**TITLE:**

Clinical outcome measures and quantitative muscle MRI in ambulant children with Duchenne muscular dystrophy

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**KEYWORDS:**

Duchenne muscular dystrophy, clinical trial, motor function measure, 6-minute walk distance, quantitative MRI, fatty muscle degeneration

**SHORT ABSTRACT:**

The aim of the study was to define the most reliable method(s) that relate to treatment efficacy by correlating different clinical assessments and quantitative muscle MRI in ambulant patients with Duchenne muscular dystrophy.

**LONG ABSTRACT:**

The number of new treatment options tested in patients with Duchenne muscular dystrophy is increasing, however, to define the most reliable assessments that reflect therapy efficacy is still an unmet need. We aimed to highlight sensitive clinical and radiologic outcome measures by analyzing standardized functional tests and quantitative muscle MRI data of ambulatory patients taking part in the “Treatment with L-citrulline and metformin in Duchenne muscular dystrophy”-study. Motor function measure, consisting of 32 items, was performed to describe standing and transfer (D1 subscore), axial and proximal motor function (D2 subscore), and distal motor function (D3 subscore). Timed function tests included the 6-minute walk test, the timed 10-meter walk/run test and the supine up time, referring to the maximal distance patients absolve in 6 minutes and the best performance of walking/running 10 meters and of standing up from supine position, respectively. These validated tests sufficiently monitor changes of muscle function, however, they still depend on the patients` collaboration and the skills of the evaluator. A low intra- and inter-rater variability can only be achieved if the used material, the examination steps and the calculation of the scores are standardized and strictly followed. Quantitative MRI is an objective and sensitive biomarker to detect even subclinical changes, though especially the examination costs might limit its use. In this study, a high correlation between all clinical assessments and the quantitative MRI was found. The combinational use of these methods provide better information about the disease progression, however, longitudinal studies are needed to validate their reliability.

**INTRODUCTION:**

Reliable outcome measures that reflect treatment response are an essential requirement of successful clinical trials. Since the rapid development of new therapeutic strategies, more and more effort has been put to define reproducible and sensitive methods that monitor clinical outcomes.

Duchenne is the most common type of muscular dystrophies with an X-linked recessive inheritance, a severe involvement of predominantly the skeletal and heart muscle, a progressive disease course with loss of ambulation around the age of 8-12 years and an early death mainly before 30 years of age1. Validated tests such as the 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 and seem to be more sensitive in ambulatory cases than quantitative muscle strength that cannot be appropriately performed in weak and non-cooperative patients2-3.

The motor function measure (MFM) consists of the detailed examination of functions of the neck, trunk, arm and leg musculature, and of the ability of standing, transferring and walking. It can be performed even in patients who lost ambulation as it assesses three dimensions of the motor performance4. The MFM (validated in Lyon, France for patients aged 6-60 years with Duchenne muscular dystrophy) was evaluated based on the User`s Manual5. It includes 32 items and is divided into 3 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 is able to sufficiently monitor changes of muscle function and to predict loss of ambulation. Moreover, it was consonant with the clinical changes perceived by patients' and physicians' in Duchenne muscular dystrophy6-7. Timed function tests are also commonly used as outcome measures, though they can be mainly performed in ambulatory patients. Among them, the 6-minute walk test (6MWT) received special attention as it shows the highest test-retest reliability, predicts clinical decline and loss of ambulation, and correlates better with muscle function measures compared to quantitative muscle strength measurements8-9. The test measures the maximal walking distance of a patient in 6 minutes (min)10. It is guided by two trained people, a “follower” who walks 1-2 m behind the patient, and an “evaluator” who records the time. Some other timed function tests have lower test-retest reliability and they don`t reflect endurance, an important marker of ambulatory function8-11. To this group belong the timed 10-meter run/walk test (10MWT) and the supine up time, that measure the best performance of walking/running 10 meters and of standing up from supine position, respectively2.

Altogether, the use of the motor function measure and timed function tests as primary or secondary endpoints in clinical trials is justified, however, a major limitation remains that none of them is independent of the patients` collaboration and the skills of the evaluator.

Quantitative MRI (QMRI) is an objective method to visualize well-described disease related morphological abnormalities of the musculature including edema, muscle degeneration and increased content of adipose and connective tissue12. The use of MRI techniques as a diagnostic tool in neuromuscular disorders has already been established, however, their 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; whereas further information about the muscle fat content can be extracted by calculating the mean fat fraction (FF). QMRI was shown to be a promising biomarker as measurements correlated with clinical outcome and disease progression, while a mean fat fraction of 50 % predicted loss of ambulation13-14. Moreover, QMRI was able to detect subclinical changes in patients with stable or even improved outcome measures15-16. QMRI data of the thigh muscle extensors were shown to be especially meaningful regarding the correlation with clinical outcomes17. QMRI is a non-invasive and sensitive method, however, its cost and the possibly reduced compliance of 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 radiologic outcome measures in ambulant children with Duchenne muscular dystrophy, as the need for standardized and disease-specific assessments is increasing in the era of clinical trials in neuromuscular disorders.

**PROTOCOL:**

Baseline data of 47 ambulatory patients (aged 6.5 to 10.8 years) with Duchenne muscular dystrophy were analyzed, who previously participated in the “Treatment with L-citrulline and metformin in Duchenne muscular dystrophy”-study. Patients were enrolled from the University Children`s Hospital Basel as well as from the patient registries of Switzerland, Germany, and Austria. Prior 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 also registered under ClinicalTrials.gov (NCT01995032). MRI examinations were blinded to clinical status and motor function tests. In all patients but one – who refused to perform the scanning – MRI of all thigh muscles was performed13,17.

**1.** **Clinical assessment of the muscle function1.1 Motor function measure**

1.1.1 Task performance5 (see Table 1 for detailed description)

**(see Table 1 for detailed description)**

1. MFM1 (D2): Place the patient on the mat in supine position. Ask the patient to hold the head in midline position and then turn it from one side to the other.

2. MFM2 (D2): Place the patient on the mat with the head in midline position. Ask the patient to raise the head and maintain the position.

3. MFM3 (D2): Place the patient on the mat in supine position with the legs resting if possible, on the thighs, calves and feet. Ask the patient to bring one knee to the chest.

4. MFM4 (D3): Place the patient on the mat in supine position with one leg flexed both at the hip and knee at approximately 90°. Place the lower part of the leg parallel to the mat with the foot in plantar flexion in the air. Ask the patient to carry out a maximal dorsiflexion of the whole foot.

5. MFM5 (D2): Place the patient on the mat 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. Ask the patient to bring one hand to the opposite shoulder.

6. MFM6 (D1): Place the patient on the mat in supine position with the lower limbs half flexed, the kneecap facing up and the feet resting on the mat slightly apart. Ask the patient to maintain this position, and then to raise the pelvis.

7. MFM7 (D2): Place the patient on the mat in supine position. Ask the patient to turn over onto the stomach and to free both upper limbs.

8. MFM8 (D1): Place the patient on the mat in supine position. Ask the patient to sit up.

9. MFM9 (D2): Place the patient on the mat 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.

10. MFM10 (D2): Place the patient on the mat in any seated position. 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. Ask the patient to lean forward to touch the ball, and then to sit back again.

11. MFM11 (D1): Place the patient in a seated position with the lower limbs in front of him. Ask the patient to stand up.

12. MFM12 (D1): Place the patient in a standing position in front of the chair. Ask the patient to sit down on the chair.

13. MFM13 (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. Ask the patient to maintain the seated position as straight as possible.

14. MFM14 (D2): Place the patient in a seated position on the chair with the head in complete flexion. Ask the patient to raise the head and maintain it raised.

15. MFM15 (D2): Place the patient in a seated position on the chair in front of a table with the forearms, but not with the elbows, on the table. Ask the patient to place both hands on top of the head.

16. MFM16 (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. Ask the patient to touch the pencil.

17. MFM17 (D3): Place the patient in a seated position on the chair in front of a table with the forearms on the table. Place 10 coins next to the hand. Ask the patient to pick up the coins, successively with one hand, one at a time, and to hold them in the same hand.

18. MFM18 (D3): 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 horizontal plane of the table and ask the patient to place one finger, preferably the index finger, in the center of the CD and go round the edge of the CD with the finger.

19. MFM19 (D3): Place the patient in a seated position on the chair in front of a table with the forearms on the table. Hold a pencil and a paper on the horizontal plane in front of him. Ask the patient to pick up the pencil and to draw inside the frame.

20. MFM20 (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 the hands. Ask the patient to tear the paper at least 4 cm.

21. MFM21 (D3): Place the patient in a seated position on the chair with the forearms on the table. Place a tennis ball next to a hand. Ask the patient to pick up the ball, to raise it, and then turn the hand.

22. MFM22 (D3): Place the patient in a seated position on the chair with the forearms on the table. Place a diagram with pictures in front of him. Ask the patient to place the finger at the center of the diagram on the word “start”, and then place the finger on the drawings.

23. MFM23 (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 his forearm when the elbow is beside the body. Ask the patient to place both hands on the table.

24. MFM24 (D1): Place the patient in a seated position on the chair with both feet on the ground slightly apart. Ask the patient to stand up.

25. MFM25 (D1): Place the patient in a standing position with the upper limbs resting on a piece of equipment for support. Ask the patient to release the support if possible and to stand straight.

26. MFM26 (D1): Place the patient in a standing position with the upper limbs resting on a piece of equipment for support. Ask the patient to release the support if possible and to raise one foot.

27. MFM27 (D1): Place the patient in a standing position, if possible without support. Ask the patient to touch the floor with one hand, and to stand up again.

28. MFM28 (D1): Place the patient in a standing position. Ask the patient to walk 10 steps on the heels.

29. MFM29 (D1): Place the patient in a standing position without support. Draw a straight line about 6 m long and 2 cm wide on the floor in front of him. Ask the patient to walk on the line.

30. MFM30 (D1): Place the patient in a standing position without support. Ask the patient to run.

31. MFM31 (D1): Place the patient in a standing position on one foot, without support, having the other foot off the ground. Ask the patient to hop in place.

32. MFM32 (D1): Place the patient in a standing position without support. Ask the patient to squat and to stand up again.

1.1.2 Calculation of the scores

Add the scores of all 32 items, divide the sum by 96 and multiply it by 100 to get the final score. To calculate the scores of the subdomains, add the score of all items in that domain, divide it by the maximum score for the domain and multiply it by 100. Note, that all scores have to be calculated in percentage.

**1.2 6-minute walk test (6MWT)**

1.2.1 Task performance

1. Let the patient rest for 10 min prior to test. Demonstrate the walking process.

2. Place the patient in a standing position at the starting line on the right side of the 0 cone. Give the instruction “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. During walking do not disrupt the patient (for instance by walking in front of him or going into conversation with him), but verbal encouragement to achieve a better performance is allowed.

4. At 6 min, stop the timer and let the patient stop walking. Count down the final seconds of the test and mark the place where the patient stopped.

1.2.2Calculation of the scores

Record each time when the patient passes a cone. 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 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. Place the patient in a standing position at the starting line. Stand at the 12 m mark and give the instruction “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 also the second foot of the patient cleared the finish line at 10 m.

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

1.3.2 Calculation of the scores

Score the patient on a 6-point scale (1-6) based on the quality of the walk/run of the fastest trial. Give 1, if he is not able to walk freely . Give 2, if he is not able to walk freely, but is able to walk if supported by a knee-ankle-foot orthosis or a person. Give 3, if he is not able to increase the walking speed and his gait remains highly adapted and lordotic. Give 4, if he is able to increase the walking speed, but is not able to run, while his gait remains moderately adapted. Give 5, if he is almost running, but cannot raise the feet from the ground. Give 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. Place the patient on the examination table in supine position. In case you have to use a mat, be sure that it is fixed and not slippery.

2. Give the instruction “ready, set – GO”. When saying GO, let the patient start getting up as fast as he can.

3. Measure the time and observe the quality of the task. Stop the timer when the patient assumed an upright position with his 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.

1.4.2 Calculation of the scores

Score the patient on a 6-point scale (1-6). Give 1, if he is not able to stand up from supine position . Give 2, if he is able to stand up from supine position by using a furniture ). Give 3, if he turns over in supine position and needs both hands “climbing up” on the legs to reach the standing position . Give 4, if he turns over in supine position and needs one hand on the leg to reach the standing position. Give 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. Give 6, if he is able to stand up without turning over or using the hands on the legs.

**2. Quantitative muscle MRI**

1. Scan all muscles of the thighs (flexors, extensor, and adductors) on a 3 Tesla scanner and perform the localizers and positioning as previously described11, 17.

2. Use a three dimensional (3D) gradient echo sequence with two different echo times for in-phase and opposed-phase imaging and a multi-contrast spin echo to quantify the transverse relaxation times.

3. Use the 2-point Dixon method and generate relative fat content maps by measuring the pixelwise fat fraction17.

4. Draw manually regions of interest (ROI) containing the whole muscle area of flexors, extensors and adductors of each leg.

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

**REPRESENTATIVE RESULTS:**

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, while **Figure 1A** illustrates the examination steps of all 32 items and **Figure 1B** the 6MWT in a selected patient with Duchenne muscular dystrophy. QMRI of the thigh muscles was performed in all but one patient, 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), of the D2 subscore 97.2% (IQR 94.4-96.6) and of the D3 subscore 90.5% (83.3-95.2), respectively. The mean distance of the 6MWT was 359 m (SD 76.4). The mean time of the 10MWT was 6.7 seconds (SD 1.8) and of the supine up test 10.2 seconds (SD 6.4), respectively. There were no correlations between the clinical assessments and the height, weight, and BMI of the patients. The total MFM, the D1 subscore, and the 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 the 10MWT were highly correlated (p < 0.001) with each other.

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

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, the specific task, and the scoring system.** Red indicates the D1, blue the D2, and yellow the D3 subdomains.

**Figure 1: Illustration of the MFM and the 6MWT in an 8 years old patient with Duchenne muscular dystrophy.** (A) All 32 items of the MFM are represented; numbers in red box indicate the D1, in blue the D2, and in yellow the D3 subscores. The first rows represent the starting positions, the second rows the tasks to perform (arrow), respectively. Note, 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 picture shows the patient performing the test on a 30 m corridor under the supervision of a physiotherapist.

**Figure 2: Representative correlation of the baseline QMRI data and the clinical assessments in two patients with Duchenne muscular dystrophy.** Patient 1 with more severe clinical involvement assessed by the MFM (in %), the 6MWT (in meter), the 10MWT (in seconds) and the supine up time (in seconds) showed a prominent fatty degeneration (FF in %) of the thigh muscles, particularly of the adductors (arrow). Patient 2 with better clinical performance had a less pronounced fatty degeneration of the adductors (arrow). For comparison, clinical assessments (median MFM in %, mean 6MWT in meter, mean 10MWT and mean 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 described to be used in clinical trials performed in patients with Duchenne muscular dystrophy. The MFM is a validated and reproducible functional test that makes a detailed examination of crucial motor functions in 32 steps4, while the 6MWT can give useful information about the patients endurance.

All currently validated tests have limitations due to their inter-rater and intra-rater variability and they all require the cooperation of the patient and the expertise of the examiner. To reduce the limitations, it is crucial that the evaluator sticks to the protocol and to the recommended examination materials and examination steps. Particularly when performing the MFM, the specified definitions of certain positions have to be considered, and the starting positions followed by the single steps of each item strictly followed and clearly presented. Any factors that can interfere with the test performance should be avoided, such as wearing uncomfortable clothes or using slippery examination materials. Also, the patients are not 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 for clinical trials. Its application is not limited to patients in adulthood, giving a unique opportunity to follow-up children from the age of 6 and to demonstrate clinical changes and therapy response throughout the years. The test is suitable for both ambulatory and non-ambulatory patients, showing its potential superiority to other tests such as the North Star Ambulatory Assessment2,8-11. Besides, the MFM is less dependent on the patient`s compliance compared to tests of the motor strength such as the manual muscle testing. Timed function tests give information about the patient`s endurance and can predict disease progression. Especially the 6MWT has been described as a reproducible outcome measure, however, it also shows an age-dependency due to the different stages of motor development. Independently of age, a rapid clinical decline could be shown in patients performing 6MWT < 350 m at inclusion, so that results of the 6MWT can even 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 put to cover 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 won increasing interest as well20,21. Quantitative MRI has also become a part of clinical trials to assess the involvement of the musculature. The fat replacement can be measured using the mean fat fraction, while the T2 relaxation time gives information about the presence of edema and inflammation. Changes on magnetic images were shown to correlate with clinical assessments to predict loss of ambulation13,22, and even with treatment response to corticosteroids23. When analyzing QMRI data, however, the non-homogenous replacement by adipose tissue has to be taken into consideration when selecting regions of interest, since higher fat contents were shown in distal and proximal parts of the musculature compared to the muscle belly, influencing the quantitative measurements24. Further, the limitations of the 2-point Dixon method to evaluate fat fraction is also of importance25. The 2-point Dixon method can lead to overestimation of the fat fraction in less affected muscles, besides, the fatty infiltration can prolong the T2-relaxation time. In the current analysis, T2-times and mean fat fraction strongly correlated in the affected muscles, and showed the same distribution of involvement15. Importantly, the existence of a second independent MRI method confirming the results of the first (Dixon) method can validate the MRI approaches used in a trial.

This cross-sectional analysis analyzed the MFM and timed function tests in correlation to QMRI regarding to treatment response and clinical decline. All timed function tests correlated significantly with each other and with the 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 imaging biomarker in clinical trials26,27. The study underscores that the combination of clinical assessments and quantitative MRI provides a better information about disease progression in patients with Duchenne muscular dystrophy, however, longitudinal confirmation of their sensitivity is still needed.

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**DISCLOSURES:**

The authors have nothing to disclose.

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