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● *Original Contribution*

MONITORING PROGRESSION OF AMYOTROPHIC LATERAL SCLEROSIS USING ULTRASOUND MORPHO-TEXTURAL MUSCLE BIOMARKERS: A PILOT STUDY

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Abstract—The need is increasing for progression biomarkers that allow the loss of motor neurons in amyotrophic lateral sclerosis (ALS) to be monitored in clinical trials. In this prospective longitudinal study, muscle thickness, echointensity, echovariation and gray level co-occurrence matrix textural features are examined as possible progression ultrasound biomarkers in ALS patients during a 5-mo follow-up period. We subjected 13 patients to 3 measurements for 20 wk. They showed a significant loss of muscle, an evident tendency to loss of thickness and increased echointensity and echovariation. In regard to textural parameters, muscle heterogeneity tended to increase as a result of the neoformation of non-contractile tissue through denervation. Considering some limitations of the study, the quantitative muscle ultrasound biomarkers evaluated showed a promising ability to monitor patients affected by ALS. (E-mail: jmartinez@ucam.edu)

Key Words: **Amyotrophic lateral sclerosis, Motor neuron disease, Neuromuscular diseases, Biomarkers, Ultrasonography, Disease progression, Image processing computer-assisted.**

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder affecting both upper motor neurons (UMNs) and lower motor neurons (LMNs), leading to gradual muscle weakness and wasting (Wijesekera and Leigh 2009). The variable degree of impairment of UMN and LMN results in pathologic and clinical heterogeneity, which hinders diagnosis, prognosis and the monitoring of progression (Kinsley and Siddique 1993). Electromyography allows LMN impairment to be detected before the onset of overt symptoms and consequently has been incorporated in successive diagnostic criteria (Brooks et al. 2000). However, despite some promising new neurophysiological techniques such as motor unit number index (MUNIX) (Neuwirth et al. 2015) and electrical impedance myography (Rutkove et al. 2012), clinical tools such as Medical Research Council (MRC) (Florence et al. 1992) and the revised ALS functional rating scale (ALSFRS-r) (Simon et al. 2014), remain the gold standard biomarkers for progression monitoring in clinical trials or clinical practice.

Muscle ultrasonography (MUS) is an accessible, painless and easy-to-perform method to detect structural muscle changes in ALS (Mayans et al. 2012). More specifically, MUS reveals marked diminished muscle thickness (MTh), increased echointensity (EI) and fasciculations (Arts et al. 2012; Martínez-Payá et al. 2017a). However, in a longitudinal study, Arts et al. (2010) observed that these ultrasound changes found in ALS patients were highly variable and did not show evident correlation with functional measures like muscle strength or disability during 6 mo of monitoring (Arts et al. 2011a).

We have previously described a new first-order ultrasound biomarker, echovariation (EV), which distinguished the muscles of ALS patients from healthy control subjects, with higher effect sizes than MTh or EI and correlating better with other clinical variables (Martínez-Payá et al. 2017a).

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Although EV is a quick and easy method to obtain information on tissue homogeneity (Aggarwal and Agrawal 2012), it does not provide information concerning the relative positions of the various grey levels within the image. This issue can be resolved by a second-order statistics feature that is based on the gray-level co-occurrence matrix (GLCM), where the pixels are considered in pairs. GLCM detects the relationship between neighboring pixel intensities and provides information about gray-level patterns (Haralick et al. 1973). Moreover, GLCM parameters showed reduced granularity in the muscles of ALS patients in comparison with control subjects and a similar discrimination capacity to EV (Martínez-Payá et al. 2017b).

However, the usefulness of the textural features of EV and GLCM as progression biomarkers has not been analyzed. Hence, we designed a prospective longitudinal study in patients with ALS to evaluate these new biomarkers and compare them with MTh and EI, during a follow-up period of 20 wk.

MATERIAL AND METHODS

Patients

Patients were recruited from the ALS Association (ADELA) of Valencia, Spain, September 2013–April 2014. We included 26 patients diagnosed with ALS, according to the revised El Escorial Criteria (Brooks et al. 2000) by an experienced neurologist (J.F.V.C.). The same cohort had been used previously to assess changes in EV and GL among patients (at recruitment) and controls (Martínez-Payá et al. 2017a, 2017b). For this longitudinal study, each patient was evaluated twice more within an interval of 10 wk ± 7 d.

Standard protocol approval, recruitment and patient consent. This study was approved by the Ethics Committee of the Universidad Católica de Murcia, Guadalupe, Spain. All participants provided written informed consent.

Recorded variables

Demographic and clinical characteristics (gender, age, weight, height, body mass index and time since diagnosis) were recorded. Muscle strength was measured using the MRC global score with a maximum value of 100 (Florence et al. 1992), as described and segmented by upper limbs, (maximum 50), lower limbs (maximum 30) and neck muscles (maximum 20). The ALSFRS-r rating scale (Cedarbaum et al. 1999) was assessed by the same investigator (J.M.P.) on the same day the MUS was performed.

Ultrasonography

MUS was performed in four muscle groups of each side by the same experienced examiner (J.M.P.), blinded for the diagnosis, with a phased array real-time scanner LOGIQe BT12 (General Electric Healthcare, Beijing, China) and a 5 – 13 MHz linear array transducer (12 L – RS) as previously published (Martínez-Payá et al. 2017a, 2017b).

Applying the standardized protocol described (Arts et al. 2011a; Martínez-Payá et al. 2017a), bilateral transverse ultrasound images of the biceps and brachialis, forearm flexors group, quadriceps femoris and tibialis anterior were obtained and measured. Three images were taken of every muscle to minimize variation in measurement parameters (Arts et al. 2008).

The resulting images had a resolution of 820 × 614 pixels (with a scale of 99.5 px/cm for tibialis anterior muscle and 83.5 px/cm for other muscles) involving 256 gray levels, and were stored as tag image file format (TIFF) files without compression or loss (Wiggins et al. 2001).

Image analysis

MTh was measured with electronic calipers as previously described (Arts et al. 2010; Martínez-Payá et al. 2017a, 2017b). This parameter was measured in all three images of each muscle group by an expert ultrasonographer (J.M.P.) and the mean of the three values was used for the corresponding analysis.

The image processing and analysis was performed by one researcher (J.R.D.), blind to diagnosis, using the ImageJ software (v.1.48 [2015], National Institutes of Health, Bethesda, MD, USA). The region of interest (ROI) was selected with the ROI Manager application for ImageJ, with a size of 71 × 40 pixels for tibialis anterior and 73 × 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 (Martínez-Payá et al. 2017a, 2017b) (Fig. 1). This ROI selection method was found to be reproducible and valid in a previous study (Martínez-Payá et al. 2017a). From the ROI we obtained EI, EV (Martínez-Payá et al. 2017a) and GLCM (Martínez-Payá et al. 2017b) textural features.

The texture analysis based on a GLCM is derived from the angular relationship between neighboring pixels (Nanni et al. 2013), among which five parameters were selected: energy or angular second moment (ASM); textural correlation (TC); homogeneity or inverse difference moment (IDM); contrast (CON) and entropy (ENT). A homogeneous image would be the result of a greater ASM, TC and IDM and a smaller CON and ENT.

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows 19.0, 2010 (IBM Corp. Armonk, NY, USA). Data were summarized by mean, standard deviations, range and 95% confidence intervals for continuous variables and absolute and relative frequencies for categorical variables. Significance was fixed at 0.05 in all the statistical tests.

The follow-up group with 3 measurements (initial, 10th wk and 20th wk) was compared with the lost to

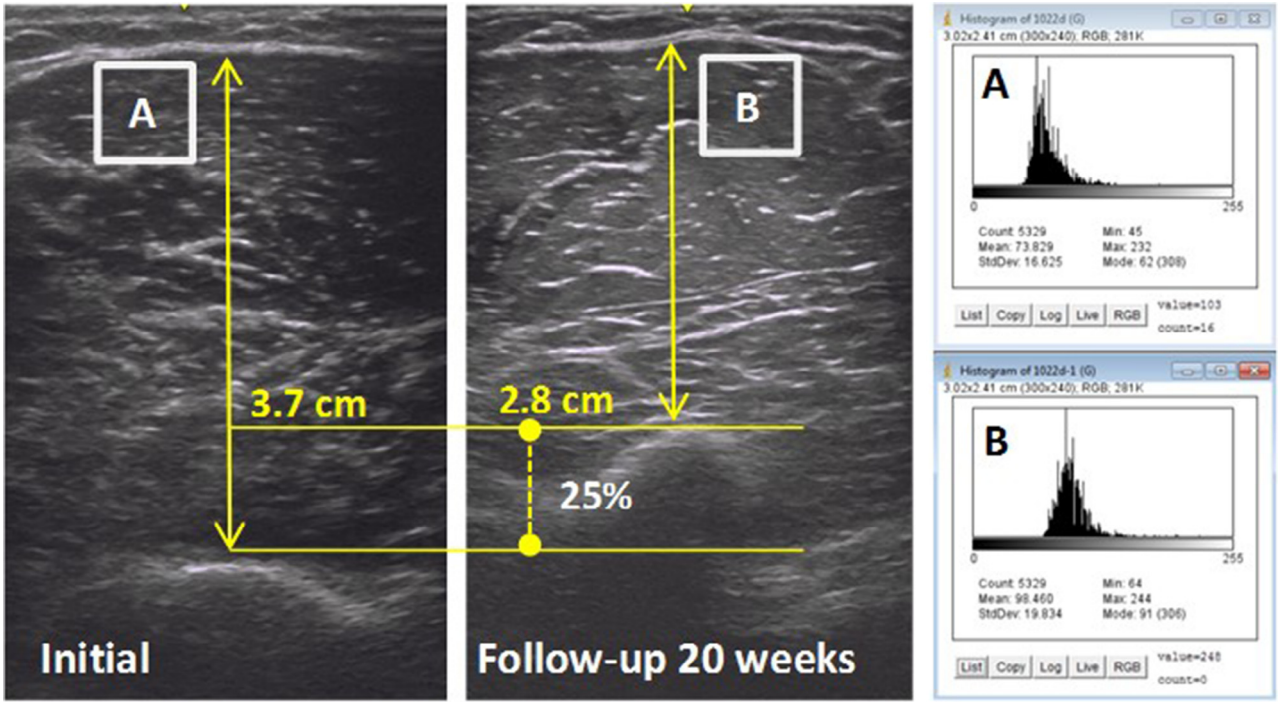

Fig. 1. Analysis of thickness and echotexture in the biceps/brachialis muscle group in ALS patients after a follow-up of 20 wk. In this patient, we observed a loss of 25% of muscle thickness and an increase of 26% of the muscle echo-intensity. ROI (a) 74 points versus ROI (b) 98 points. ALS = amyotrophic lateral sclerosis; ROI = region of interest.

follow-up group at baseline with a one-way analysis of variance (ANOVA) for continuous variables and chi-squared test for categorical variables.

All continuous variables were distributed normally and thus the ANOVA assumptions were not violated. A repeated measures ANOVA was used to compare the effect of time (three levels) on MRC, ALSFRS-r, EI, EV and the five GLCM textural features. The Mauchly test was used to evaluate the assumption of sphericity, in case it was violated; a Greenhouse-Geisser corrected test for degrees of freedom was performed. Univariate paired *post hoc* t-test (with Bonferroni correction) analyses for each dependent measure with respect to baseline were performed.

Cohen's d statistic (taking the SD at the baseline as a reference) was used to determine the effect size in pairwise comparisons (<0.1 small, 0.25 medium and >0.4 large effect size) (Kelley and Preacher 2012). To compare ultrasound parameters of various muscles, in addition to the ALSFRS-R and MRC scores for the 20th wk, the percentage change from the baseline was calculated for each parameter.

RESULTS

Patients' characteristics

Included in this study were 26 patients with ALS (8 women) and 13 patients were lost to follow-up (6 deaths and 7 because of severe disability that prevented the ultrasound study). Therefore, 13 patients were followed for 20 wk (2 women, mean age 56.3 y, SD 10.41) (Table 1).

Follow-up versus lost to follow-up cohort

The clinical and ultrasound characteristics of the first measurement were compared between patients who finished all three measurements and the patients lost to follow-up.

Variations in demographic and clinical characteristics between both cohorts can be found in Table 1. As expected, patients lost to follow-up were more disabled, had less muscle strength and a longer disease duration since diagnosis.

Quantitative muscle ultrasound biomarkers and progression monitoring in ALS

A significant decrease in muscle strength and ALSFRS-r rating scale was evident at the first and second follow-up (Table 2). The mean of the MRC global values and ALSFRS-r score had fallen by about 20% at the 20th wk (approx. 1%/wk) with a rate of change of –0.55 points/wk (maximum 100) and –0.33 points/wk (maximum 48), respectively. The decrease in MRC in lower limbs was similar in both follow-up measurements. Conversely, in upper limbs, the decrease in MRC occurred predominantly between the 10th and 20th wk (Table 2).

Table 1. Baseline differences between patients subjected to three measurements and lost patients*

Baseline characteristics	Follow-up cohort (n = 13)	Lost to follow-up cohort (n = 13)	*p* value
Women, n (%)	2 (15.4%)	6 (46.2%)	0.016
Age, y	56.3 (10.41); 52.1–60.5	60.9 (11.46); 56.3–65.6	0.137
Weight, kg	66.9 (14); 61.3–72.6	68.6 (17.22); 61.7–75.6	0.699
Height, m	1.7 (0.075); 1.67–1.73	1.62 (0.074); 1.59–1.65	0.001
BMI, kg/m^2	23 (3.6); 21.6–24.5	25.8 (4.98); 23.7–27.8	0.027
Disease onset-diagnosis, mo	14.1 (9.01); 10.5–17.8	18.5 (10.43); 14.3–22.7	0.115
Region of onset, n (%)			
Right lower limb	3 (23.1%)	6 (46.2%)	0.321
Left lower limb	3 (23.1%)	2 (15.4%)	
Right upper limb	1 (7.7%)	0 (0%)	
Left upper limb	2 (15.4%)	2 (15.4%)	
Bulbar	4 (30.8%)	3 (23.1%)	
ALFSFR-r (max 48)	29.3 (11.92); 24.534.1	23 (10.72); 18.7–27.3	0.050
MRC (max 100)	62.9 (19.72); 54.970.8	54.2 (28.66); 42.6–65.8	0.210
MRC upper limbs (max. 50)	29.6 (13.79); 24.–35.2	27.5 (18.53); 20–35	0.636
MRC lower limbs (max 30)	17.9 (5.42); 15.7–20	13 (11.03); 8.5–17.4	0.048

BMI = body mass index; ALFSFR-r = amyotrophic lateral sclerosis functional rating scale; max = maximum; MRC = Medical Research Council Scale for muscular strength.

* Data are presented as mean (SD); 95% CI. *p* value for chi-square (gender), and Student *t*-test for independent samples. *p* value for chi-square test for gender differences and Student *t*-test for independent samples for age, weight, height, and body mass index differences.

We observed consistent changes with time in the first-order MUS biomarkers (Table 2). MTh showed a trend to decrease in all muscle groups but with a low effect size (≤0.43) and rate of change (–0.4%/wk). Moreover, this decrease was not linear and occurred late (between the second and third measurements). Conversely, EI increased and showed a greater rate of change (1.1%–1.3%/wk) and effect sizes (1.22–1.49) than clinical variables, except for tibialis anterior. Moreover, changes in EI were similar in both follow-up measurements, suggesting a linear increase. EV tended to increase in all muscle groups, except biceps brachialis. However, greater heterogeneity in effect sizes and rates of change was found depending on muscle groups and the follow-up time.

Regarding GLCM textural parameters, most of them showed significant changes with time: ASM, TC and IDM decreasing and CON and ENT increasing (Table 3). Although effect sizes and rate of change of each parameter varied as per muscle group, overall CON was the parameter showing the greatest effect sizes (0.35–1.08) and rates of change (between 1.4 and 3.3%/wk).

MTh of all muscles showed similar or worse size effects in both measurements than the respective clinical variables. However, compared with ALSFRS-r, all biomarkers except IDM showed greater effect sizes in at least one muscle of the lower and upper limbs. Compared with the MRC in upper limbs, all biomarkers except EV and CON, showed better effect sizes in both upper limb muscles. Finally, in lower limbs, all biomarkers except IDM showed better effect sizes than MRC in quadriceps, but not in tibialis anterior, suggesting that muscle changes are more evident in those more preserved ones.

Differences in first-order MUS variables between cohorts were also analyzed (Supplementary Table S1, online only, available at https://doi.org/10.1016/j.ultrasmedbio.2017.09.013). We observed a lower MTh and a greater EI in most evaluated muscles in the lost to follow-up cohort. Quadriceps femoris was the muscle showing the greatest differences, whereas no relevant differences were found in forearm flexor. Greater EV in the quadriceps femoris was found in the lost to follow-up group, but no differences in EV in other muscle groups.

Analyses of GLCM textural features showed that, again, quadriceps femoris presented the greatest differences (lower ASM, TC and IDM and a greater CON and ENT in the lost to follow-up group), whereas no differences were found in the upper limb muscle groups (Supplementary Table S2, online only, available at https://doi.org/10.1016/j.ultrasmedbio.2017.09.013).

DISCUSSION

We have previously described an increased EI and reduced MTh, EV and granularity (*i.e.,* reduced muscle heterogeneity) in ALS patients compared with healthy subjects (Martínez-Payá et al. 2017a, 2017b). MTh is a measurement of muscle atrophy, which is a well-known feature of ALS. EI measures the mean pixel intensity of an ROI and has been shown to increase in ALS patients (Arts et al. 2008; Martínez-Payá et al. 2017a) probably because of the infiltration of fatty and connective tissue after neurogenic denervation (Pillen et al. 2009). EV is a parameter that quantifies the deviation of the level of gray from the average and is the result of dividing the SD by the mean pixel intensity (EI), thus providing information on tissue homogeneity (*i.e.,* greater EV reflects greater tissue heterogeneity). GLMC variables investigate the relationship between neighboring pixel intensities (Haralick et al.

Table 2. Evolution in clinical and first order statistic ultrasound variables

US parameters	Time	Mean (SD)	95% CI	Minimum	Maximum	*p* value*	Effect size†	Mean % change
Clinical variables								
MRC total	Initial	62.9 (19.72)	54.9–70.8	31.4	94.6			
(max 100)	10th wk	58.3 (23.13)	48.9–67.6	12.0	94.6	0.006	0.22	–8.2
	20th wk	51.8 (25.46)	41.5–62.0	12.0	94.6	0.001	0.56	–20.8
MRC upper limbs	Initial	29.6 (13.79)	24.1–35.2	2.0	46.7			
(max 50)	10th wk	27.5 (15.11)	21.4–33.6	0.0	46.7	0.009	0.16	–9.4
	20th wk	22.9 (16.68)	16.2–29.7	0.0	46.7	0.007	0.48	–34.7
MRC lower limbs	Initial	17.9 (5.42)	15.7–20	6.0	28.0			
(max 30)	10th wk	15.9 (7.51)	12.9–19	0.0	28.0	0.005	0.35	–15.7
	20th wk	14.5 (8.02)	11.2–17.7	0.0	28.0	<0.001	0.62	–26.5
ALSFRS–r	Initial	29.3 (11.92)	24.5–34.1	5.0	48.0			
(max 48)	10th wk	26.5 (12.53)	21.5–31.6	5.0	48.0	0.012	0.23	–11.2
	20th wk	22.7 (11.34)	18.1–27.3	5.0	46.0	<0.001	0.55	–21.3
Muscle thickness (MTh; mm)								
Biceps/brachialis	Initial	31.1 (6.13)	28.5–33.7	21.4	41.1			
	10th wk	30.1 (7.24)	27.0–33.1	10.0	42.5	0.847	0.20	–6.3
	20th wk	28.9 (5.38)	26.6–31.1	18.0	39.1	<0.001	0.40	–7.6
Forearm flexor	Initial	31.1 (7.89)	27.8–34.4	16.4	44.2			
	10th wk	31.0 (6.63)	28.2–33.8	15.6	44.2	1.000	0.05	0.4
	20th wk	27.8 (6.55)	25.0–30.6	19.2	39.8	0.177	0.43	–7.2
Quadriceps femoris	Initial	26.5 (7.84)	23.2–29.8	15.6	40.7			
	10th wk	26.4 (7.08)	23.4–29.4	10.4	41.5	1.000	0.04	–0.9
	20th wk	24.1 (7.67)	20.8–27.3	10.8	35.6	0.095	0.32	–8.5
Tibialis anterior	Initial	21.8 (5.73)	19.4–24.2	11.3	32.4			
	10th wk	19.8 (3.83)	18.2–21.4	12.4	25.4	0.318	0.32	–1.5
	20th wk	19.5 (4.04)	17.7–21.2	12.1	27.4	0.034	0.39	–7.1
Echointensity (EI; 0–255 levels)								
Biceps/brachialis	Initial	87.7 (13.72)	81.9–93.5	63.3	120.3			
	10th wk	102.4 (16.67)	95.4–109.5	76.4	143.1	0.001	0.99	17.5
	20th wk	108.0 (14.98)	101.7–114.4	88.1	151.4	<0.001	1.49	25.8
Forearm flexor	Initial	99.3 (16.49)	92.4–106.3	66.5	130.1			
	10th wk	109.2 (14.68)	103.0–115.4	82.0	142.1	0.115	0.61	12.0
	20th wk	119.0 (13.26)	113.4–124.6	94.8	140.9	<0.001	1.22	23.3
Quadriceps femoris	Initial	95.6 (15.38)	89.1–102.1	74.4	123.9			
	10th wk	103.0 (13.22)	97.4–108.6	79.6	123.2	0.069	0.53	10.3
	20th wk	114.3 (15.55)	107.7–120.9	81.5	143.8	<0.001	1.22	22.3
Tibialis anterior	Initial	110.1 (14.28)	104.1–116.1	75.1	129.8			
	10th wk	114.3 (13.25)	108.7–119.8	94.0	137.4	0.618	0.29	4.2
	20th wk	116.3 (11.36)	111.5–121.1	100.0	134.8	0.096	0.48	7.6
Echovariation (EV; 0–100 points)								
Biceps/brachialis	Initial	24.5 (7.65)	21.3–27.8	9.5	38.3			
	10th wk	21.1 (4.89)	19.0–23.2	11.0	30.5	0.089	0.42	–7.4
	20th wk	22.4 (5.47)	20.1–24.7	12.6	31.3	0.577	0.27	–0.2
Forearm flexor	Initial	19.0 (4.42)	17.1–20.9	10.6	26.5			
	10th wk	21.3 (4.11)	19.6–23.1	14.3	29.0	0.173	0.50	9.8
	20th wk	22.7 (4.02)	21.0–24.4	14.3	31.2	<0.001	0.77	21.6
Quadriceps femoris	Initial	16.9 (3.42)	15.5–18.4	11.0	24.2			
	10th wk	21.5 (5.35)	19.3–23.8	12.8	33.2	0.005	1.24	22.5
	20th wk	22.7 (4.18)	20.9–24.4	14.8	29.8	<0.001	1.61	37.7
Tibialis anterior	Initial	17.2 (3.92)	15.6–18.9	11.5	25.2			
	10th wk	19.1 (4.45)	17.2–21.0	12.3	28.1	0.181	0.54	17.1
	20th wk	18.4 (4.35)	16.5–20.2	12.8	29.1	0.447	0.40	13.9

SD = standard deviation; 95% CI = 95% confidence interval; US = ultrasound; MRC = Medical Research Council scale for muscular strength; max = maximum; ALSFRS-r = amyotrophic lateral sclerosis functional rating scale.

* The reference is the initial exploration.

† Effect size was estimated with Cohen's d Statistic (<0.1 small, 0.25 medium and >0.4 large effect size).

1973) and provide information about gray level patterns (granularity) (Gdynia et al. 2009).

Study design

In this longitudinal study, the evolution of all these quantitative MUS parameters as the disease progressed was analyzed in the same cohort of ALS patients (Martínez-Payá et al. 2017a, 2017b).

Half of the patients were lost to follow-up because of poor short-term prognosis (death or increasing disability that prevented continuing in the study). As expected, patients lost to follow-up were more disabled at recruitment. Interestingly, they

Table 3. Evolution of second order statistical ultrasound variables through gray-level co-occurrence matrix (GLCM) textural features

US parameters	Time	Mean (SD)	95% CI	Minimum	Maximum	*p* value*	Effect size†	Mean % change
Angular Second Moment								
Biceps/brachialis	Initial	19.4 (8.71)	15.8–23.1	8.2	44.0			
	10th wk	14.7 (6.84)	11.8–17.6	6.1	41.0	0.083	0.55	–11.8
	20th wk	12.8 (4.04)	11.1–14.5	6.3	21.0	0.001	0.76	–25.6
Forearm flexor	Initial	15.4 (6.57)	12.6–18.1	7.7	31.0			
	10th wk	11.1 (4.00)	9.4–12.8	5.2	21.0	0.040	0.65	–7.5
	20th wk	9.8 (2.54)	8.8–10.9	6.1	16.0	0.005	0.84	–25.6
Quadriceps femoris	Initial	18.3 (8.56)	14.7–21.9	7.2	48.0			
	10th wk	14.0 (5.77)	11.5–16.4	6.4	29.0	0.154	0.50	–5.5
	20th wk	11.4 (3.65)	9.8–12.9	5.7	18.0	0.007	0.81	–25.9
Tibialis anterior	Initial	19.4 (8.71)	15.8–23.1	8.2	44.0			
	10th wk	14.7 (6.84)	11.8–17.6	6.1	41.0	0.061	0.65	–14.4
	20th wk	12.8 (4.04)	11.1–14.5	6.3	21.0	0.018	0.65	–18.1
Contrast								
Biceps/brachialis	Initial	194.0 (99.06)	152.2–235.8	84.1	517.6			
	10th wk	229.3 (71.16)	199.3–259.4	75.3	365.9	0.087	0.36	39.9
	20th wk	228.4 (91.28)	189.9–267.0	127.0	535.9	0.540	0.35	32.8
Forearm flexor	Initial	210.3 (73.38)	179.4–241.3	87.3	335.0			
	10th wk	247.1 (77.88)	214.2–280.0	105.8	447.4	0.388	0.50	24.4
	20th wk	289.5 (92.40)	250.4–328.5	145.9	486.6	0.010	1.08	58.6
Quadriceps femoris	Initial	203.5 (84.39)	167.9–239.2	107.5	394.6			
	10th wk	227.9 (94.05)	188.2–267.6	89.4	508.3	1.000	0.29	23.2
	20th wk	288.0 (150.67)	224.4–351.6	151.9	852.8	0.076	1.00	65.6
Tibialis anterior	Initial	263.2 (100.28)	220.9–305.6	78.3	476.2			
	10th wk	304.6 (111.42)	257.5–351.6	142.3	554.0	0.100	0.41	21.8
	20th wk	312.2 (139.01)	253.5–370.9	140.8	601.4	0.192	0.49	28.3
Textural Correlation								
Biceps/brachialis	Initial	18.7 (9.1)	14.9–22.6	7.3	47.0			
	10th wk	16.0 (7.65)	12.8–19.2	6.1	38.0	0.639	0.30	1.0
	20th wk	14.2 (5.43)	11.9–16.5	5.3	26.0	0.085	0.50	–10
Forearm flexor	Initial	24.4 (10.21)	20.1–28.7	10.3	46.5			
	10th wk	16.0 (6.25)	13.3–18.6	6.3	28.0	0.056	0.59	–7.3
	20th wk	12.1 (5.54)	9.8–14.5	4.4	30.5	<0.001	0.94	–38
Quadriceps femoris	Initial	20.2 (9.31)	16.2–24.1	9.3	44.0			
	10th wk	14.2 (5.13)	12–16.3	6.5	27.5	0.005	0.90	–13.9
	20th wk	10.6 (3.28)	9.2–12	6.0	20.0	<0.001	1.31	–40.2
Tibialis anterior	Initial	17.2 (8.16)	13.7–20.6	8.0	49.0			
	10th wk	13.8 (5.21)	11.6–16.0	5.8	32.0	0.350	0.41	–9.1
	20th wk	14.0 (5.05)	11.8–16.1	6.6	24.0	0.268	0.40	–10.2
Inverse Difference Moment								
Biceps/brachialis	Initial	25.7 (5.28)	23.5–28.0	17.7	34.5			
	10th wk	22.6 (4.50)	20.7–24.5	16.2	37.5	0.005	0.60	–9.6
	20th wk	22.0 (3.72)	20.4–23.6	15.4	28.9	<0.001	0.71	–13.0
Forearm flexor	Initial	21.5 (3.97)	19.8–23.1	15.7	29.0			
	10th wk	20.3 (4.76)	18.3–22.3	15.2	32.6	1.000	0.29	2.7
	20th wk	19.5 (3.35)	18.0–20.9	14.5	25.4	0.333	0.51	–5.7
Quadriceps femoris	Initial	22.5 (4.12)	20.8–24.3	15.5	30.7			
	10th wk	22.3 (4.65)	20.3–24.2	14.0	32.3	1.000	0.06	5.6
	20th wk	21.0 (3.84)	19.3–22.6	15.7	27.0	0.567	0.38	–4.7
Tibialis anterior	Initial	22.3 (4.54)	20.4–24.3	14.6	31.4			
	10th wk	20.0 (4.33)	18.2–21.9	14.3	28.6	0.014	0.51	–7.5
	20th wk	20.0 (3.57)	18.5–21.5	14.6	25.7	0.015	0.52	–10.0
Entropy								
Biceps/brachialis	Initial	7.0 (0.41)	6.8–7.1	5.9	7.7			
	10th wk	7.1 (0.38)	7.0–7.3	6.0	7.8	0.148	0.44	2.5
	20th wk	7.2 (0.33)	7.1–7.4	6.6	7.9	0.004	0.63	3.7
Forearm flexor	Initial	7.1 (0.38)	6.9–7.2	6.3	7.6			
	10th wk	7.3 (0.34)	7.2–7.5	6.6	8.0	0.097	0.63	2.1
	20th wk	7.4 (0.24)	7.3–7.5	6.9	7.9	0.005	0.96	5.3
Quadriceps femoris	Initial	6.9 (0.40)	6.7–7.1	5.9	7.7			
	10th wk	7.2 (0.38)	7.0–7.3	6.4	7.8	0.139	0.59	2.5
	20th wk	7.3 (0.34)	7.2–7.5	6.8	7.9	0.004	1.01	6.1
Tibialis anterior	Initial	6.9 (0.27)	6.8–7	6.2	7.5			
	10th wk	7.1 (0.29)	7.0–7.2	6.3	7.5	0.026	0.78	3.0
	20th wk	7.1 (0.24)	7.0–7.2	6.7	7.5	0.016	0.71	3.4

SD = standard deviation; 95% CI = 95% confidence interval; US = ultrasound.

* The reference is the initial exploration.

† Effect size was estimated with Cohen's d Statistic (<0.1 small, 0.25 medium and >0.4 large effect size).

showed lower MTh and greater EI than the follow-up cohort. Although our study was not designed to evaluate MUS as a prognosis biomarker, these results reinforce previous findings by Arts et al. (2011b) who found that EI predicts prognosis (Arts et al. 2011b). Greater differences between groups were found in the lower limb muscles, which could be because more patients in the lost to follow-up group had a lower limb onset. No SDs between groups were found in EV and GLMC, except as regards quadriceps femoris, where the follow-up cohort showed reduced heterogeneity. However, the effect sizes of each biomarker varied considerably according to the muscle group, suggesting that differences both in the muscle characteristics and in the degree of impairment influence MUS biomarkers.

Quantitative MUS parameters as progressions biomarkers

ALS is characterized by a great heterogeneity in the disease onset and spread (Ravits and La Spada 2009), which results in a variable degree of muscle impairment, depending on the region of onset, the spread of the disease and the muscle group studied in each single region. This hinders an accurate measurement of disease progression in clinical trials. The patients followed in this longitudinal study are representative of those usually included in clinical trials, namely patients in a moderate stage of the disease with at least two body regions affected. Despite this disease-related heterogeneity and the low number of studied patients, we were able to find consistent changes as the disease progressed in all the studied MUS biomarkers. Moreover, although decreases in MTh were mild and late, a linear variation with greater effect sizes than that of clinical variables (ALSFRS-r and MRC) was observed in most first- and second-order parameters. Among all participants, EI showed the greater effect sizes in most muscles. This suggests that EI (a measure of fatty and connective tissue infiltration) is more sensitive to changes with disease progression than clinical variables. Surprisingly, Arts et al. (2011a) previously failed to find changes in EI and MTh with disease progression (Arts et al. 2011a); whereas Lee et al. (2010) found changes in MTh but not in EI. Both studies included a small number of patients and any divergences from our results are likely attributable to methodological differences.

Regarding EV, we found a gradual increase in forearm flexor and quadriceps femoris muscle groups but not in biceps or brachialis and tibialis anterior. This finding reflects an increase in tissue heterogeneity with disease progression and is in agreement with the increased muscle heterogeneity reported in qualitative visual assessment after neurogenic denervation (Pillen et al. 2008).

Similar to EV, GLMC parameters showed a progressive increase in tissue granularity and contrast with disease progression. The textural variable most sensitive to changes was CON, although, as with EV, differences were not statistically significant in biceps brachialis and tibialis anterior.

These results appear to conflict with our previous findings of reduced EV, granularity and contrast in ALS patients compared with healthy subjects (Martínez-Payá et al. 2017a, 2017b). These apparently contradictory results suggest that changes in these biomarkers (unlike EI) vary with disease progression, with an initial decrease of tissue heterogeneity and granularity, followed by a gradual increase. Increases in EI found in ALS patients have been previously interpreted as a result of fibrotic and fatty tissue formation after muscle denervation, because these tissues are more echointense than muscle (Caresio et al. 2015). However, the first muscle change during denervation, is an edema-like phase. This phase is characterized by an increase of both T1 and T2 relaxation times in muscle MRI (Bryan et al. 1998). With advanced denervation, muscle is replaced by fat and fibrotic tissue, which is characterized by a decrease in T1 relaxation time, and a further increase in the T2 relaxation time. Thus, during the progressive stages of muscle denervation, the T2 relaxation time increases continuously; whereas the T1 relaxation time increases and then decreases (Bryan et al. 1998). We believe that something similar happens with muscle biomarkers. The initial increase in EI and decrease in homogeneity and granularity are because of this edema-like phase, which explains that ALS patients show less homogeneity and granularity than controls. However, as the disease progresses and muscle tissue is progressively replaced by fat and fibrotic tissue, the formation of both non-contractile tissues would decrease the homogeneity, while the EI continues to increase. Moreover, the fatty tissue is more echointense than the fibrotic one (Walker and Cartwright 2011), which means that the degree of irregular distribution of both non-contractile tissues would affect muscle texture at a different degree in each muscle group. These phenomena would explain the diversity of changes in EV, granularity and contrast found in various muscle groups, because each might be in a different denervation stage and with a variable proportion of fatty and fibrotic tissue infiltration.

Our results reported here, together with those previously published (Martínez-Payá et al. 2017a, 2017b), suggest that EI would be the best muscle biomarker to monitor disease progression changes (at least in late disease stages), because it continuously increases throughout the disease course. However, textural parameters would discriminate better early muscle changes and would therefore perform better as diagnostic biomarkers (Martínez-Payá et al. 2017a, 2017b).

Strengths and limitations

To the best of our knowledge, our study represents the most thorough analysis to date of muscle biomarkers to assess disease progression in ALS patients. Moreover, the highly reliable methodology identified consistent changes in muscle biomarkers with disease progression,

where others did not succeed (Arts et al. 2011a; Lee et al. 2010). However, the studied sample was small, patients were in a moderate to advanced stage of the disease and follow-up was limited to 20 wk. Therefore, larger studies assessing longitudinal changes in muscle EV from diagnosis to an advanced stage are warranted to confirm our results and hypotheses and to establish the role of EI, EV and GLMC as progression and prognostic biomarkers.

CONCLUSIONS

Our pilot study suggests that, unlike previously reported, quantitative MUS parameters are feasible progression biomarkers in ALS and could be more sensitive than clinical variables for monitoring progression. In moderately disabled ALS patients, disease progression results in a decrease in MTh and an increase in EI and in muscle heterogeneity (measured with both EV and GLMC monitoring parameters). Overall, EI appears to be the most sensitive and reliable biomarker for progression in a moderate to advanced disease stage. Changes in muscle homogeneity and granularity vary according to muscle group and disease stage, which could reflect distinct denervation phases.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ultrasmedbio.2017.09.013.

REFERENCES

Aggarwal N, Agrawal RK. First and second order statistics features for classification of magnetic resonance brain images. JSIP 2012;3:146–153.

Arts IMP, Overeem S, Pillen S, Kleine BU, Boekestein WA, Zwarts MJ, Jurgen Schelhaas H. Muscle ultrasonography: A diagnostic tool for amyotrophic lateral sclerosis. Clin Neurophysiol 2012;123:1662–1667.

Arts IMP, Overeem S, Pillen S, Schelhaas HJ, Zwarts MJ. Muscle changes in amyotrophic lateral sclerosis: A longitudinal ultrasonography study. Clin Neurophysiol 2011a;122:623–628.

Arts IMP, Overeem S, Pillen S, Schelhaas HJ, Zwarts MJ. Muscle ultrasonography to predict survival in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2011b;82:552–554.

Arts IMP, Pillen S, Schelhaas HJ, Overeem S, Zwarts MJ. Normal values for quantitative muscle ultrasonography in adults. Muscle Nerve 2010; 41:32–41.

Arts IMP, van Rooij FG, Overeem S, Pillen S, Janssen HMHA, Schelhaas HJ, Zwarts MJ. Quantitative muscle ultrasonography in amyotrophic lateral sclerosis. Ultrasound Med Biol 2008;34:354–361.

Brooks BR, Miller RG, Swash M, Munsat TL. El Escorial revisited: Revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 2000;1:293–299.

Bryan WW, Reisch JS, McDonald G, Herbelin LL, Barohn RJ, Fleckenstein JL. Magnetic resonance imaging of muscle in amyotrophic lateral sclerosis. Neurology 1998;51:110–113.

Caresio C, Molinari F, Emanuel G, Minetto MA. Muscle echo intensity: Reliability and conditioning factors. Clin Physiol Funct Imaging 2015; 35:393–403.

Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B, Nakanishi A. The ALSFRS-R: A revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J Neurol Sci 1999;169:13–21.

Florence JM, Pandya S, King WM, Robison JD, Baty J, Miller JP, Schierbecker J, Signore LC. Intrarater reliability of manual muscle test (Medical Research Council scale) grades in Duchenne's muscular dystrophy. Phys Ther 1992;72:115–122.

Gdynia H-J, Müller H-P, Ludolph AC, Köninger H, Huber R. Quantitative muscle ultrasound in neuromuscular disorders using the parameters "intensity", "entropy", and "fractal dimension". Eur J Neurol 2009;16:1151–1158.

Haralick RM, Shanmugam K, Dinstein I. Textural features for image classification. IEEE Trans Syst Man Cybern 1973;3:610–621.

Kelley K, Preacher KJ. On effect size. Psychol Methods 2012;17:137–152.

Kinsley L, Siddique T. Amyotrophic lateral sclerosis overview. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJ, Bird TD, Ledbetter N, Mefford HC, Smith RJ, Stephens K, (eds). GeneReviews. Seattle: University of Washington; 1993.

Lee CD, Song Y, Peltier AC, Jarquin-Valdivia AA, Donofrio PD. Muscle ultrasound quantifies the rate of reduction of muscle thickness in amyotrophic lateral sclerosis. Muscle Nerve 2010;42:814–819.

Martínez-Payá JJ, Del Baño-Aledo ME, Ríos-Díaz J, Tembl-Ferrairó JI, Vázquez-Costa JF, Medina-Mirapeix F. Muscular echovariation: A new biomarker in amyotrophic lateral sclerosis. Ultrasound Med Biol 2017a;43:1153–1162.

Martínez-Payá JJ, Ríos-Díaz J, del Baño-Aledo ME, Tembl-Ferrairó JI, Vázquez-Costa JF, Medina-Mirapeix F. Quantitative muscle ultrasonography using textural analysis in amyotrophic lateral sclerosis. Ultrason Imaging 2017b;39:357–368.

Mayans D, Cartwright MS, Walker FO. Neuromuscular ultrasonography: Quantifying muscle and nerve measurements. Phys Med Rehabil Clin N Am 2012;23:133–148.

Nanni L, Brahnam S, Ghidoni S, Menegatti E, Barrier T. Different approaches for extracting information from the co-occurrence matrix. PLoS ONE 2013;8:e83554.

Neuwirth C, Barkhaus PE, Burkhardt C, Castro J, Czell D, de Carvalho M, Nandedkar S, Stålberg E, Weber M. Tracking motor neuron loss in a set of six muscles in amyotrophic lateral sclerosis using the Motor Unit Number Index (MUNIX): A 15-month longitudinal multicentre trial. J Neurol Neurosurg Psychiatry 2015;86:1172–1179.

Pillen S, Arts IMP, Zwarts MJ. Muscle ultrasound in neuromuscular disorders. Muscle Nerve 2008;37:679–693.

Pillen S, Tak RO, Zwarts MJ, Lammens MMY, Verrijp KN, Arts IMP, van der Laak JA, Hoogerbrugge PM, van Engelen BGM, Verrips A. Skeletal muscle ultrasound: Correlation between fibrous tissue and echo intensity. Ultrasound Med Biol 2009;35: 443–446.

Ravits JM, La Spada AR. ALS motor phenotype heterogeneity, focality, and spread: Deconstructing motor neuron degeneration. Neurology 2009;73:805–811.

Rutkove SB, Caress JB, Cartwright MS, Burns TM, Warder J, David WS, Goyal N, Maragakis NJ, Clawson L, Benatar M, Usher S, Sharma KR, Gautam S, Narayanaswami P, Raynor EM, Watson ML, Shefner JM. Electrical impedance myography as a biomarker to assess ALS progression. Amyotroph Lateral Scler 2012;13:439–445.

Simon NG, Turner MR, Vucic S, Al-Chalabi A, Shefner J, Lomen-Hoerth C, Kiernan MC. Quantifying disease progression in amyotrophic lateral sclerosis. Ann Neurol 2014;76:643–657.

Walker F, Cartwright M. Neuromuscular ultrasound. Madrid: Elsevier; 2011.

Wiggins RH, 3rd, Davidson HC, Harnsberger HR, Lauman JR, Goede PA. Image file formats: Past, present, and future. Radiographics 2001; 21:789–798.

Wijesekera LC, Leigh PN. Amyotrophic lateral sclerosis. Orphanet J Rare Dis 2009;4:3.