Semi-automated volumetry of MRI serves as a biomarker in neuromuscular patients

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Abstract

Background: Muscle MRI is of increasing importance for neuromuscular patients to detect changes in muscle volume, fat-infiltration, and edema. We developed a method for semi-automated segmentation of muscle MRI datasets.

Methods: An active contour-evolution algorithm implemented within the ITK-SNAP software was used to segment T1-weighted MRI, and to quantify muscle volumes of neuromuscular patients (n = 65).

Results: Semi-automated compared with manual segmentation was shown to be accurate and time-efficient. Muscle volumes and ratios of thigh/lower leg volume were lower in myopathy patients than in controls (P < .0001; P < .05). We found a decrease of lower leg muscle volume in neuropathy patients compared with controls (P < .01), which correlated with clinical parameters. In myopathy patients, muscle volume showed a positive correlation with muscle strength (rleft = 0.79, pleft < .0001). Muscle volumes were independent of body mass index and age.

Conclusions: Our method allows for exact and time-efficient quantification of muscle volumes with possible use as a biomarker in neuromuscular patients.

KEYWORDS
biomarker, MR imaging, muscle dystrophy, neuromuscular disorders, segmentation, thresholding

1 | INTRODUCTION

Neuromuscular disorders are a diverse group of diseases that are characterized by muscle wasting and weakness, intramuscular fat-accumulation, and an often chronic progressive disease course.1,2 Due to slow progression and phenotypic variability of several neuromuscular disorders, clinical trials remain difficult to conduct.1,3 Neurological examination and functional tests are often not sensitive enough to measure potential slowing of disease progression in clinical studies.4 To monitor treatment effectiveness of approved therapies and to facilitate the development of novel therapies in clinical trials, there is a need for reliable, valid, and objective treatment response and disease progression measurements.5 Muscle MRI is increasingly used to detect pathological muscle changes in neuromuscular disorders such as Duchenne muscular dystrophy,7 limb-girdle muscular dystrophy (LGMD),8 and...
Charcot–Marie-Tooth (CMT) neuropathy.9 Previous studies have found that intramuscular fat accumulation detected in MRI correlates with functional measurements.1 In some cases, the detection of early disease changes in MRI was possible before subjective, clinical, or functional changes emerged.10,11 This method of in vivo detection using a noninvasive imaging marker might expedite reliable treatment response measurements and diagnostic options in neuromuscular disorders. To date, many studies on muscle MRI were done using single or a few consecutive slices.12-14 However, due to the highly variable degree to which muscle changes in neuromuscular disorders can occur,15,16 single-slice measurements could over- or underestimate the extent of muscle changes along the leg or even the whole body.

In this study, we performed 3D semi-automated segmentation of standardized muscle MRI datasets to analyze whole muscle volumes of thighs and lower legs.

2 | METHODS

2.1 | Study design

In this retrospective study, all clinical and MRI data were obtained at the Unit for Neuromuscular Disorders and Clinic for Diagnostic and Interventional Radiology at RWTH Aachen University Hospital. Every patient treated between September 2012 and October 2016 with complete MRI of the lower limbs and an established diagnosis was offered enrollment. Due to incompleteness of MRI datasets 11 myopathy and 2 neuropathy patients were excluded. Because the diagnosis remained unclear, 4 patients with suspected myopathy and 1 with suspected neuropathy were omitted.

Diagnoses were proven by molecular genetics, histological examination of muscle and nerve tissue obtained by biopsies, and/or clinical tests based on electrophysiological, clinical, and laboratory parameters.17,18 In controls, the absence of neuromuscular disorders was determined by clinical evaluation including muscle strength using the Medical Research Council (MRC) grading scale,19 sensory examination, deep tendon reflexes and blood tests. In each patient, detailed neurological examinations, general medical history and blood parameters were analyzed. Every patient underwent an assessment of knee extension strength by MRC scale. The vibration perception level was assessed in the neuropathy subgroup by using a 64 Hz Rydell-Seiffer tuning fork. In neuropathy patients, electrophysiological investigations included sensory and motor nerve conduction studies of the sural and tibial nerves bilaterally. Sural nerves were examined by antidromic stimulation and recording by needle electrodes inserted in the vicinity of the sural nerve lateral to the Achilles tendon. Tibial nerves were examined by orthodromic stimulation in the popliteal region and behind the medial ankle and recording using surface electrodes over the muscle bulk of the abductor hallucis muscle.

The study was approved by the local ethical committee (approval number: EK 278/15) and performed in adherence to the guidelines of the Declaration of Helsinki.

2.2 | MRI

All MRI examinations were performed on a 1.5Tesla system (Achieva; Philips Healthcare, Best, the Netherlands) using a 16-channel receiver coil. The MRI protocol comprised a T1-weighted turbo spin echo (TSE) sequence (repetition time/echo time [TR/TE] = 902/17; TSE factor 6; slice thickness 6 mm; voxel size 1.1 × 1.35 mm) and a T2-weighted TSE sequence with spectral fat-suppression (TR/TE = 3971 / 64; TSE factor 6; slice thickness 6 mm; voxel size 1.47 × 1.98 mm). Whole-body scans were performed in transverse orientation using up to six stacks.

FIGURE 1  Comparison of manual and semi-automated segmentation. A, 1–15 represent lower leg muscle volumes; 16–30 represent thigh muscle volumes. B, Differences versus averages are presented by means of a Bland–Altman plot with 95% confidence intervals showing an absence of proportional bias. The differences were calculated by subtracting the manually calculated volumes from the automated ones.
2.3 Image analysis

MRI images were pseudonymized and converted from DICOM (digital imaging and communications in medicine) to NIFTI (Neuroimaging Informatics Technology Initiative) using the software dcm2niix. Muscle volumes of left and right lower legs and thighs were then semi-automatically segmented using an active contour evolution algorithm implemented in the ITK-SNAP software. In the ITK-SNAP interface, a single observer defined two regions of interest for the upper and lower legs. The first region was defined from the femoral head to the cranial tip of the kneecap, the second between the medial tibial condyle and the inferior articular surface of the tibia. The semi-automatic active contour segmentation was initialized by defining image intensity thresholds that provided the best separation between muscle and surrounding tissue considering the individual grey levels of muscle, fat, and connective tissue. On conventional T1-weighted TSE pulse sequences, the signal intensities of fat or muscle and connective tissue, respectively, exhibit high intrinsic differences. Thus, a clear differentiation of these tissues was achieved even without the application of quantitative measurement techniques, such as 3-point Dixon method.

One or more spherical “seeds” were placed on the thresholded image, and the active contours segmentation approach was started (Supporting Information Figure S1). The surface of the spheres then

FIGURE 2 Examples of muscle MRI and 3D segmentations. Lower leg and thigh muscle volumes are each presented in 2D (A) and 3D (B) for controls, myositis, LGMD, other myopathies, and neuropathy patients. The left leg muscle volume is represented in red and the right in green (3D), whereas intra- and extra-muscular fat remains unsegmented.
evolved under the control of the contour evolution algorithm until the segmentation was completed (Supporting Information Figure S1). Finally, each segmentation was manually checked and corrected if necessary.

The muscle volumes of each side were analyzed separately, and the ratio of thigh/lower leg was calculated for each side. To evaluate the reliability of semi-automated segmentation, a parallel manual slice-by-slice segmentation was performed using ITK-Snap software in five randomly chosen controls, neuropathy and myopathy patients each. These 30 manually determined volumes of lower legs and thighs were compared with the respective muscle volumes obtained by semi-automated segmentation. Moreover, to assess the time-efficiency of semi-automated segmentation, we also measured the time to segment the leg muscles by either method. The whole measurement was done blinded by the same person, who was experienced with manual and semi-automatic image segmentation.

2.4 | Statistics

Statistical analysis was performed using IBM-SPSS Statistics version 20 (IBM, Armonk, NY) and GraphPad Prism version 7.03 (GraphPad Software, San Diego, CA). To test for normal distribution, all data underwent the Kolmogorov–Smirnov, Shapiro–Wilk, and D’Agostino normality test as appropriate. Due to absence of normal distribution of thigh/lower leg ratios, log-transformation of these data was performed. Thereafter, this data still failed normality tests.

Differences between muscle volumes, comparing myopathy and neuropathy patients and controls were analyzed using one-way analysis of variance (ANOVA). Significance of thigh/lower leg ratios was tested using nonparametric Kruskal-Wallis tests.

To correct for multiple comparisons, each assessment underwent the Tukey–Kramer posttest after one-way ANOVA, and the Dunn’s test correction after performing Kruskal-Wallis tests. To keep the sample size statistically independent, only muscle volumes of one side (left) were compared. To investigate differences in muscle strength between the leg sides of myopathy patients, the nondominant and dominant sides were correlated separately with the respective muscle volumes.

Correlations between muscle strength, vibration sense, age, body mass index (BMI), and electrophysiological data with muscle volumes were determined with Pearson correlation tests and linear regression analyses. Missing data from electrophysiological examinations were omitted. Differences between manual and semi-automated segmentation were assessed with the Bland–Altman method and paired t-tests with testing the effectiveness of pairing.

The threshold of significance for all examinations was \( P < .05 \). Data are given as means ± SD.

3 | RESULTS

The group of myopathy patients consisted of 11 males and 13 females (mean age, 52 ± 16.2 years; BMI 27.5 ± 5.1 kg/m²), the group of

![FIGURE 3](image-url) Comparisons of semi-automatically quantified muscle volumes between patients and controls. A, Myopathy, but not neuropathy patients showed significant decreases of thigh muscle volume compared with controls. B, Patient lower leg muscle volumes were decreased compared with controls. C, The ratio of thigh/lower leg muscle volume was smaller in myopathy patients compared with controls, whereas in neuropathy patients, the ratio was higher. Whiskers represent range, boxes show median and interquartile range. *\(P < .05\); **\(P < .01\); ****\(P < .0001\)
neuropathy patients of 9 men and 4 women (mean age, 66 ± 10.1 years; BMI 25.7 ± 3.1 kg/m²) and the control group of 15 men and 13 women (mean age, 38 ± 10.2 years; BMI 26.2 ± 4.6 kg/m²). Among the myopathy patients, 6 had a diagnosis of inflammatory myopathy (3 inclusion body myositis, 2 necrotizing myositis and 2 polymyositis), 7 had LGMD and 11 had a wide range of other myopathies. Among the neuropathy patients, 7 had chronic inflammatory demyelinating polyneuropathy (CIDP) and 6 other neuropathies such as CMT or diabetic neuropathy. Diagnoses and relevant blood test results are presented in Supporting Information Table S1, which is available online.

3.1 | Accuracy and time-efficiency of semi-automated vs manual segmentation

Muscle volumes measured by semi-automatic and manual segmentation were similar (semi-automated: 2613 cm³ vs manual: 2594 cm³; mean and standard deviation of differences: 25.1 ± 70.6 cm³) (P > .05; effectiveness of pairing: P < .0001; r = 1.00) (Figure 1A,B). This was the case for myopathy and neuropathy patients as well as for controls. In semi-automated segmentation, there were some male patients in which the algorithm ran over the cremaster muscle and included it into the thigh muscle volume. This was the major point that had to be corrected manually. The time-saving effect of semi-automated compared with manual segmentation was strong (29.7 ± 2.7 vs 399.3 ± 9.2 min per patient, P < .0001). There were no significant differences of time needed among neuropathy, myopathy, or control subjects.

3.2 | Comparison of muscle volumes between patients and controls

Representative MR images and segmentations of patients of different disease groups and controls as well as the corresponding 3D reconstructions are shown in Figure 2.

In myopathy patients, the total muscle volumes of thighs were lower than in controls and neuropathy patients (Figure 3A). There was a smaller, but significant difference between lower leg muscle volumes of myopathy patients and controls (Figure 3B). In neuropathy patients, the muscle volumes of lower legs were smaller than in controls (Figure 3B). The ratio of thigh/lower leg muscle volumes was smaller in myopathy patients than in controls and neuropathy patients (Figure 3C). The differences in thigh/lower leg ratios were significant comparing neuropathy with myopathy patients. Numerical data and P-values are presented in Table 1.

### TABLE 1  Summary of muscle volumes and ratios

|                | Thigh muscle volume (cm³) | Lower leg muscle volume (cm³) | Ratio of thigh/lower leg |
|----------------|---------------------------|-------------------------------|--------------------------|
| Control        | 4240.2 (1139.7)           | 1421.1 (320.9)               | 3.0 (0.5)                |
| Myopathy       | 2551.3 (1025.2) ***       | 1128.8 (392.9) *             | 2.5 (1.3) *              |
| Neuropathy     | 3599.1 (1164.0) **        | 1017.2 (320.8) **            | 3.6 (1.0)                |

Note: Data are presented as mean (SD).
*P < .05; **P < .01; ***P < .001; each in relation to the control group.

![FIGURE 4](image_url)  Correlations between functional parameters and muscle volumes. A, Thigh muscle volume strongly correlated with strength of knee extension in myopathy patients. B.C, Positive correlations were seen among vibration perception level (B), CMAP (C), and NCV (C) and the respective muscle volume in neuropathy patients.
3.3 | Correlations of muscle volumes with clinical and paraclinical parameters

In myopathy patients, the strength of knee extension showed a strong positive correlation with the thigh muscle volume of the left side and a moderate positive correlation on the right side. (Figure 4A). In neuropathy patients, linear regression analyses showed lower leg muscle volume to be strongly correlated with both vibration sense and tibial compound muscle action potential (CMAP) amplitude, and moderately correlated with tibial nerve conduction velocity (NCV) lower leg muscle volumes (Figure 4B,C). Our analyses showed that there was no correlation between BMI or age with total muscle volume the thigh or lower leg (P > .05).

4 | DISCUSSION

This proof-of-concept study demonstrates that semi-automated quantification of muscle MRI was reliable and time-efficient in determination of muscle volumes of neuromuscular patients. This new application enabled us to rapidly and accurately determine differences between myopathy, neuropathy, and control subjects and to correlate muscle changes with (para)clinical parameters.

4.1 | Semi-automatic muscle quantification

In particular, the muscle volumes of the thighs of myopathy patients were markedly lower than those of controls. The concomitant muscle wasting in lower legs was observable but smaller than in thighs of myopathy patients. This led to smaller thigh/lower leg-ratios in myopathy patients compared with controls and neuropathy patients, indicating that thigh muscles were more severely affected. This corresponds well to the typical clinical phenotype of predominant proximal atrophy, but relatively normal or even hypertrophic lower legs.22 Notably, our study did not exclude distal myopathies, but they represent a rarer clinical phenotype.24

In neuropathy patients, the accordingly higher thigh/lower leg-ratio leads to the conclusion that lower leg muscles were more affected by atrophy, which agrees with the typical clinical phenotype of predominant distal involvement in neuropathies.

Compared with other studies, our findings in the double track test allow the conclusion that disease-related changes are not only validly detectable by the more time-consuming examination of single MRI slices,13,25,26 but also by semi-automatic segmentation and quantification of muscle volumes.

In addition to previous studies revealing the fat-fraction as a promising biomarker,1,13 we suggest that muscle volume as detected by semi-automatic segmentation may serve as a useful biomarker in neuromuscular disease, as it is a reliable and time-efficient method and correlates well with clinical markers of disease severity. Another valuable topic for future studies could be the influence of activity levels on muscle volume in neuromuscular diseases. Furthermore, we are currently working on a protocol for semi-automated segmentation of the fat-fraction.27

4.2 | Correlation of muscle volumes with age, BMI, and functional parameters

Muscle volume was shown to be negatively correlated with age, especially in the quadriceps femoris muscle, by other studies using single slice MRI quantification.29-30 In our data using more than single slices, there was no correlation between muscle volume of the thigh or lower leg with BMI or age. The different methodology may explain this discrepancy. It is also possible that our sample was of insufficient size to detect associations between age and muscle volume, especially in the presence of neuromuscular disease. A further confounder could be the inhomogeneous age distribution in our study, as neuropathy patients were on average the oldest group, followed by myopathy patients and then controls. Unmeasured confounding factors cannot be excluded.

In myopathy patients, muscle strength was negatively correlated with muscle volume. Interestingly, there was a difference between the correlation coefficients of the right and left sides when comparing muscle volume and knee strength. This may be explained by the highly variable range of right-sided muscle volumes seen among the subjects with 5/5 strength, ie, constitutional differences or training effects.

While considering these factors, further research with larger numbers of subjects should be done to establish regression lines more resistant to extreme values. Furthermore, a worthwhile approach for further investigations could be to assess differences between dominant and nondominant sides in myopathy patients with respect to disease progression and the effects of exercise as a modifying factor.

In neuropathy patients, the electrophysiological correlations were in accordance with a previous imaging study on familial amyloid polyneuropathy.31

Perhaps a combined marker that includes fat and remaining muscle volume change would have great potential as a precise and non-subjective biomarker of disease progression.

5 | LIMITATIONS OF THE STUDY

There are some limitations to our study. As muscle MRI is more commonly done in myopathy patients, this group is overrepresented in our study compared with neuropathy patients. Furthermore, our patient cohort was heterogeneous, as we did not focus on specific disease entities but rather concentrated on the inclusion of a variety of patients. Focusing on more specific disease groups would likely produce more homogenous data, possibly leading to more statistical significance. However, our approach is more realistic in terms of the clinical setting of a neuromuscular in- or outpatient unit.

A limitation of the method used in this study is that it does not identify and segment individual muscles. Because several neuromuscular disorders show preferential involvement of specific muscles or muscle groups, it would be a valuable future direction to segment individual muscles and compare them between specific neuromuscular diseases.

It could be seen as a limitation that our study used T1-weighted images, rather than 3-point DIXON images, which are the current...
standard in muscle MRI. However, T1-weighted muscle MRI images display good contrast between fat and muscle tissue and can thus be used well for the segmentation protocol introduced here.

It may be seen as a limitation that the comparison of time taken for manual versus semi-automatic segmentation was done on 15 randomly chosen subjects rather than the whole group of 65 subjects. This was due to the very time-consuming work of manual segmentation of more than 100 slices per subject, and the clear and unambiguous result in these 15 subjects. It is very unlikely that extending this analysis to the whole 65 subjects would yield a different result. As this is a retrospective proof-of-concept-study, there are no longitudinal data with sequential MRI studies of patients yet.

A further limitation is the focus on remaining muscle volume, rather than fat fraction, because the latter has been shown to be a good biomarker in neuropathy progression. However, we do not yet know which parameter, either the fat fraction or the change in muscle volume, is more sensitive to general disease status, correlation with clinical parameters or disease progression. One benefit of measuring remaining muscle volume could be to pinpoint disease progression in long-term studies more accurately or even before the fat fraction shows any significant differences in the course. Nevertheless, muscle volume alone does not distinguish between muscle loss due to atrophy or dystrophy and is, therefore, less specific than fat fraction. However, with the fat fraction only, information on disease progression may be inaccurate as it does not represent atrophy. Eventually, a combination of both, fat fraction and remaining muscle volume, may be the best imaging biomarker in neuromuscular patients.

In conclusion, semi-automated segmentation of muscle MRI allows for exact, noninvasive, and time-saving quantification of muscle volumes in neuromuscular patients. This method may provide a reliable and objective biomarker for clinical studies. Further studies should also address the sensitivity to change in muscle volume over time, evaluating its potential as a marker of disease progression or monitoring of the efficacy of therapy in clinical studies.

CONFLICT OF INTEREST
None of the authors has any conflict of interest to disclose.

ETHICAL PUBLICATION STATEMENT
We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this article is consistent with those guidelines.

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

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