Video Article

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

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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.

Video Link

The video component of this article can be found at https://www.jove.com/video/58784/

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 old[1]. 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 patients[2,3].

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 performance[4]. The MFM (validated for patients aged 6-60 years with DMD) was evaluated based on the MFM User's Manual[5]. 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 DMD[6,7]. 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 measures[8,9]. The test measures the maximal walking distance of a patient in 6 minutes[10]. 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 function[8,9,10,11]. 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 position[2]. 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 tissue[12]. 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 ambulation[13,14]. Moreover, QMRI has been able to detect subclinical changes in patients with stable or even improved outcome measures[15,16]. QMRI data of extensors muscles has also shown to be meaningful regarding its correlation with clinical outcomes[17]. 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 dystrophy[18]. 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. **Motor function measure (MFM)**
    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 patient[5]. See **Table 1** for a detailed description of each task and scoring.
        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.
        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.
        3. Ask the patient to lie down on his back. Ask him to bring one knee to the chest.
        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.
        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.
        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.
        7. Ask the patient to lie down on his back. Ask him to turn over onto the stomach and free both upper limbs.
        8. Ask the patient to lie down on his back. Ask him to sit up.
        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.
        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.
        11. Ask the patient to sit on the mat with the lower limbs in front of him. Ask him to stand up.
        12. Ask the patient to stand in front of the chair. Ask him to sit down on the chair.
        13. Ask the patient to sit on the chair. Ask him to maintain the seated position as straight as possible.
        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.
        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.
        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.
        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.
        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.
        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.
        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.

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.
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.
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.
24. Ask the patient to sit on the chair with both feet on the ground. Ask him to stand up.
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.
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.
27. Ask the patient to stand without support. Ask him to touch the floor with one hand and stand up again.
28. Ask the patient to stand and walk 10 steps on his heels.
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.
30. Ask the patient to stand without support. Ask him to run.
31. Ask the patient to stand on one foot without support with the other foot off the ground. Ask him to hop in place.
32. Ask the patient to stand without support. Ask him to squat and stand up again.

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

2. **6-minute walk test (6MWT)**
    1. Task performance
        1. Let the patient rest for 10 min prior to testing. Demonstrate the walking process.
        2. Ask the patient to stand at the starting line on the right side of the 0 cone. Give the instructions, “Ready, set, go”.
        3. When saying “go”, let the patient start walking around the cones without crossing the middle and, if possible, without slowing down or stopping.
        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.

    2. Calculation of the scores
        1. Record each timepoint at which the patient passes a cone.
        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).

3. **Timed 10-meter walk/run test (10MWT)**
    1. Task performance
        1. Ask the patient to stand at the starting line. Stand at the 12-meter mark and give the instructions, “Ready, set, go”.
        2. When saying “go”, let the patient start walking/running.
        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.
        4. Repeat the test three times, and use the fastest performance to calculate the score.

    2. Calculation of the scores
        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.
        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.
        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.

4. **Supine-up time**
    1. Task performance
        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.
        2. Give the instructions, “Ready, set, go”. When saying “go”, let the patient start standing up as fast as he can.
        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.
        4. Repeat the test three times, and use the fastest performance to calculate the score.

    2. Calculation of the scores

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.
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.
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

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. 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.
3. Manually draw regions of interest (ROI) on the MR images containing the whole muscle area of flexors, extensors, and adductors of each leg.
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 images[17].
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 publication[17].

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. Please click here to download this file.

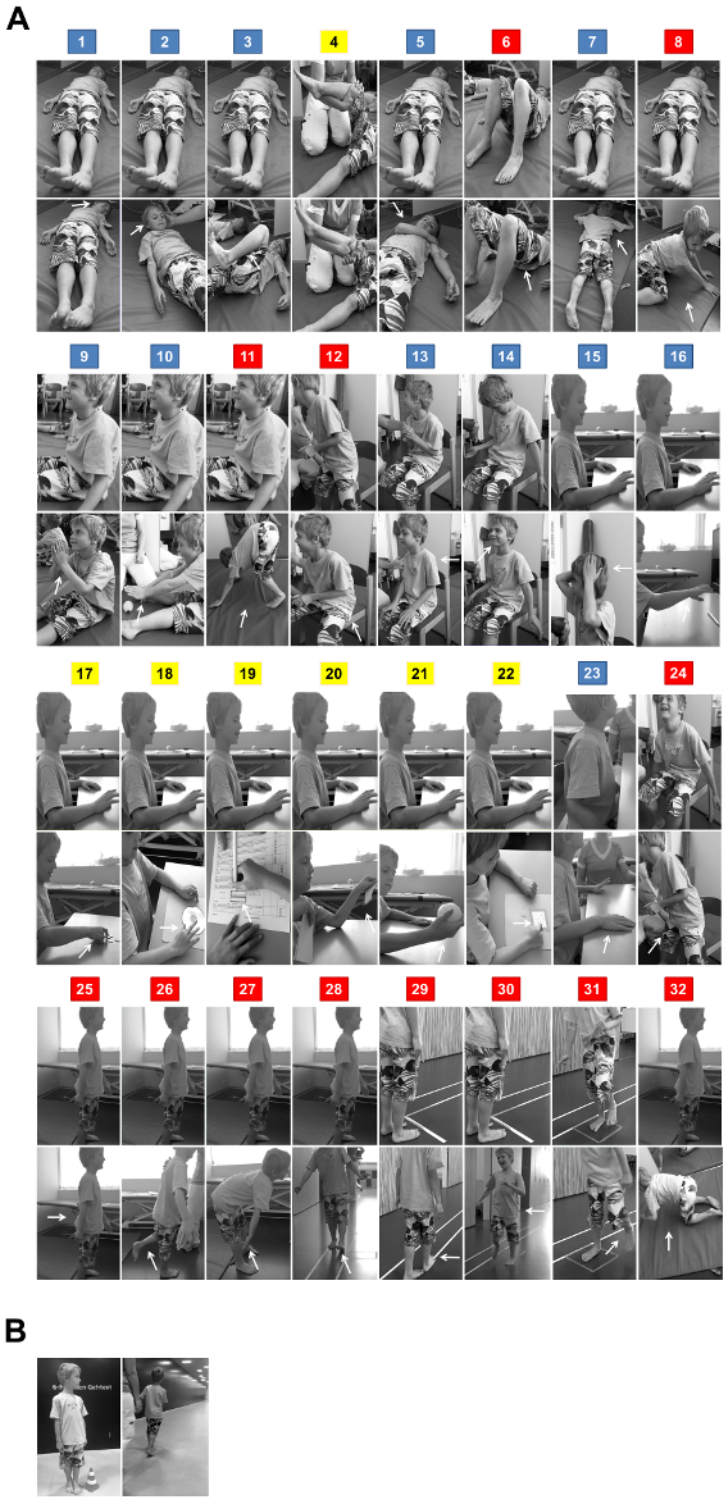
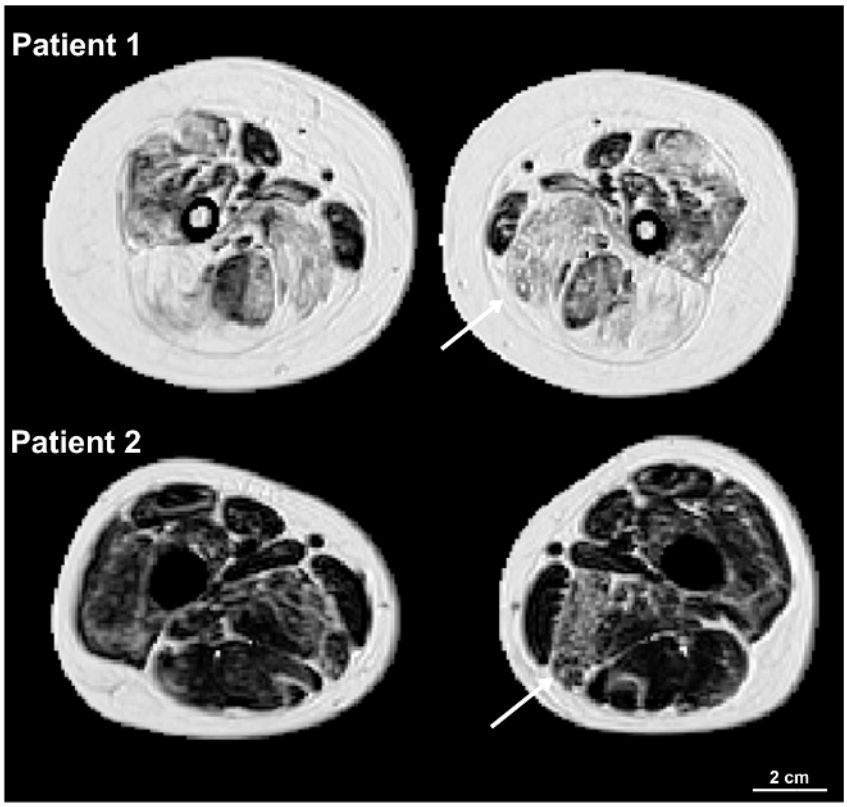


Figure 1: Illustration of the MFM and 6MWT in an 8-year-old patient with DMD. (A) All 32 items of the MFM are represented; numbers in red box = D1, blue = D2, and yellow = D3 subscores. The first rows represent the starting positions and second rows represent the tasks to perform (arrow). It should be 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. Please click here to view a larger version of this figure.



	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

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. Please click here to view a larger version of this figure.

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 steps[4], 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[2,8,9,10,11]. 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 parameters[11].

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 questionnaires[19]. 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 corticosteroids[23]. 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 measurements[24]. Furthermore, limitations of the two-point Dixon method to evaluate fat fraction is also of importance[25]. 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 involvement[15]. 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.

Disclosures

The authors have nothing to disclose.

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