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Abstract: The purpose is to assess the characteristics of echovariation in amyotrophic lateral sclerosis (ALS) compared with other MUS parameters. Twenty-six ALS patients (8 women, mean age 58.9 years, SD 12.02 yr) and 26 healthy controls (17 women; mean age 59.6 years, SD 6.41 yr) were included in this observational study. They underwent bilateral and transverse ultrasound of the biceps/brachialis, forearm flexor group, quadriceps femoris and tibialis anterior. Muscular thickness, echointensity and echovariation were analyzed. Muscles affected by ALS showed increased echointensity, decrease in thickness, and decrease echovariation. Echovariation in all muscles but quadriceps femoris, strongly correlated with muscle strength (explained variance between 21.8% in the biceps/brachialis and 37.5% in tibialis anterior) and the ALSFRS-R score (explained variance between 26% in the biceps/brachialis and 36.7% in the forearm flexor group). Echovariation is an easy to obtain QMUS parameter that could distinguish ALS from healthy controls more accurately than previous described biomarkers.

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ABSTRACT

The purpose is to assess the characteristics of echovariation in amyotrophic lateral sclerosis (ALS) compared with other MUS parameters. Twenty-six ALS patients (8 women, mean age 58.9 years, SD 12.02 yr) and 26 healthy controls (17 women; mean age 59.6 years, SD 6.41 yr) were included in this observational study. They underwent bilateral and transverse ultrasound of the biceps/brachialis, forearm flexor group, quadriceps femoris and tibialis anterior. Muscular thickness, echointensity and echovariation were analyzed. Muscles affected by ALS showed increased echointensity, decrease in thickness, and decrease echovariation. Echovariation in all muscles but quadriceps femoris, strongly correlated with muscle strength (explained variance between 21.8% in the biceps/brachialis and 37.5% in tibialis anterior) and the ALSFRS- R score (explained variance between 26% in the biceps/brachialis and 36.7% in the forearm flexor group). Echovariation is an easy to obtain QMUS parameter that could distinguish ALS from healthy controls more accurately than previous described biomarkers.

Keywords: clinical neurology examination; observational study; ultrasound; amyotrophic lateral sclerosis.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disease involving the upper and lower motor neuron (LMN) that produces muscle fasciculations, weakness and atrophy. Clinical monitoring of LMN is usually based on testing muscular strength with the Medical Research Council scale (MRC) (Florence et al. 1992) and the revised ALS functional rating scale, (ALSFRS-R) (Cedarbaum et al. 1999). However, these tools show limited sensitivity for measuring changes and, consequently, clinical endpoints usually require prolonged follow up (Simon et al. 2014; Turner and Benatar 2015). In clinical trials, therefore, reliable biomarkers measurable over the short term are desirable (Turner and Benatar 2015).

Although several neurophysiological tools have been developed as progression biomarkers in ALS (Simon et al. 2014), they require wide operator experience, are time consuming and pain is a considerable limiting factor (Simon et al. 2014).

Current ALS diagnostic criteria require the detection by electromyography (EMG) of denervation signs or fasciculations in muscles with neurogenic changes (Costa et al. 2012). A fasciculation is an involuntary synchronous contraction of all the skeletal muscle fibers within a single motor unit, which arises as a result of spontaneous depolarization of a lower motor neuron. Fasciculation potentials of abnormal morphology in the EMG recording are a characteristic clinical feature of ALS, but fasciculation potentials of normal morphology can occur in healthy subjects (Brooks et al. 2000).

Muscle ultrasonography (MUS) is an accessible, painless and easy to perform method to detect fasciculations and structural muscle changes in ALS (Arts et al. 2011a; Simon et al. 2014). MUS has been shown more sensitive than EMG for detecting fasciculations, especially in the bulbar region (Grimm et al. 2015; Misawa et al. 2011) and structural changes such as decrease of muscle thickness and increase of echointensity (EI) have been found (Arts et al. 2011a; Misawa et al. 2011; Simon et al. 2014).

However, their role as diagnostic or progression biomarkers is limited, because of the high interindividual variability in structural parameters (Arts et al. 2011a; Misawa et al. 2011; Pillen et al. 2008). Therefore, it would be a major advance to find a MUS biomarker not influenced by other factors than disease ones. Echovariation (EV) has been previously established to characterize plantar fasciitis and it is a reproducible, short and easy to carry out procedure (Ríos-Díaz et al. 2015).

We hypothesize that EV could act as a more reliable biomarker in ALS since it is an adimensional parameter (Arts et al. 2011a). Therefore, we compared muscle thickness, EI and EV in four muscle groups in ALS patients and age matched controls and assessed how time and other clinical variables influenced these parameters.

MATERIAL AND METHOD

Subjects selection

Patients (n=26) were recruited from the Valencia ALS Association (ADELA) between September 2013 and April 2014. The patients were diagnosed as having ALS by an experienced neurologist (JFVC), according to the revised El Escorial Criteria (Brooks et al. 2000).

Twenty-six healthy volunteers without a history of hereditary neuromuscular disease were recruited as control group.

Standard protocol approvals, registrations, and patient consents

This study was approved by the ethics committee of the Universidad Católica de Murcia (Spain) and performed following the Helsinki Declaration principles. All participants provided written informed consent.

Recorded clinical variables

Demographical and clinical characteristics (sex, age, weight, height, body mass index -BMI-, time of evolution from symptoms onset and date of diagnosis) were recorded. ALSFRS-R score (Cartwright et al. 2011) and muscle strength, measured with the Medical Research Council (MRC) rating scale (Arts et al. 2011a), were assessed by the same researcher (JMP) on the same day as the MUS was performed.

Ultrasonography

MUS was performed in four muscle groups of each side in patients and controls by the same experienced examiner (JMP) with the participants sitting and completely relaxed. A LEbt12 phased array real-time scanner (software 2014) from General Electric Company with a 5–13 MHz linear array transducer (12L–RS) was used for MUS. All system-setting parameters, such as gain (98 dB), time gain compensation (in neutral position), depth (5 cm for tibialis anterior and 6 cm for the other muscle groups), frequency (12 Mhz), compression and focus (two focal points at 1.8 and 2.6 cm) were kept constant throughout the study.

To avoid oblique scanning angles the angle of the probe was adjusted until the best muscle EI was obtained in every image.

Applying the standardized protocol described by Arts et al. (2008) bilateral transverse ultrasound images of the biceps/brachialis group (2/3 distance acromion – antecubital crease), forearm flexors group (2/5 distance antecubital crease – distal end radius), quadriceps (1/2 distance anterior superior iliac spine – superior aspect patella) and tibialis anterior (1/4 distance inferior aspect patella – lateral malleolus) were obtained and measured. Three images were taken of every muscle in order to minimize variation in muscle thickness, EI and EV.

The resulting bitmaps had a resolution of 820 x 614 pixels (7.6 px/mm) with 256 grey levels and were stored as .TIFF files without compression or losses (Wiggins et al.

2001).

Image analysis

Fasciculations as well as quantitative MUS variables (QMUS), including muscle thickness, EI and EV, were obtained for each muscle group.

Muscle thickness was measured with electronic calipers as previously described (Arts et al. 2010). The thickness of the biceps/brachialis group was measured between the uppermost part of the bone echo of the humerus and the superficial fascia of the biceps; the forearm flexor group between the interosseous membrane (next to the radius) and the superficial fascia of the most ventral flexors; the quadriceps femoris between the uppermost part of the bone echo of the femur and the superficial fascia of the rectus femoris (which includes the vastus intermedius); and the tibialis anterior between the interosseous membrane (next to the tibia) and the ventral fascia of the tibialis anterior (Figure 1).

Fasciculations were registered in each muscle for 10 seconds as previously published (Arts et al. 2008).

Thickness was measured in all three images of each muscle group by an expert ultrasonographist (JMP) and the mean of the three values was used for the corresponding analysis.

Image processing and analysis was performed by one researcher (JRD) using the ImageJ (v.1.48) software. This researcher, who was blind for diagnosis, selected the region of interest (ROI) the ROI Manager application for ImageJ, with a size of 71 x 40 pixels for tibialis anterior and 73 x 73 pixels for other muscle groups on an 8-bit gray scale. The ROI was defined as the muscle region without bone and fascia with the best reflection (Figure 1).

EV, a parameter that can be interpreted as a measure of intensity range (Ríos-

Díaz et al. 2015), was determined by the relation between standard deviation and mean of pixel intensity obtained from the histogram:

$$EV = \sigma/\mu \cdot 100$$

where σ is the standard deviation of the image intensities and μ is the mean value of intensity in each ROI.

EI and EV were obtained from the ROIs of three images of each muscle and the mean of the three values was used for analysis. Sets of 20 images for each muscular group were analyzed by another researcher (MEDBA) who was blinded to the previous results to analyze inter-observer reliability in thickness measurement and ROI selection. EI and EV of each ROI and muscle thickness were calculated.

Statistics

Data were analyzed using IBM SPSS Statistics for Windows 19.0 (IBM Company, 2010).

Variables were checked for normality by the Kolmogorov-Smirnov test and for the homogeneity of variances by the Levene test. In addition, we analyzed the asymmetry and kurtosis coefficients and the normality Q-Q plots.

Data were summarized as mean and standard deviations (SD), with 95% confidence intervals (CI) for continuous variables, and absolute and relative frequencies for categorical variables.

Intraclass correlation coefficient (ICC) for 2-way mixed effect model and absolute agreement was calculated to determine inter-observer reliability of thickness measure, EI and EV. Next criteria were used to judge the reliability coefficients: very low (<0.20), low (0.21-0.40), moderate (0.41-0.60), good (0.61-0.80) and very good (0.81-1.00).

Unpaired t-tests were used to compare continuous variables and chi- square test to compare categorical variables at baseline between ALS patients and controls.

Paired t-tests were used to assess right-left differences in muscle thickness, EI and

EV measurements (Armitage et al. 2002).

QMUS variables in patients and controls

One-way ANCOVA was used to compare QMUS variables in patients and controls, controlling for effects of clinical and demographical covariates (Feinstein 2002)

Cohen's d statistic was used to evaluate the effect sizes, in this case by dividing mean score differences between patient and control group by the initial standard deviation of the control group (Cohen 1988; Kelley and Preacher 2012). A Cohen's d statistic of <0.1 corresponds to a small size effect, around 0.3 to a medium size effect and > 0.5 a large size effect.

Influence of time of evolution.

Regression models were used to study the relationship between time of evolution (logarithmic transform was applied to this variable to correct the absence of a normal distribution) as independent variable and the QMUS parameters as dependent variables (Kleinbaum et al. 1998).

Relations between QMUS parameters and MRC- ALSFRS-R

MRC and ALSFRS-R were taken as dependent variables to check the relationship with QMUS parameters (thickness, echointensity and echovariation as independent variables).

All regressions were carried out with a fixed inclusion of ultrasonographics parameters to obtain a raw model and the stepwise inclusion of sex, age, and BMI. The P-in and P-out values were 0.05 and 0.10, respectively. To control for possible collinearity, the tolerance to enter in the model was fixed at 0.01 (Hair et al. 2010).

The presence of influential observations was checked with the Cook distance (any influential observation was considered a Cook distance of >1). Collinearity for independent variables was evaluated with the tolerance and variance inflation factors (Kleinbaum et al. 1998). Data are presented as b coefficient and 95% CI. The relation

between variables was studied with a partial correlation coefficient that adjusts the linear relation between the dependent and independent variables. In addition, we calculated the goodness of fit with the partial determination coefficient (r-squared in %).

P values of < 0.05 were considered statistically significant for all the tests.

RESULTS

Study subjects characteristics

Twenty-six participants with ALS (8 women, mean age 58.9 years, SD 12.02 yr) and 26 healthy controls (17 women; mean age 59.6 years, SD 6.41 yr) were included in this study. No significant differences in age, height and weight were found. BMI was slightly different and sex distribution was significantly different. The clinical characteristics along with their mean and standard deviation (SD) are shown in Table 1.

Ultrasound variables

Fasciculations were detected in 15 of the 26 patients (57.7%) and none of the healthy controls. Altogether, they were detected in 30 out of 52 (57.7%) of the biceps/brachialis group, 26 (50%) of the forearm flexor group, 22 (42.3%) of the quadriceps femoris and 13 (25.7%) of tibialis anterior.

QMUS variables for each muscle and group are shown in Table 2. There were no significant right–left differences in thickness, EI or EV in the four studied muscle groups, so only one sample of each right/left muscle group was selected for further analysis (52 ultrasonograms for each group).

The inter-observer reliability ICCs for thickness measurement were over 0.97 for all the muscular groups that revealed a very good reliability. Inter-observer measures of echointensity revealed very good reliability too; the highest ICC was obtained for quadriceps femoris (ICC=0.98; 95% CI= 0.95 to 0.99) and the lowest for tibialis anterior (ICC=0.95; 95% CI= 0.87 to 0.98). As regards the reliability of echovariation, the inter-

observer ICCs were over 0.80 for all the muscular groups.

When analyzing QMUS differences between patients and controls, mean comparisons were made with the corresponding corrections for sex and BMI (see footnotes in tables for details). As expected, muscle thickness in the ALS group was significantly lower than in the healthy controls for all analyzed muscles groups. EI was higher in patients than in controls, except in the case of quadriceps femoris where no differences between patients and controls were found. Finally, EV was significantly lower in patients for all muscles, with a strong size effect (higher than 1.0), except for quadriceps femoris which showed a moderate size effect of 0.55.

Relation between QMUS parameters and time from symptoms onset.

Table 3 shows the results of the raw and adjusted regression analysis for age, sex and BMI, when necessary.

No significant relationship was found between time of evolution and QMUS for any muscle group, although in the biceps/brachialis group a 6.71% of the EV variance could be explained by time of evolution, which was nearly significant.

BMI, age and sex showed an interaction with muscle thickness and EI, but not with EV.

Relation between QMUS parameters and MRC-ALFSRS-R

Table 4 shows the relationships between the QMUS parameters and MRC ad ALFSRS-R scores. Correlations are expressed as partial correlations, which provide information on the explained variance about the individual variable without the effect of the others.

A significant and positive relationship was found between thickness and EV, and the MRC score in all regions, i.e. the greater the thickness or EV the higher the MRC score. The explained variance for thickness was between 24.8% in the biceps/brachialis

group and 7.4% in the forearm flexor group. The explained variance for EV was between 26.5% in the tibialis anterior and 4.5% in the quadriceps femoris. The EI showed no significant correlation with MRC in any muscular group.

Analysis of the relationship between the ALSFRS-R score and QMUS parameters showed similar results. Thickness and EV were significantly and directly correlated with the ALSFRS-R score: the greater the thickness or EV the higher the ALSFRS-R score. The explained variance for thickness was between 26.9% in the biceps/brachialis group and 7.5% in the forearm flexor group. The explained variance for EV was between 36.7% in the forearm flexor group and 8.9% in the quadriceps femoris.

The EI showed an inverse correlation with the ALSFRS-R score only in tibialis anterior (4.0% of explained variance).

DISCUSSION

We describe a new QMUS parameter (EV) that could be useful as diagnostic and/or progression biomarker in ALS, and compared its characteristics with those of previously described QMUS parameters (muscle thickness and EI).

Previous studies have shown that muscle thickness decreases and EI increases even in clinically unaffected muscles (Arts et al. 2011a; Arts et al. 2012; Grimm et al. 2015), reflecting the progressive muscular atrophy and fibrosis that occurs in ALS as a result of muscle denervation (Pillen et al. 2009). Recently, MUS has been proposed to increase the sensitivity of EMG if both the presence of fasciculations and EI are taken into account (Arts et al. 2012; Grimm et al. 2015), suggesting their role as diagnostic biomarker. However, EMG remains necessary for ALS diagnosis, since decreased muscle thickness and increased EI are age-dependent, show high interindividual variability, and can also be observed in other neuromuscular diseases (Pillen et al. 2008).

The major advantage of MUS is that it is a painless technique. Consequently, its

role in monitoring progression in clinical trials could be of great value to avoid the repetition of painful techniques such as EMG. However, correlation between EI and muscle thickness and muscle strength and disability is only weak (Arts et al. 2011a; Grimm et al. 2015) and disease progression measured as changes in MRC or ALSFRS- R does not correlate with any of them (Arts et al. 2011a). The ultimate cause of this remains unknown but suggests that factors other than disease related ones could influence changes in EI or muscle thickness. Another reason may be that the reproducibility of these measurement techniques is limited because of the ROI selection or because very important adjustments such as frequency and depth of ultrasonography are not taken into account or two different US devices are used (Arts et al. 2011b; Florence et al. 1992; Pillen et al. 2009). Whatever the reason, it implies a strong limitation and a major challenge of MUS when used as a progression biomarker.

Second order statistical methods of texture analysis have also been proposed to improve accuracy and precision of these measures (Molinari et al. 2015). However, in comparison to EI or muscle thickness they require more complex post-processing operations.

Consequently, reproducibility supposes a strong limitation and a major challenge of MUS when used as a progression biomarker. To minimize this risk, we performed all studies with the same US device, we strictly defined the acquisition parameter and the mean of three images of each muscle was calculated for each parameter. Moreover, the inter-observer reliability in ROI selection for EI and EV and thickness measure were vey good, suggesting a high reproducibility of our results.

Fasciculations

As expected, MUS identified frequent and widespread fasciculations. However, they were less frequent than previously reported (Grimm et al. 2015; Misawa et al. 2011), especially in lower limbs. Most patients in our study had lower limb onset and were at a

moderate to advanced stage of the disease. Fasciculations are an early finding in ALS and their frequency diminishes with time (de Carvalho and Swash 2013), which might explain the relatively low frequency of fasciculations in our cohort, especially where the disease started.

Muscle thickness

As described in previous studies (Arts et al. 2008; Arts et al. 2012; Grimm et al. 2015), a decrease in muscle thickness was observed in ALS patients compared to healthy controls. The highest size effect of this difference was obtained for quadriceps femoris. As mentioned above, this finding could be related to the fact that in more than 50% of subjects with ALS in our sample, symptoms started in lower limbs.

As previously published, muscle strength and disability showed a weak correlation with muscle thickness (Arts et al. 2011a) except for the quadriceps femoris where this correlation was moderate. No clear correlation was found between EV and disease duration.

Echointensity

EI can be estimated using either a subjective visual grading scale (Grimm et al. 2015) or a quantitative methodology with an average gray-scale analysis that provides more objective and potentially more sensitive information, but which does not contain any data about tissue homogeneity (Pillen et al. 2008). Quantitative analysis of the EI is dependent on settings, the ultrasound device and on ROI selection, so that to record EI, a standardized protocol and some degree of experience are necessary. New methods of computer-assisted texture analysis, such as gray level co-occurrence matrix (Pillen et al. 2008; Ríos-Díaz et al. 2010) and fractal analysis (Gdynia et al. 2009), have been proposed to quantify muscle alterations in neuromuscular disorders. However, these procedures

require expert handling of image analysis software and involve extra time for exportation and subsequent computer analysis, which could hinder their application in clinical practice.

In the present study we analyzed EI using the Arts protocol (Arts et al. 2008), but system-setting parameters such as frequency, gain, compression, time gain compensation, focus (number and position) and depth were kept constant throughout our study to ensure measurement reliability (Mayans et al. 2012). As suggested by Gdynia et al. (2009), we selected a small ROI, unlike other authors who selected the largest ROI possible (Arts et al. 2008; Arts et al. 2011a; Arts et al. 2011b). In this last approach, large muscle areas are evaluated, combining areas of maximum reflection with anisotropic areas, which cause a decrease in EI. In our study, a ROI of the most reflective muscle segment, avoiding anisotropic muscle areas was obtained. This ensures that zones are selected in which muscle tissue presents maximum brightness.

EI was found to increase in all the muscles studied of ALS patients except, intriguingly, the quadriceps femoris. Considering that symptoms in most patients started in the lower limbs and that EI significantly interacted with sex, age and BMI, we suggest that EI (at least in quadriceps femoris) can be influenced by non-disease related factors, limiting its usefulness. Moreover, as previously reported (Grimm et al. 2015), we found no significant correlation between EI and time of evolution. Likewise, no correlation was found with muscle strength or disability. Others have found a weak correlation with these variables (Arts et al. 2011a; Grimm et al. 2015); however, our statistical analysis (partial correlations) was stronger than those, to avoid confounding variables.

Echovariation

We propose a new MUS biomarker, EV, which is a first order statistical measure that quantifies the deviation of the level of gray from the average. It is a fast and easy method to obtain information on tissue homogeneity (Aggarwal and Agrawal 2012) and

an adimensional parameter, reducing the chance of the heterogeneity.

In our study, we observed a significantly lower level of EV in the muscles of ALS patients compared to healthy controls, with higher effect sizes than those found for muscle thickness or EI except for the quadriceps femoris, in which muscle thickness showed greater effect. Unlike EI, EV did not interacted with non-disease related factors such as age, sex or BMI, further suggesting its potential as biomarker. Moreover, muscle strength and disability strongly correlated with EV except, once again, for the quadriceps femoris. Conversely, no clear correlation was found between EV and disease duration.

There are some limitations in our study. First, the study is transversal and the number of studied subjects was limited. Moreover, our cohort of ALS patients was in a moderate to advanced disease stage and no ALS mimics subjects were studied. Therefore, in order to establish the role of EV as a diagnostic, progression or prognostic biomarker, these results must be replicated in a larger, prospective and longitudinal cohort of suspected ALS patients.

In conclusion, we describe a new, easy to obtain QMUS parameter that seems to distinguish ALS from healthy controls more accurately than previous described biomarkers. It also seems to correlate better with strength and disability, limiting the influence of non-disease related factors.

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FIGURE LEGENDS

Figure 1. The ultrasound measurement of echointensity and muscle thickness. Ultrasonographic scans of the biceps/brachialis group (A-B), forearm flexor group (C-D), quadriceps (E-F), and tibialis anterior (G-H). The left panel depicts muscle thickness measured through electronic calipers in healthy subjects and the right panel represents the region of interest for echointensity in subjects with ALS using the ImageJ (v.1.48) software.

Figure 2. Comparison of quantitative muscular US parameters in controls and ALS patients. Mean and 95% confidence interval for BBr (biceps/brachialis group), FFG (forearm flexors group), QF (quadriceps femoris) and TB (tibialis anterior). * $p<0.005$; ** $p\leq0.001$. n.s=non-significative.

Table 1. Baseline characteristics of the study sample.

Baseline characteristics	ALS Patients (n=26)	Healthy (n=26)	p-value
Females (n) (%)	8 (30.8 %)	17 (65.4 %)	<0.001
Age (years)	58.9 (12.02); 55.8 to 62.0	59.6 (6.41); 57.9 to 61.4	0.570
Weight (kg)	69.9 (17.42); 65.4 to 74.4	72.4 (17.19); 67.6 to 77.2	0.154
Height (m)	1.67 (0.086); 1.65 to 1.69	1.66 (0.08); 1.63 to 1.68	0.773
BMI (kg/m^2)	24.9 (5.13); 23.6 to 26.3	26.2 (4.87); 24.9 to 27.6	0.050
Time from symptoms onset (months)	26.1 (15.77); 21.72 to 30.5		
Symptoms onset-diagnosis (months)	16.3 (9.89); 13.5 to 19.1		
Disease onset (n) (%)			
Right Lower Limb	9 (34.6 %)		
Left Lower Limb	5 (19.2 %)		
Right Upper Limb	1 (3.8 %)		
Left Upper Limb	4 (15.4 %)		
Bulbar	7 (26.9 %)		
ALSFRS-r (max 48)	26.2 (11.67); 22.9 to 29.4		
MRC (max 100)	58.5 (24.75); 51.7 to 65.4		

Data are presented as mean (Standard deviation) and 95% of confidence interval for quantitative variables and as absolute frequencies (relative frequencies). BMI: Body Mass Index. ALSFRS-r: Amyotrophic lateral sclerosis functional rating scale. MRC: Medical Research Council Scale for muscular Strength. P-value for Chi-square test for sex differences and T-Student test for independent samples for age, weight, height and body mass index differences.

Table 2. Quantitative muscular differences between controls and ALS patients.

QMUS parameters	Patients (n=52)		Controls (n=52)		p-value	Size effect
	Mean (SD)	95% C.I.	Mean (SD)	95% C.I.		
			Biceps/brachialis group ‡			
Thickness	28.6 (6.34)	26.8 to 30.3	32.8 (6.25)	31.1 to 34.6	<0.001	0.90
Echointensity	92.2 (14.4)	88.2 to 96.2	86.7 (8.78)	84.2 to 89.1	0.001	0.67
Echovariation	23 (7.33)	21 to 25	29.2 (4.24)	28 to 30.4	<0.001	1.08
			Forearm flexor group ‡‡			
Thickness	30 (9.69)	27.3 to 32.7	31.3 (5.96)	29.7 to 33	0.016	0.42
Echointensity	101.4 (15.25)	97.2 to 105.7	90.7 (15.07)	86.5 to 94.9	<0.001	0.88
Echovariation	19.3 (4.55)	18.1 to 20.6	25.5 (4.22)	24.4 to 26.7	<0.001	1.09
			Quadriceps femoris §			
Thickness	22.9 (8.97)	20.4 to 25.4	29.4 (6.06)	27.7 to 31.1	<0.001	1.00
Echointensity	100.6 (18.03)	95.5 to 105.6	97 (12.77)	93.4 to 100.5	0.245	0.23
Echovariation	18.9 (4.46)	17.6 to 20.1	21.7 (5.66)	20.2 to 23.3	0.005	0.55
			Tibialis anterior §§			
Thickness	19.1 (5.59)	17.5 to 20.6	21.7 (4.91)	20.3 to 23	<0.001	0.91
Echointensity	116 (16.36)	111.5 to 120.6	105.1 (14.63)	101.1 to 109.2	<0.001	1.03
Echovariation	16.5 (4.31)	15.3 to 17.7	25 (4.85)	23.6 to 26.3	<0.001	1.35

SD: Standard Deviation. CI 95%.: Confidence Interval. p-value for independent-samples T-Student test. ‡ Thickness ANCOVA corrected by sex and BMI, Echointensity and Echovariation corrected for sex. ‡‡ Thickness ANCOVA corrected for sex and BMI, Echointensity corrected for sex and Echovariation corrected for BMI. § Thickness ANCOVA corrected for sex. *§§* Thickness and Echointensity ANCOVA corrected for sex. † Effect size was estimated with Cohen's d (low<0.2; moderate=0.5; large>0.80).

Table 3. Relation between QMUS parameters and time from symptoms onset.

	Raw				Adjusted			
QMUS	**B coefficient (SE)**	**95% CI for B**	**p-value**	**Rp (Rp-squared %)***	**B coefficient (SE)**	**95% CI for B**	**p-value**	**Rp (Rp-squared %)**
Biceps/brachialis group								
Thickness	-0.045 (0.057)	-0.16 to 0.070	0.431	-0.112 (1.25%)	-0.007 (0.047)	-0.10 to 0.09	0.881	-0.022 (0.05%)
Echointensity	-0.050 (0.129)	-0.31 to 0.21	0.702	-0.054 (0.29%)	-0.133 (0.118)	-0.37 to 0.10	0.263	-0.161 (2.6%)
Echovariation	-0.12 (0.064)	-0.25 to 0.010	0.064	-0.259 (6.71%)	--	--	--	--
Forearm flexor group								
Thickness	-0.146 (0.084)	-0.315 to 0.02	0.091	-0.237 (5.61%)	-0.115 (0.072)	-0.26 to 0.03	0.116	-0.225 (5.08%)
Echointensity	-0.139 (0.135)	-0.411 to 0.13	0.308	-0.144 (2.07%)	-0.183 (0.133)	-0.45 to 0.08	0.175	-0.193 (3.73%)
Echovariation	-0.002 (0.041)	-0.084 to 0.08	0.954	-0.008 (0.01%)	--	--	--	--
Quadriceps femoris								
Thickness	-0.052 (0.08)	-0.213 to 0.11	0.519	-0.092 (0.84%)	-0.015 (0.075)	-0.17 to 0.14	0.844	-0.028 (0.08%)
Echointensity	-0.218 (0.159)	-0.536 to 0.1	0.177	-0.19 (3.62%)	--	--	--	--
Echovariation	0.003 (0.04)	-0.165 to 0.14	0.949	0.009 (0.01%)	--	--	--	--
Tibialis anterior								
Thickness	0.01 (0.05)	-0.091 to 0.11	0.841	0.029 (0.08%)	0.042 (0.042)	-0.04 to 0.13	0.319	0.142 (2.02%)
Echointensity	0.004 (0.147)	-0.291 to 0.3	0.980	0.004 (0%)	-0.089 (0.125)	-0.34 to 0.16	0.480	-0.101 (1.02%)
Echovariation	0.011 (0.039)	-0.066 to 0.09	0.771	0.041 (0.17%)	--	--	--	--

The regression models for thickness were adjusted for sex in biceps/brachialis group, quadriceps femoris, and tibialis anterior and for sex and BMI in forearm flexors. The regression models for echointensity were adjusted for sex and BMI in biceps/brachialis group, and for sex in forearm flexors and tibialis anterior. The regression models for echovariation did not need corrections. SE: Standard Error for coefficient B. 95% CI: 95% Confidence Interval. *Rp: partial correlation coefficient and partial determination coefficient in brackets. Notice that in these analyses, the independent variable was the time from symptoms onset, and the dependent variables were QMUS parameters.

Table 4. Relations between QMUS parameters and MRC score.

QMUS-MRC	Raw				Adjusted			
	B coefficient (SE)	95% CI for B	p-value	Rp (Rp-squared %)*	B coefficient (SE)	95% CI for B	p-value	Rp (Rp-squared %)
Biceps/brachialis group (Model Goodness= 58.3%)								
Thickness	1.38 (0.338)	0.71 to 2.05	<0.001	0.378 (14.3%)	1.98 (0.347)	1.29 to 2.67	<0.001	0.498 (24.8%)
Echointensity	-0.32 (0.186)	-0.69 to 0.05	0.087	-0.17 (2.9%)	-0.34 (0.173)	-0.68 to 0	0.053	-0.193 (3.7%)
Echovariation	1.67 (0.317)	1.04 to 2.3	<0.001	0.466 (21.8%)	1.51 (0.297)	0.92 to 2.1	<0.001	0.454 (20.6%)
Forearm flexor group (Model Goodness= 48.8%)								
Thickness	0.92 (0.278)	0.37 to 1.47	0.001	0.315 (9.9%)	--	--	--	--
Echointensity	-0.25 (0.145)	-0.54 to 0.04	0.087	-0.17 (3.7%)	--	--	--	--
Echovariation	2.65 (0.383)	1.89 to 3.41	<0.001	0.569 (32.4%)	--	--	--	--
Quadriceps femoris (Model Goodness= 31.2%)								
Thickness	1.59 (0.295)	1 to 2.17	<0.001	0.475 (22.5%)	--	--	--	--
Echointensity	-0.13 (0.166)	-0.46 to 0.2	0.451	-0.075 (0.6%)	--	--	--	--
Echovariation	0.95 (0.466)	0.03 to 1.87	0.044	0.2 (4.0%)	--	--	--	--
Tibialis anterior (Model Goodness= 54.9%)								
Thickness	1.15 (0.408)	0.34 to 1.96	0.006	0.272 (7.4%)	--	--	--	--
Echointensity	-0.17 (0.132)	-0.43 to 0.09	0.201	-0.128 (1.6%)	--	--	--	--
Echovariation	2.49 (0.321)	1.85 to 3.12	<0.001	0.612 (37.5%)	--	--	--	--

The regression models were adjusted for sex in biceps/brachialis group. SE: Standard Error for coefficient B. 95% CI: 95% Confidence Interval. *Rp: partial correlation coefficient and partial determination coefficient (Rp-squared) in brackets. The independent variables were the QMUS parameters and the dependent variable was MRC score.

Table 5. Relations between QMUS parameters and ALSFRS-r score.

QMUS-ALSFRS	Raw				Adjusted			
	B coefficient (SE)	**95% CI for B**	**p-value**	**Rp (Rp-squared %)***	**B coefficient (SE)**	**95% CI for B**	**p-value**	**Rp (Rp-squared %)**
Biceps/brachialis group (Model Goodness= 54.9%)								
Thickness	0.69 (0.169)	0.35 to 1.02	<0.001	0.375 (14.1%)	1.03 (0.17)	0.69 to 1.36	<0.001	0.519 (26.9%)
Echointensity	-0.1 (0.093)	-0.29 to 0.08	0.270	-0.11 (1.2%)	-0.11 (0.085)	-0.28 to 0.05	0.183	-0.134 (1.8%)
Echovariation	0.94 (0.159)	0.63 to 1.26	<0.001	0.51 (26%)	0.85 (0.146)	0.56 to 1.14	<0.001	0.506 (25.6%)
Forearm flexor group (Model Goodness= 46.1%)								
Thickness	0.39 (0.138)	0.12 to 0.67	0.005	0.275 (7.5%)	--	--	--	--
Echointensity	-0.12 (0.072)	-0.26 to 0.02	0.104	-0.162 (2.6%)	--	--	--	--
Echovariation	1.45 (0.19)	1.07 to 1.83	<0.001	0.606 (36.7%)	--	--	--	--
Quadriceps femoris (Model Goodness= 29.9%)								
Thickness	0.73 (0.149)	0.44 to 1.03	<0.001	0.441 (19.4%)	0.68 (0.151)	0.38 to 0.98	<0.001	0.414 (17.2%)
Echointensity	-0.1 (0.084)	-0.26 to 0.07	0.250	-0.115 (1.3%)	-0.12 (0.082)	-0.28 to 0.05	0.156	-0.143 (2%)
Echovariation	0.67 (0.235)	0.2 to 1.13	0.006	0.273 (7.4%)	0.71 (0.228)	0.25 to 1.16	0.003	0.299 (8.9%)
Tibialis anterior (Model Goodness= 57.4%)								
Thickness	0.56 (0.205)	0.15 to 0.96	0.008	0.263 (6.9%)	0.82 (0.219)	0.39 to 1.26	<0.001	0.355 (12.6%)
Echointensity	-0.13 (0.066)	-0.27 to 0	0.045	-0.199 (4.0%)	-0.16 (0.065)	-0.29 to -0.03	0.014	-0.246 (6%)
Echovariation	1.2 (0.161)	0.88 to 1.52	<0.001	0.598 (35.8%)	0.98 (0.167)	0.65 to 1.31	<0.001	0.509 (25.9%)

The regression models ALSFRS-r were adjusted for sex in biceps/brachialis group, for sex and BMI in quadriceps femoris and tibialis anterior. SE: Standard Error for coefficient B. 95% CI: 95% Confidence Interval. *Rp: partial correlation coefficient and partial determination coefficient (Rp-squared) in brackets. The independent variables were the QMUS parameters and the dependent variable was ALSFRS-r score

Figure 1

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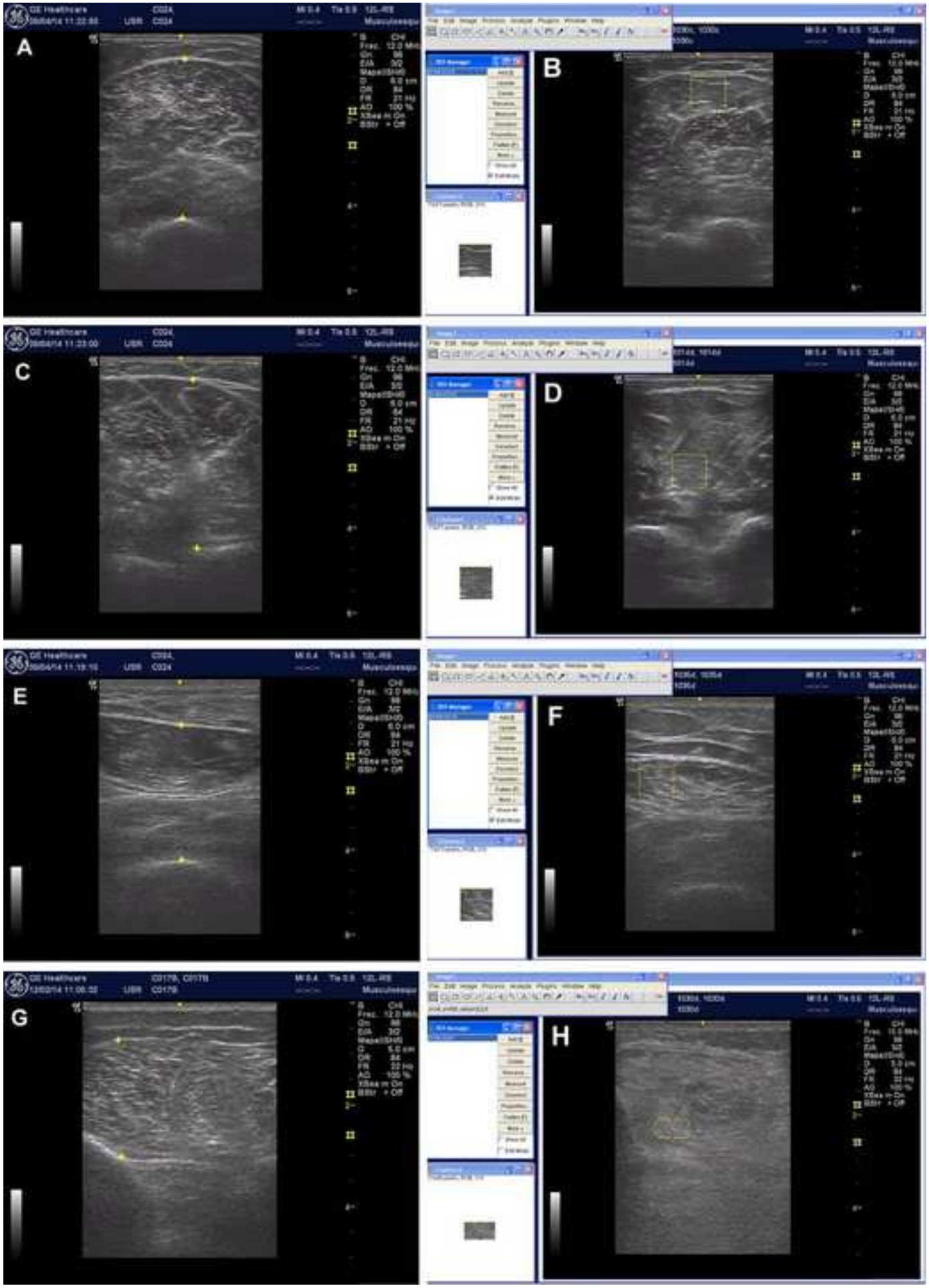

Figure 2

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