation. Changes on magnetic images were shown to correlate with clinical assessments and predict loss of ambulation^{13,22} and treatment response to corticosteroids²³. Nevertheless, when analyzing QMRI data, non-homogenous replacement by adipose tissue must be taken into consideration when selecting regions of interest, since higher fat contents have been shown in distal and proximal parts of the musculature compared to the muscle belly, influencing quantitative measurements²⁴. Furthermore, limitations of the two-point Dixon method to evaluate fat fraction is also of importance²⁵. The two-point Dixon method can lead to overestimation of the fat fraction in less affected muscles; besides, fatty infiltration can prolong the T2-relaxation time. In the current analysis, T2-times and mean fat fraction show strong correlation in the affected muscles and exhibit the same distribution of involvement¹⁵. Accordingly, the existence of a second independent MRI method confirming results of the first (Dixon) method can validate the MRI approaches used in a given trial.

This cross-sectional analysis looked at the MFM and timed function tests in correlation to QMRI regarding treatment response and clinical decline. All timed function tests correlated significantly with each other and with motor function measure; moreover, all clinical assessments correlated highly with QMRI data. The extensor muscle of the thigh showed the strongest correlation with motor function tests; accordingly, it could serve as an imaging biomarker in clinical trials^{26,27}.

This study illustrates that the combination of clinical assessments and quantitative MRI provides a stronger understanding about disease progression in patients with Duchenne muscular dystrophy; however, longitudinal confirmation of the sensitivity of these measures is still needed.

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DISCLOSURES:

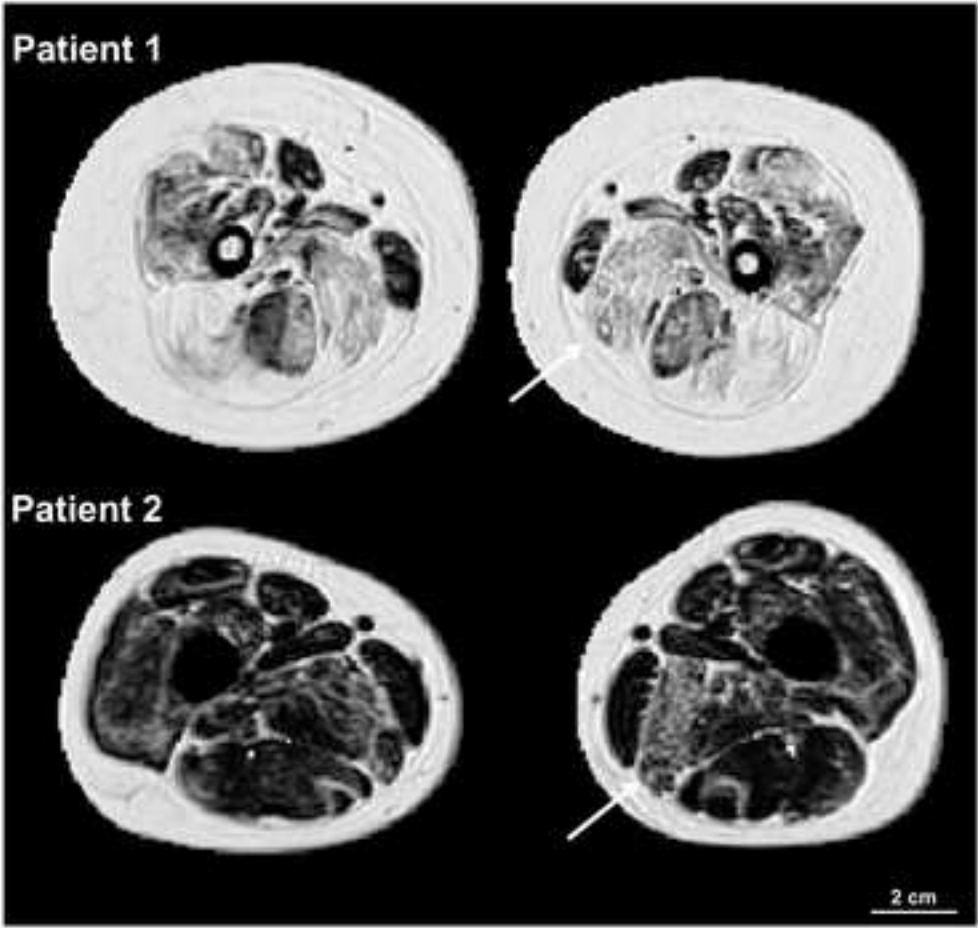
The authors have nothing to disclose.

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A**B**




	Age	MFM total	D1	6MWT	10MWT	Supine up	FF all muscles
Patient 1	9.3	75.0	43.6	227	11.6	28.1	47.2
Patient 2	7.4	88.5	76.9	509	4.1	3.8	16.3
All patients	8.2	78.1	56.4	359	6.7	10.2	26.9







MFM item	MFM subscore	Starting position	Description of the task	Definition of the scores
1	D2	Place the patient on the mat/wide examination table in supine position.	To hold the head in the midline position and then to turn it from one side to the other.	<p>0: cannot hold the head for 5 seconds in the midline position and/or cannot turn it</p> <p>1: holds the head for 5 seconds in the midline position and turns it partially, at least to one side</p> <p>2: holds the head for 5 seconds in the midline position and turns it completely from one side to the other, with difficulty</p> <p>3: holds the head for 5 seconds in the midline position and turns it completely from one side to the other</p>
2	D2	Place the patient on the mat/wide examination table with the head in midline position.	To raise the head and to maintain the position.	<p>0: cannot initiate the movement</p> <p>1: cannot lift the head but initiates the movement</p> <p>2: raises the head, but cannot maintain the raised position for 5 seconds</p> <p>3: raises the head and maintains the raised position for 5 seconds</p>
3	D2	Place the patient on the mat/wide examination table in supine position, with the legs resting, if possible, on the thighs, calves and feet.	To bring one knee to the chest.	<p>0: cannot initiate the movement</p> <p>1: initiates the movement at the hip and/or knee</p> <p>2: partially flexes the hip and/or the knee (<90°) or the foot remains in contact with the mat or table</p> <p>3: flexes the hip and knee more than 90° by raising the foot for the whole movement</p>
4	D3	Place the patient on the mat/wide examination table in supine position with one leg flexed both at the hip and knee at approximately 90°, while the lower part of the leg should be parallel to the mat and the foot should be in plantar flexion in the air.	To carry out a maximal dorsiflexion of the whole foot.	<p>0: cannot initiate the movement</p> <p>1: only extends the toes</p> <p>2: partially dorsiflexes the foot but does not reach 90° in relation to the leg</p> <p>3: from the plantar flexion, dorsiflexes the foot to at least 90° in relation to the leg</p>
5	D2	Place the patient on the mat/wide examination table in supine position with the upper limb to be tested positioned alongside the body with the hand or the fingers in contact with the mat.	To bring one hand to the opposite shoulder.	<p>0: cannot raise the hand and elbow</p> <p>1: partially achieves the movement by raising at least the hand and the elbow</p> <p>2: raises the hand and moves it to the opposite shoulder using compensatory movements</p>

				3: raises the hand and moves it to the opposite shoulder
6	D1	Place the patient on the mat/wide examination table in supine position with the lower limbs half flexed, the kneecap facing up and the feet resting on the mat slightly apart. The upper limbs are in a comfortable position, not in contact with the body.	To maintain the starting position then to raise the pelvis.	<p>0: cannot maintain the starting position for 5 seconds</p> <p>1: maintains the starting position for 5 seconds</p> <p>2: maintains the starting position for 5 seconds then partially raises the pelvis</p> <p>3: maintains the starting position for 5 seconds then raises the pelvis; the lumbar spine, the pelvis and the thighs are aligned and the feet are slightly apart</p>
7	D2	Place the patient on the mat/wide examination table in supine position.	To turn over onto the stomach and to free both upper limbs.	<p>0: cannot initiate rolling</p> <p>1: rolls partially</p> <p>2: turns over into prone with difficulty and compensatory movements and/or cannot free the upper limbs from under the trunk</p> <p>3: turns over into prone and frees both upper limbs from under the trunk</p>
8	D1	Place the patient on the mat/wide examination table in supine position.	To sit up.	<p>0: cannot initiate the movement</p> <p>1: initiates the movement, or turns over into prone to sit up</p> <p>2: with upper limb support, sits up</p> <p>3: without upper limb support, sits up</p>
9	D2	Place the patient on the mat/wide examination table in any seated position without trunk support. Ask the patient to maintain the seated position then to keep the hands in contact in front of the trunk.	To maintain the seated position then keep the hands in contact in front of the trunk.	<p>0: cannot maintain the seated position for 5 seconds</p> <p>1: with support of one or both upper limbs, maintains the seated position for 5 seconds</p> <p>2: without upper limb support, maintains the seated position for 5 seconds, but does not maintain contact between the two hands for 5 seconds</p> <p>3: without upper limb support, maintains the seated position for 5 seconds and is then capable of maintaining contact between the two hands for 5 seconds</p>
		Place the patient on the mat/wide examination table in any seated position. The subject must be reasonably stable in this position in order to	To lean forward to touch the	<p>0: cannot lean forward far enough</p> <p>1: with upper limb support, leans forward, touches the ball, but does not sit back again</p>

10	D2	attempt this item. Place a tennis ball in front of him at a distance that he has to lean his trunk forward about 30 ° in relation to the starting position to be able to touch it.	To lean forward to touch the ball, then to sit back again.	<p>2: with upper limb support, leans forward, touches the ball and sits back again</p> <p>3: without upper limb support, leans forward, touches the ball and sits back again</p>
11	D1	Place the patient in a seated position on the mat/wide examination table with the lower limbs in front of him.	To stand up.	<p>0: cannot stand up</p> <p>1: stands up with upper limb support on a piece of equipment</p> <p>2: stands up with upper limb support on the mat and/or on himself</p> <p>3: without upper limb support, stands up</p>
12	D1	Place the patient in a standing position in front of the chair. Leaning against the chair to support oneself using any other part of the body is not allowed.	To sit down on the chair.	<p>0: cannot sit down on the chair</p> <p>1: with upper limb support, sits down on the chair or lets himself fall into the chair</p> <p>2: without upper limb support, sits down on the chair using compensatory movements or poor control of movement</p> <p>3: without upper limb support, sits down on the chair with the feet slightly apart</p>
13	D2	Place the patient in a seated position on the chair or at the edge of the table, with the feet on the floor, the upper limbs alongside the body. This item should not be performed with the subject in the wheelchair.	To maintain the seated position as straight as possible.	<p>0: cannot maintain the seated position for 5 seconds</p> <p>1: with upper limb support and/or leaning against the back of the chair, maintains the seated position for 5 seconds</p> <p>2: without upper limb support nor leaning against the back of the chair, maintains the seated position for 5 seconds, but the head and/or trunk are not in midline position</p> <p>3: without upper limb support or leaning against the back of the chair, maintains the seated position for 5 seconds, with the head and trunk in midline position</p>
14	D2	Place the patient in a seated position on the chair with the head in complete flexion.	To raise the head and to maintain this position.	<p>0: cannot raise the head</p> <p>1: raises the head partially</p> <p>2: from head in complete flexion, raises the head but during the movement and/or while holding the position, the head is not in midline position</p>

		head in complete flexion.		3: from head in complete flexion, raises the head then maintains it raised for 5 seconds, the head stays in midline position throughout the movement and the holding position
15	D2	Place the patient in a seated position on the chair in front of a table with the forearms, but not the elbows on the table. If the patients seats in the wheelchair, the armrests should be removed.	To place both hands on top of the head.	<p>0: cannot raise both hands from the table</p> <p>1: raises both hands from the table, but the forearms can remain in contact with the table</p> <p>2: raises both forearms from the table but does not manage to place both hands on top of the head at the same time. The hands must at least reach the level of the mouth</p> <p>3: places both hands on top of the head at the same time while the head remains in midline position</p>
16	D2	Place the patient in a seated position on the chair in front of a table with the forearms on the table. Place a pencil on the table at a distance equal to the length of his upper limb.	To touch the pencil	<p>0: cannot move forward the hand</p> <p>1: reaching the pencil with the hand without a maximum extension of the elbow or not reaching the pencil</p> <p>2: reaches the pencil with one hand, with the elbow in maximal available or full extension, but slowly or with compensatory movements</p> <p>3: without moving the trunk, reaches the pencil with one hand, the forearm and hand off the table with the elbow in full extension at the end of the movement</p>
17	D3	Place the patient in a seated position on the chair in front of a table with the forearms on the table. Place ten coins next to his hand.	To pick up the coins successively with one hand, one at a time, and to hold them in the same hand.	<p>0: cannot pick up a single coin during the 20-second period</p> <p>1: successively picks up and holds 1 to 5 coins in one hand during the 20-second period</p> <p>2: successively picks up and holds 6 to 9 coins in one hand during the 20-second period</p> <p>3: successively picks up and holds 10 coins in one hand during the 20-second period</p>
		Place the patient in a seated position on the chair in front of a table with the forearms on the table. Hold a CD glued to a piece of cardboard on the		<p>0: cannot go round the small circle of the CD with a finger</p> <p>1: goes round the small circle of the CD with a finger</p>

18	D3	horizontal plane of the table. One finger of the patient, preferably the index finger, is placed in the center of a CD glued to a piece of cardboard. The position of the cardboard is chosen by the patient.	To go round the edge of the CD with a finger.	<p>2: goes round the edge of the CD with a finger with compensatory movements or difficulty</p> <p>3: goes round the edge of the CD with the same finger without hand support on the table</p>
19	D3	Place the patient in a seated position on the chair in front of a table with the forearms on the table. The patient chooses the position of the pencil and the paper held by the examiner on a horizontal plane.	To pick up the pencil and draw inside the frame.	<p>0: cannot pick up the pencil or cannot make a written mark</p> <p>1: picks up the pencil and makes a written mark, but cannot draw one loop inside the frame touching the top and bottom lines of the frame</p> <p>2: picks up the pencil, and draws at least one loop inside the frame touching the top and bottom lines of the frame, but cannot draw a continuous series of loops in the frame touching the top and bottom lines of the frame</p> <p>3: picks up the pencil and draws a continuous series of loops over the full length of the frame, touching the top and bottom line of the frame</p>
20	D3	Place the patient in a seated position on the chair in front of a table with the forearms on the table. Place a sheet of paper in his hands.	To tear the paper at least 4 cm.	<p>0: cannot tear the sheet of paper</p> <p>1: tears the unfolded sheet of paper</p> <p>2: tears the sheet of paper folded in 2, beginning from the folded edge</p> <p>3: tears the sheet of paper folded in 4, beginning from the folded edge</p>
21	D3	Place the patient in a seated position on the chair with the forearms on the table. Place a tennis ball next to the hand chosen by him. The position of the ball is chosen by the patient.	To pick up the ball, to raise it, and then to turn the hand.	<p>0: cannot pick up the ball</p> <p>1: picks up the ball but cannot turn over the hand</p> <p>2: picks up the ball, and turns the hand over, but incompletely or completely with compensatory movements</p> <p>3: picks up the ball, and turns the hand over completely holding the ball</p>
22	D3	<p>Place the patient in a seated position on the chair with the forearms on the table, having a diagram with pictures in front of him. One finger, chosen by the subject (or the thumb) is placed at the center of the diagram below, on the word "start".</p> 	To place the finger on the drawings.	<p>0: cannot raise the finger, nor slide it onto a drawing</p> <p>1: cannot raise the finger to place it on a drawing, but can slide it on at least one drawing</p> <p>2: raises the finger and places it imprecisely on 1 to 8 drawings of the diagram</p>

		     		3: raises the finger and places it successively on the 8 drawings of the diagram without touching the lines
23	D2	Place the patient in a seated position on the chair with the arms alongside the body and the table at a distance equivalent to the length of the subject's forearm when the elbow is beside the body. If the subject is seated in the wheelchair, the arm rests should be removed.	To place both hands on the table.	0: cannot touch the top of the table with one finger 1: achieves the movement with only one upper limb, or only the fingers of one or both hands placed on the table 2: places the two forearms and/or the hands on the table slowly or with compensatory movements 3: places the two forearms and/or the hands on the table at the same time without moving the trunk
24	D1	Place the patient in a seated position on the chair with both feet on the ground slightly apart.	To stand up.	0: cannot stand up 1: stands up with support using the nearby table or the chair and/or the body 2: without upper limb support, stands up with compensatory movements 3: without upper limb support, stands up with the feet slightly apart
25	D1	Place the patient in a standing position with upper limbs resting on a piece of equipment for support.	To release the support if possible and to stand straight.	0: cannot maintain a standing position for 5 seconds 1: with support of one or both upper limbs, maintains the standing position for 5 seconds 2: without upper limb support, maintains a standing position for 5 seconds with poor alignment or compensatory movements 3: without upper limb support, maintains a standing position for 5 seconds with the feet slightly apart, the head, trunk and limbs in midline position
26	D1	Place the patient in a standing position with upper limbs resting on a piece of equipment for support.	To release the support if possible, then to raise one foot.	0: with support from one or both upper limbs, cannot raise one foot for 3 seconds 1: with support from one or both upper limbs, raises one foot for 3 seconds 2: without upper limb support, raises one foot between 3 to 9 seconds 3: without upper limb support, raises one foot for 10 seconds
				0: cannot touch the floor with one hand or cannot stand up again

27	D1	Place the patient in a standing position, if possible without support.	To touch the floor with one hand, and to stand up again.	<p>1: with support, touches the floor with one hand and stands up again</p> <p>2: without support, touches the floor with one hand and stands up again, slowly or with compensatory movements</p> <p>3: without support, touches the floor with one hand and stands up again</p>
28	D1	Place the patient in a standing position. To perform this task, the patient must be able to walk without support.	To walk 10 steps on the heels.	<p>0: takes less than 10 steps forward, lifting the toes of one or both feet</p> <p>1: takes 10 steps forward lifting all the toes of one or both feet</p> <p>2: takes less than 10 steps forward on both heels, or 10 steps forward, including 5 on only one heel</p> <p>3: takes 10 steps forward on both heels</p>
29	D1	Place the patient in a standing position without support, in front of a straight line about 6 m long and 2 cm wide, drawn on the floor. To perform this task, the patient must be able to take at least one step without support.	To walk on the line.	<p>0: cannot take 1 step forward on a line</p> <p>1: takes 1 to 3 steps forward on a line</p> <p>2: takes 4 to 9 steps forward on a line</p> <p>3: takes 10 steps forward on a line</p>
30	D1	Place the patient in a standing position without support. To perform the task, the patient must be able to walk without support.	To run.	<p>0: cannot increase walking speed</p> <p>1: increases walking speed but cannot run 10 meters</p> <p>2: runs 10 meters with compensatory movements</p> <p>3: runs 10 meters</p>
31	D1	Place the patient in a standing position on one foot, without support, having the other foot off the ground. To perform this task, the patient must be able to walk without support.	To hop in place.	<p>0: cannot initiate hopping</p> <p>1: initiates a hop, the heel is raised from the floor but the toes remain in contact with the floor</p> <p>2: hops 1 to 9 times</p> <p>3: hops 10 times in place</p>
32	D1	Place the patient in a standing position without support.	To squat and to stand up again.	<p>0: with upper limb support, cannot squat or cannot get up</p> <p>1: with upper limb support, manages to squat and gets up once</p> <p>2: without upper limb support, manages to squat and gets up once</p> <p>3: without upper limb support, manages to squat and gets up twice in a row</p>

Name of Material/ Equipment	Company	Catalog Number
Physiotherapy mat	-	-
Cushions	-	-
Table	-	-
Chair	-	-
Stopwatch	-	-
CD or CD-ROM glued onto a piece of cardboard	-	-
10 coins	-	-
Lead pencil	-	-
Tennis ball	-	-
Sheets of A4 paper or equivalent	-	-
Clipboard	-	-
Two small traffic cones	-	-
Tape	-	-
Line traced on the floor	-	-
Corridor	-	-

Comments/Description

should not be slippery; alternatively use a wide examination table

-

with adjustable height; it should allow the patient to rest forearms while seated and elbows flexed at 90°

with adjustable height if possible; it should allow the patient to touch the floor with the feet while seated with tl

-

-

dimensions: 20 mm wide and 2 mm thick (10 euro cents or equivalent)

-

-

weight: 70-80g

-

-

for marking arrows and stop

2 centimeters wide and 6 meters long

indoor, straight, up to 30 meters long

he hips and knees flexed at 90°



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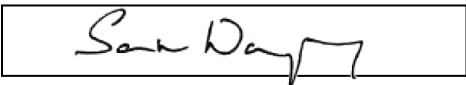
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Authors`s reply

We thank you very much for further editorial comments on our manuscript.

- 1. The manuscript will benefit from thorough language revision as there are a number of grammatical errors throughout. Please thoroughly review the manuscript and edit any errors (preferably use a proficient English speaker).**

The final version was reviewed by a professional English speaker and grammatical errors corrected.

- 2. Males and females?**

Only male patients attended the study, we added this information.

- 3. This para fits better in the results section.**

We added the indicated paragraph to the results section.

- 4. The JoVE protocol should be almost entirely composed of numbered short steps (2-3 related actions each) written in the imperative voice/tense (as if you are telling someone how to do the technique, i.e. "Do this", "Measure that" etc.). Please re-write your ENTIRE section 1.1.1. There should be logical flow between steps. Currently, these are definitions of actions and not a protocol.**

Thank you for your comment.

Writing the manuscript, we decided to focus on the motor function measure and to support a video recording of it which has not been published before. Based on the character of this clinical test, there is no "obvious logical flow" between the 32 individual steps; however, the main idea/logic behind this order is to avoid an unnecessary repositioning and exhausting of the patient. This explanation we included in the current description of the task. The 32 items are not to be regarded as separate definitions, but as a complete and validated protocol of a single clinical testing method. Therefore, we would very much appreciate it, if this structure would be acceptable for you.

We also adapted some of the remaining sentences to have an imperative voice in each item. We made adaptations to have as less actions as possible in each item (2-3). Please note that some steps can not be deleted; otherwise the item is not performed correctly.

- 5. The highlighted portions (max 2.75 pages) must best represent the title and abstracts. It currently does not meet this requirement.**

We adapted our title and the short and long abstracts. In order to achieve coherence throughout the manuscript and not to exceed the 2.75 pages, we decided to focus only on the MFM in the video recording. Therefore, only the protocol of the MFM was highlighted.

6. The numbering here is off. Please follow 1, 1.1, 1.1.1 etc. for Levels 1,2,3 etc.

We adapted the numbering of the whole protocol section, also based on the publications you gave us as examples.

7. Several of these definitions show a high degree of overlapping text with previous publications. I suggest avoiding these definitions and leaving them in Table 1, because the specifics are redundant. However, any steps to be filmed must be described fully in the text.

Thank you for your comment.

We deleted some of the less significant aspects of each item, but these aspects are included and can be found in the table. Small changes in the description were also done; however, we are not allowed to reformulate the items completely, since the test was validated using these exact words. Also our co-author, Mrs. Carole Vuillerot, who was part of the team writing the User's Manual of the MFM, asked us not to do so. We hope that this is acceptable for you. All relevant steps for the filming were left.

8. Please avoid these recurring headings one Lines 148-163.

The recurring headings (MFM item, D subdomains) were deleted.

9. Standing up is the task here? Or is it sitting up?

The patient has to stand up in this test, this we clarified in the current version.

10. Scan the patient? You don't excide any tissue so this is misleading. Please mention all MRI acquisition parameters.

The suggested corrections were made.

11. Mention statistical test?

A short description of the used statistical tests was added to the representative results part.

12. Please add scale bars.

Scale bars were added to Figure 2.

06/09/2018