MUSCLE ULTRASOUND QUANTIFIES THE RATE OF REDUCTION OF MUSCLE THICKNESS IN AMYOTROPHIC LATERAL SCLEROSIS

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ABSTRACT: Sensitive biomarkers are lacking in amyotrophic lateral sclerosis (ALS). Muscle ultrasound (MUS) can quantify muscle thickness and echointensity (EI). We evaluated the rate of muscle atrophy in ALS using MUS. Ten patients had serial unilateral MUS examination of biceps, wrist flexors, and tibialis anterior over 6 months. The rates of change of muscle thickness and EI were determined. Muscle thickness declined at a mean rate of −0.663 mm/month ($P = 0.0014$), greatest in biceps. Muscle thickness correlated moderately with ALSFRS-R, grip dynamometry, and body weight. There was no change in EI. MUS can quantify the rate of reduction in muscle thickness in ALS patients. The lack of strong correlation between muscle thickness and standard ALS measures may reflect limited sensitivity in these conventional measures. The rate of change of muscle thickness merits further study as a potential biomarker in ALS, particularly when considering biceps brachii as a candidate.

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Because it is a uniformly fatal and debilitating disease, there is tremendous interest in the development of effective therapies to slow or halt the progression in amyotrophic lateral sclerosis (ALS). Riluzole is the only U.S. Food and Drug Administration (FDA)-approved drug treatment for ALS, and its effects are modest.[1] Current study designs often use a primary endpoint of either death from ALS or initiation of long-term mechanical ventilation (LTMV). This design requires a relatively long observation time to determine whether there is a positive treatment effect. Although several drugs have demonstrated efficacy in murine models, nearly all have failed in human trials.[2] The availability of a sensitive biomarker to determine the activity of disease in ALS would accelerate the process of determining the efficacy of therapeutic agents.

The revised ALS Functional Rating Scale (ALSFRS-R),[3] manual muscle strength testing (MMT), or motor unit number estimation (MUNE)[4–6] are currently used in clinical studies in addition to the primary endpoint of mortality. Each of these secondary endpoints has potential drawbacks as biomarkers, which include potential lack of objectivity in a questionnaire, variable effort when assessing direct strength measurements, and considerable discomfort in MUNE testing. Trials of potential serologic, cerebrospinal fluid (CSF), and skin biopsy–derived biomarkers in ALS have failed to correlate with disease activity or to change continuously as the disease progresses.[4–5] More recently, certain CSF glial markers have been correlated with survival in ALS, but repeated measures have not yet been published for these markers.[6] Muscle impedance electromyography has also been hypothesized as a biomarker,[7] but it has not been shown to be useful in any longitudinal trials.

Muscle atrophy is a disease-defining feature of ALS and, in clinical experience, atrophy correlates with progressive weakness. As such, muscle atrophy is a qualitative marker of disease progression, although there is no clear quantitative marker of atrophy. Muscle ultrasound (MUS) is a safe, noninvasive, and rapid method of measuring muscle volume or thickness.[8] Arts et al. presented an assessment of MUS in ALS subjects, in which they found lower muscle thickness and higher ultrasound echointensity (EI) when compared with normal controls.[9,10] Their results showed the most significant thickness abnormalities in the biceps brachii and wrist flexors. Normal values for muscle thickness and EI have been generated, with norms varying by age and weight.[11,12]

The earlier results suggest both muscle thickness and EI change over time in ALS, but the rates of these changes are unknown. In addition, it is not known whether these changes continue throughout the course of the illness in a quantifiable manner. We sought to determine whether MUS is sensitive enough to quantitatively detect changes in muscle thickness over time and to determine the biomarker potential of the calculated rate in change of muscle thickness, as defined by MUS.

Abbreviations: ALS, amyotrophic lateral sclerosis; ALSFRS-R, revised Amyotrophic Lateral Sclerosis Functional Rating Scale; BMI, body mass index; EI, echointensity; FDA, U.S. Food and Drug Administration; FVC, forced vital capacity; LTMV, long-term mechanical ventilation; MMT, manual motor testing; MRC, Medical Research Council; MUNE, motor unit number estimation; MUS, muscle ultrasound

Key words: amyotrophic lateral sclerosis, biomarker, clinical trial design, muscle thickness, muscle ultrasound

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METHODS

Subjects. Ten patients were recruited between January and March 2009 from the Vanderbilt ALS

Clinic. They gave voluntary, written informed consent to participate. The study was approved by the Vanderbilt University Institutional Review Board and was registered at clinicaltrials.gov with identifier NCT00838617. Each subject had serial unilateral MUS examinations every 3 months at regularly scheduled clinic appointments in the Vanderbilt ALS Clinic, and a 6-month follow-up period was planned for each patient. As a pilot study, 10 patients were thought adequate for proof-of-concept purposes. Inclusion criteria were fulfillment of El Escorial criteria for ALS and age at least 18 years. Exclusion criteria were a concomitant alternate neurologic disorder causing weakness, prior forced vital capacity (FVC) of <50% predicted, and patients without measurable strength in any limbs.

Ultrasound Measurements. Muscles examined included biceps brachii, wrist flexors, and tibialis anterior. Measurement sites were the same as those described by Arts et al.[9] In their study, the biceps and wrist flexors had the largest mean reduction in muscle thickness compared with controls, so these muscles were studied. In addition, we studied the tibialis anterior in order to include a lower extremity muscle. The biceps brachii was measured at two-thirds the distance from the acromion to the antecubital crease, with the borders of the muscle defined between the humerus and the ventral surface of the muscle. The brachialis is included in this measurement. Wrist flexors were measured at a site two-fifths the distance from the antecubital fossa to the radial head. Muscle thickness was measured from the interosseous membrane to the ventral muscle surface, including the flexor carpi radialis and superficial and deep flexor digitorum muscles. The tibialis anterior was measured at one-fourth the distance from the inferior edge of the patella to the lateral malleolus. The muscle definition is clear between the deep fascial border to the ventral surface of the muscle. Two transverse images of each muscle were obtained with the transducer perpendicular to the direction of muscle fiber orientation, and two longitudinal images of each muscle were obtained with the transducer parallel to the muscle fiber direction. Generous amounts of ultrasound gel were used, and the least possible pressure was applied to each muscle to avoid compression.

In subjects with nearly normal strength, the more symptomatic side was studied and, in subjects with severe weakness, the stronger side was chosen. Real-time ultrasound measurements were conducted (Titan; SonoSite, Inc., Bothell, Washington) with a linear-array 5–10-MHz L38 transducer. The analog video signal was analyzed, and still images were stored digitally as high-quality jpeg files of 640 × 480 pixels. Ultrasound settings were maintained at the same default "soft-tissue" settings at each muscle site and at each visit, with the depth setting the only change permitted to include the entire muscle in the image. MUS, MMT, and dynamometry were performed by the same investigator for each patient (C.D.L.) at every visit to preclude interrater variability.

Traditional measurements in ALS included ALSFRS-R, MMT, FVC, body weight, and quantitative strength testing with hand-grip dynamometry, using the best result from three attempts in each limb. Repeat measurements were conducted 3 and 6 months afterwards. Images and patient data were stored on REDCap, Vanderbilt University's secure, web-based database application.[13]

Data Analysis. After collection of all the MUS data, labels on the data images were masked to limit bias. Muscle thickness was measured in each image with digital calipers to calculate pixel distance, which was converted into centimeters using the scale on each ultrasound image. EI was measured in each image of the biceps brachii and tibialis anterior with the largest feasible representative rectangular region of interest computed into a gray-scale histogram (8-bit, black = 0, white = 255), each of which were performed using Adobe Photoshop CS3, version 10 (Adobe Systems, Inc., San Jose, California). With several smaller muscles in the wrist flexor group, there are a few layers of fascia that prohibit a large region of interest for EI analysis. Therefore, EI was not analyzed in wrist flexors, as in prior studies.[14,17]

Due to the longitudinal nature of the study, the linear regression analysis chosen was a mixed-effect model, which was used to estimate and compare changes in muscle thickness. The model was first fitted with fixed effects of the mode of ultrasound scanning, muscle, and any two-way interaction with time. The random effects included patients and time. The mode of ultrasound scanning and the mode of ultrasound scanning by time interaction were highly nonsignificant. Both terms were removed from the regression model, as the mode of ultrasound scan did not affect the primary outcome variable, muscle thickness rate of change. The final model was a three-intercept and three-slope random-effects model, which was used to estimate the rate of change in muscle thickness over time for each muscle type. Patient was inserted as a random rather than fixed effect to allow for dependency of repeated measures for each individual and to allow inferences to be made for a general population of patients with ALS from which we sampled. Including time as a random effect allowed

Table 1. Baseline characteristics of ALS patients enrolled ($n = 10$).

Variable	Mean ± standard deviation
Age at enrollment (years)	54.7 ± 14.07
Body mass index (kg/m^2)	24.06 ± 4.31
Age at symptom onset (years)	53.5 ± 13.91
Baseline FVC (% predicted)	69.1 ± 22.35
Baseline ALSFRS	25.5 ± 7.93

estimation of individual patient muscle thickness change over time for specific muscles.[14]

EI change over time, the second outcome variable, was also analyzed with a mixed-effect model with time both as a fixed and random effect, and patient as a random effect. Spearman correlations were used to assess the relationship between muscle thickness and MMT, grip dynamometry, body weight, ALSFRS-R, and FVC. For MMT, elbow flexion was compared with biceps thickness, wrist flexion was compared with wrist flexor thickness, and foot dorsiflexion was compared with tibialis anterior thickness. R software (version 2.9.1) and SAS (version 9.1; SAS Institute, Cary, North Carolina) were used for data analysis, and a two-sided 5% significance level was used for all statistical inferences.

RESULTS

Baseline Characteristics. Ten subjects were recruited, of whom 8 were able to complete follow-up imaging. One subject died of ALS complications, and another became too weak to travel for follow-up. Of the 8 subjects who completed 6-month follow-up imaging, only 2 patients missed the interim 3-month follow-up imaging appointment. Missing data from interim visits were excluded from our analyses. Subjects without any follow-up data were excluded from the analysis of changing muscle thickness and EI over time, but these subjects were included in correlations between ultrasound measurements and other clinical characteristics. Baseline characteristics are reported in Table 1 and were similar to those of many patients in our ALS Clinic.

The mean age of the participants was 54.7 years. Onset of symptoms was approximately 1 year prior to enrollment. Age at symptom onset ranged from 28 to 74 years. Two patients had bulbar-onset ALS, with nearly normal strength in the arms and legs, and 1 patient had weakness confined to one hand. The remainder had mixed spinal and bulbar weakness. Eight of 10 patients were taking riluzole, and 1 was also taking lithium.

Muscle Thickness Results. Based on the original mixed-effect model, muscle type by time interaction was significant ($P = 0.0014$), which indicates that muscle thickness declined over the study period. The final model mean muscle thickness change over time was −0.663 mm/month (95% confidence interval: −0.969 to −0.357). There was a greater decline in biceps brachii thickness compared with other muscles (Table 2). Graphical representations of the muscle thickness plots for the 8 subjects with follow-up data are shown in Figure 1. There was a trend toward reduction in most of the individual muscles studied.

Table 2. Results of mixed-effect model of change in thickness and mean EI over time.

Variable	Mean	95% confidence interval
Overall muscle thickness change*	−0.663 mm/month	(−0.969 to −0.357)
Biceps brachii thickness*	−1.062 mm/month	(−1.509 to −0.561)
Wrist flexors thickness*	−0.477 mm/month	(−0.951 to −0.003)
Tibialis anterior thickness	−0.474 mm/month	(−0.951 to 0.003)
Mean EI	0.5391 units/month	(−2.3736 to 3.468)

*$P < 0.05$.

Echointensity Results. We collected mean, median, and standard deviation data for echointensity. Mean and median had a Spearman correlation of 0.9895, and therefore median was dropped from the model as exchangeable with mean. Based on the mixed-effect model, mean and standard deviation EI did not change over time, with a 95% confidence interval of −2.374 to 3.468 units/month (Fig. 2).

Clinical Correlations. Spearman correlations between muscle thickness and ALSFRS-R, FVC,

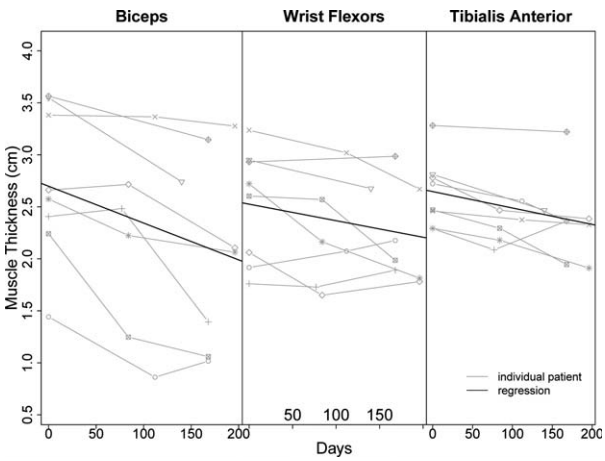

FIGURE 1. Changes in muscle thickness over time in 8 ALS subjects with follow-up data. Each box represents one muscle, with each subject plotted with connected gray lines. The bold line is the regression line of all patients analyzed together for each muscle. In most muscles of most subjects, there is a decline in muscle thickness over time.

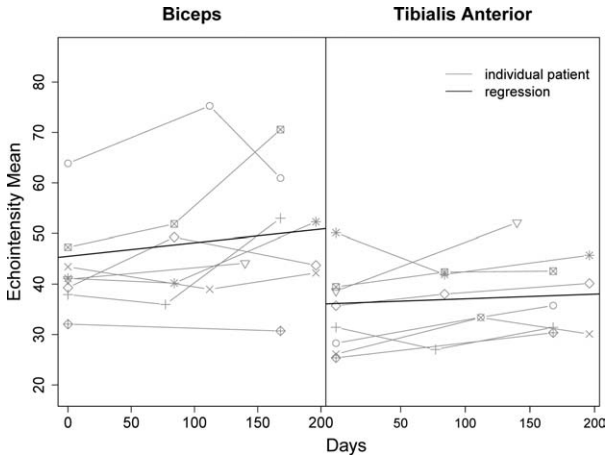

FIGURE 2. Change in mean EI in biceps and tibialis anterior over time in 8 ALS subjects with follow-up data. Each subject's values are connected with a gray line. The bold line is the regression line of all patients analyzed together. In most subjects, an increase in mean EI is apparent; however, with wide standard errors, this is not statistically significant. EI, echointensity.

strength, dynamometry, body weight, and EI are presented in Table 3. Correlation values were moderate for conventional measures, with most values near 0.3. The Spearman correlation for muscle thickness and EI was −0.4614, indicating moderate correlation between these measures.

The planned correlations compared 12 muscle thickness measurements to each corresponding ALS parameter, except for MMT, in which there were site-specific measurements to compare, as described in the Methods section. This imbalance limits the strength of correlation. When we restricted Spearman correlations to biceps, which showed the most significant results, we found stronger results, as demonstrated in Table 3.

DISCUSSION

MUS has previously detected reduced muscle thickness and increased EI in ALS patients when compared with normal controls.[9] A correlation between MUS-defined biceps size and strength in ALS patients has also been shown.[15] To our knowledge, our study is the first to determine the rate of change in muscle thickness over time in ALS patients. We found a definite reduction in muscle size in the 8 subjects who completed this trial, and there was more reduction in the biceps brachii than in wrist flexors or tibialis anterior. The average change of −0.663 mm/month may appear to be small, but over 3 months this decrease was nearly 2 mm. Over 1 year, this accounted for about a 25% loss of thickness in a muscle if the initial thickness was 3 cm. This is equivalent to about 45% loss of muscle volume in a cylindrical model of muscle. Figure 1 shows substantial reductions in muscle thickness in some subjects, and it also shows that muscle loss did not always occur at a constant rate. Figure 3 shows two examples of the loss of muscle thickness evident in several months in 2 patients, each of whom had no change in strength on direct muscle testing. This may indicate improved ability to detect changes in ALS with ultrasound compared with MMT. It is not clear why the overall rate of change in biceps was larger than in the other muscles studied, although it did not seem to be a significant outlier. We hypothesize that this was due to higher rates of baseline hypertrophy in the biceps, and subsequent denervation in ALS causes a greater degree of muscle bulk loss.

We did not find a significant change in EI over time. This was surprising, given the prior finding that EI is abnormal in patients with ALS, and several patients in our study showed increases in EI over time. Further, the correlation between muscle thickness and EI was stronger than that between muscle thickness and many other variables. In a review of other investigators' methods, we found that our method differed from theirs in that we

Table 3. Spearman correlations between various clinical measures and ultrasound-defined muscle thickness in ALS patients.*

Variable	Comparison	Spearman rho
Median echointensity	Overall muscle thickness	−0.4614
ALSFRS score	Overall muscle thickness	0.3265
Body weight	Overall muscle thickness	0.3153
Grip dynamometry	Overall muscle thickness	0.2953
Manual motor testing	Overall muscle thickness	0.1903
Forced vital capacity (% predicted)	Overall muscle thickness	0.2541
Median echointensity	Biceps only	−0.6843
ALSFRS score	Biceps only	0.5042
Body weight	Biceps only	0.4767
Grip dynamometry	Biceps only	0.5413
Manual motor testing	Biceps only	0.5344
Forced vital capacity (% predicted)	Biceps only	0.3402

**In the top section, characteristics are compared with all muscle thickness measurements, and in the bottom section these characteristics are compared with biceps thickness only.*

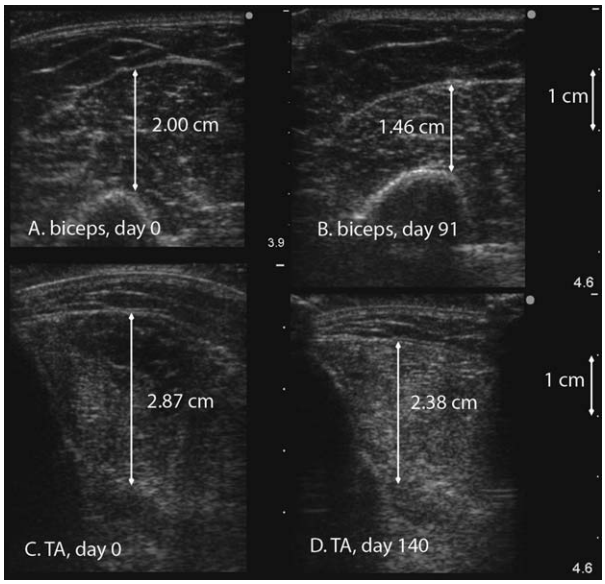

FIGURE 3. Right biceps brachii ultrasound images on initial examination **(A)** and at follow-up examination approximately 3 months later **(B)** in a subject with ALS. Muscle thickness decreased by 27%, from 2.00 cm to 1.46 cm. EI does not appear to have changed. Biceps strength was 5/5 on the MRC scale on both occasions. In a different subject with ALS, right tibialis anterior ultrasound images are shown on initial examination **(C)** and at follow-up examination approximately 5 months later **(D)**. Muscle thickness decreased by 17%, from 2.87 cm to 2.38 cm. EI increased somewhat in this example, with whiter-appearing muscle tissue in **(D)** compared with **(C)**. Foot extension strength was 4^{+}/5 on the MRC scale on both occasions. Ultrasound images have been rescaled to equilibrate the scales of the images. MRC, Medical Research Council.

did not keep ultrasound depth constant in images for EI analysis. There were also larger standard deviations in EI measurements compared with muscle thickness standard deviations, which would reduce statistical power to find a difference. We believe these elements could account for failure to find a change in EI over time. This result also highlights the technical limitations of EI. Differences in ultrasound settings, such as gain, power, and angle of insonation, can all dramatically alter EI calculations, whereas these factors do not have an impact on thickness measurements.

Spearman correlations indicate only moderate correlation between muscle thickness and most conventional measures of ALS progression. There was a stronger correlation between muscle thickness and median EI of −0.4614. MMT had a less significant correlation value. This may be due in part to the heterogeneous group of patients studied, which included 2 patients without significant limb weakness. It may also be due in part to the wide variation of muscle size and strength among different individuals. If we are correct in our belief that MUS is sensitive to early changes in muscle size in ALS when strength is still normal, then it is true that correlations with less sensitive markers will be reduced. Because ALS is heterogeneous, often affecting different muscles at different rates, these correlations are reduced. We hypothesize that there is less variation in the rate of change of muscle thickness of a given patient than in muscle size across a population. Therefore, the rate of change of muscle thickness in individual patients can be more sensitive to changes as a clinical parameter than muscle thickness itself.

A separate analysis of biceps thickness compared with dynamometry shows a substantially improved Spearman correlation of 0.5413. Other correlations with biceps alone showed stronger correlations. This may add to evidence that the biceps is particularly sensitive to muscle mass loss that corresponds to loss of strength in the affected limb. We recognize that the inference of this retrospective subgroup analysis is limited.

These findings suggest that the rate of change of ultrasound-defined muscle thickness may be more sensitive than other markers in this illness and, as a continuous parameter, the rate of change in ultrasound-defined muscle thickness has potential as a biomarker in ALS. Biomarkers ideally should have high interrater correlations, and there should be consistency with different machines and settings. This is true for MUS-defined muscle thickness, but not for EI. Larger studies with longer follow-up time are needed, which we hypothesize will show a relatively constant rate of decline in muscle thickness until end-stage atrophy. Alternatively, a sigmoid-shaped curve may be evident if patients can be studied very early in the course of their disease. It seems clear that muscle atrophy reaches an end stage, after which the atrophy does not continue to worsen. The rate of change in muscle thickness very early in the disease is unknown. A longer duration study may also correlate the rate of change in muscle thickness with eventual mortality.

A limitation of the study is the heterogeneity of ALS itself. This creates a dilemma in terms of what muscles to study, in that some patients have a prolonged course with only bulbar weakness, and others may have weakness and atrophy initially limited to a single limb. Our study suggests that biceps brachii may be a suitable candidate for study, but it seems that the study of multiple muscles would be required to account for such heterogeneity. Another limitation of our study is the potential imprecision in the site of ultrasonography. Although MUS sites were measured from bony and reliable skin landmarks, measurement lines could have been angled differently from one measurement to the next, potentially producing error.

In conclusion, this study has provided data suggesting that ultrasound-defined muscle thickness decreases over time in ALS patients. Further, MUS can quantify this rate. These results will prompt future studies into the utility of ultrasound-defined rate of change of muscle thickness as a biomarker in ALS.

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