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● *Original Contribution*

SONOELASTOGRAPHY FOR THE ASSESSMENT OF MUSCLE CHANGES IN AMYOTROPHIC LATERAL SCLEROSIS: RESULTS OF A PILOT STUDY

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Abstract—The purpose of this study was to assess the sonoelastographic features of four different muscles in patients with amyotrophic lateral sclerosis compared with healthy controls and to evaluate the relationship of these features to muscle strength and other ultrasonographic variables. Fourteen patients with amyotrophic lateral sclerosis and 20 controls were examined using strain sonoelastography scanning. The RGB channel fraction ratio was analyzed with ImageJ software (Version 1.48). Two main sonoelastographic patterns could be distinguished in the controls: a clear predominance of the blue channel (hard areas) and a more heterogeneous pattern with predominance of the green channel (intermediate stiffness). These patterns were also observed in patients, although a higher green channel score was observed in mildly impaired muscles, whereas a higher blue channel score was observed in the most severely impaired muscle. Sonoelastography may be a good complementary biomarker in the detection and monitoring of muscle changes in amyotrophic lateral sclerosis. (E-mail addresses: jrios@nebrija.es, jriosmetinv@gmail.com)

Key Words: **Amyotrophic lateral sclerosis, Atrophy, Fasciculations, Peripheral nervous system diseases, Biomarkers, Elasticity imaging techniques, Sonoelastography, Motor neuron disease, Neuromuscular diseases, Ultrasonography.**

INTRODUCTION

B-Mode ultrasonography (US) allows the measurement of thickness, echo-intensity (EI), homogeneity and textural properties of tissues. These biomarkers applied to muscular tissue have been seen to be useful for the diagnosis, prognosis and progression monitoring of amyotrophic lateral sclerosis (ALS) (Arts et al. 2011a; Martínez-Payá et al. 2017a, 2017b, 2017c).

Strain elastography (EL), a complementary technique to conventional US, analyzes the mechanical properties of the examined tissue (Paluch et al. 2016). EL, which is based on Hooke's law for the calculation of Young's elastic modulus, estimates the stiffness of a given tissue (Carlsen et al. 2013; Drakonaki et al. 2012). Strain EL is applied through the transducer by repetitive manual pressure and the displacement is calculated from the return velocities of the tissues with respect to time. Given the same amount of applied stress, softer tissue in the elastogram has more deformation and therefore experiences greater strain than stiffer tissue (Winn et al. 2016). This displacement is then converted into a color-coded strain distribution map ("the elastogram"), which is superimposed over the conventional B-mode image or displayed next to it (Klauser et al. 2014). EL has previously been used in the evaluation of damaged muscles in some musculoskeletal diseases, which appear harder in patients with muscular dystrophy, myositis or children with cerebral palsy (Klauser et al. 2014; Ríos-Díaz et al. 2015).

Denervation caused by ALS causes fibrotic and adipose tissue infiltration (Arts et al. 2011a), which can lead to changes in muscle stiffness. However, this parameter has not been studied in ALS patients to date.

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Therefore, we aimed to study the differences in muscle stiffness as measured by EL in ALS patients versus healthy controls and compare the results with differences in muscle atrophy.

METHODS

Patients

Patients with ALS were recruited from the Department of Neurology at Hospital Universitario y Politécnico La Fe (Valencia, Spain). Patients were diagnosed with ALS by an experienced neurologist (J.F.V.C.).

Healthy volunteers without neuromuscular diseases or family history of the same were recruited as the control group.

Standard protocol approval, recruitment and patient consent

This study was approved by the ethics committees of the Universidad Católica de Murcia (Guadalupe, Spain) and Hospital Universitario y Politécnico La Fe (Valencia, Spain). All participants provided written informed consent.

Recorded variables

Demographic and clinical characteristics (sex, age, weight, height, body mass index (BMI), time from diagnosis and site of disease onset) were recorded. Muscle strength was measured using the Medical Research Council (MRC) rating scale (ranging from 0 to 5) in a total of 26 muscles throughout the body (including US-examined muscles), and a global score with a maximum of 130 points was calculated (Florence et al. 1992). The ALSFRS-r (a revised ALS Functional Rating Scale) global score (Cedarbaum et al. 1999) was assessed by the same investigator (J.F.V.C.) on the same day the ultrasound tests were performed.

Ultrasonography

Strain EL was performed in four muscle groups bilaterally in patients and controls by the same experienced examiner (J.M.P.) (Martínez-Payá et al. 2017a, 2017c; Ríos-Díaz et al. 2015) with the GE Logiq S8 (General Electric Healthcare, Beijing, China) ultrasound machine and a 4−15 MHz linear array transducer (ML6-15). All B-mode and sonoelastographic system-setting parameters, such as gain (41 dB), time gain compensation (in neutral position), depth (6 cm), frequency (15 MHz), pulse repetition frequency (0.10), compression and focus were kept constant throughout the study.

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

Elastography recordings were acquired by applying gentle compression with the transducer using a freehand technique. Pressure was adjusted according to the visual indicator for compression on the video screen, and a quality factor $\geq$60 indicated the optimal compression force (Wu et al. 2011). To avoid pitfalls, the explorations were perpendicular to the objectives because the non-linear compression force could misread the stiffness (McNally 2011). According to the recommendations of Klauser et al. (2014) three compression−relaxation cycles were carried out and the best fit B-mode−elastogram image pairs were selected for evaluation.

Finally, an elastogram superimposed over the convectional B-mode image was obtained with the following spectrum of colors: blue representing hard areas, red representing soft areas and green representing areas of intermediate stiffness. The resulting images had a resolution of 1552 × 970 pixels with 256 gray levels and were stored as .TIFF files without compression or losses.

Image analysis

Strain EL included assessment of fasciculations and muscle thickness (MTh), using previously described methodology (Martínez-Payá et al. 2017a).

ImageJ 1.48 software (National Institutes of Health, USA) was used to make a quantitative analysis of the color histogram of the elastogram. For this analysis, a researcher (J.R.D.), who was blind to the diagnosis, selected a region of interest (ROI) visible on the EL screen that included the largest muscle mass within the epimysium without subcutaneous tissue or cortical bone (Fig. 1).

RGB channel fraction ratios (FRs) were analyzed in each selected ROI. The fraction ratio is the percentage representation of each color extension, in the form RGB (R%, G%, B%), where R, G and B are the fraction values for the red, green and blue values of the color, respectively, ranging from 0 to 100. RGB channel FRs were obtained using the "color threshold" plugin (Fig. 1).

The FR and MTh were measured in all three images of each muscle group, and the mean of the three values was used for the corresponding analysis.

Statistical analysis

An Excel (Microsoft Office 365 ProPlus 2016) database was created to store and debug data. Statistical analyses were carried out using the R software (Version 3.4.3), clickR (Version 0.3.43) and glmmADMB (Version 0.8.3.3) packages.

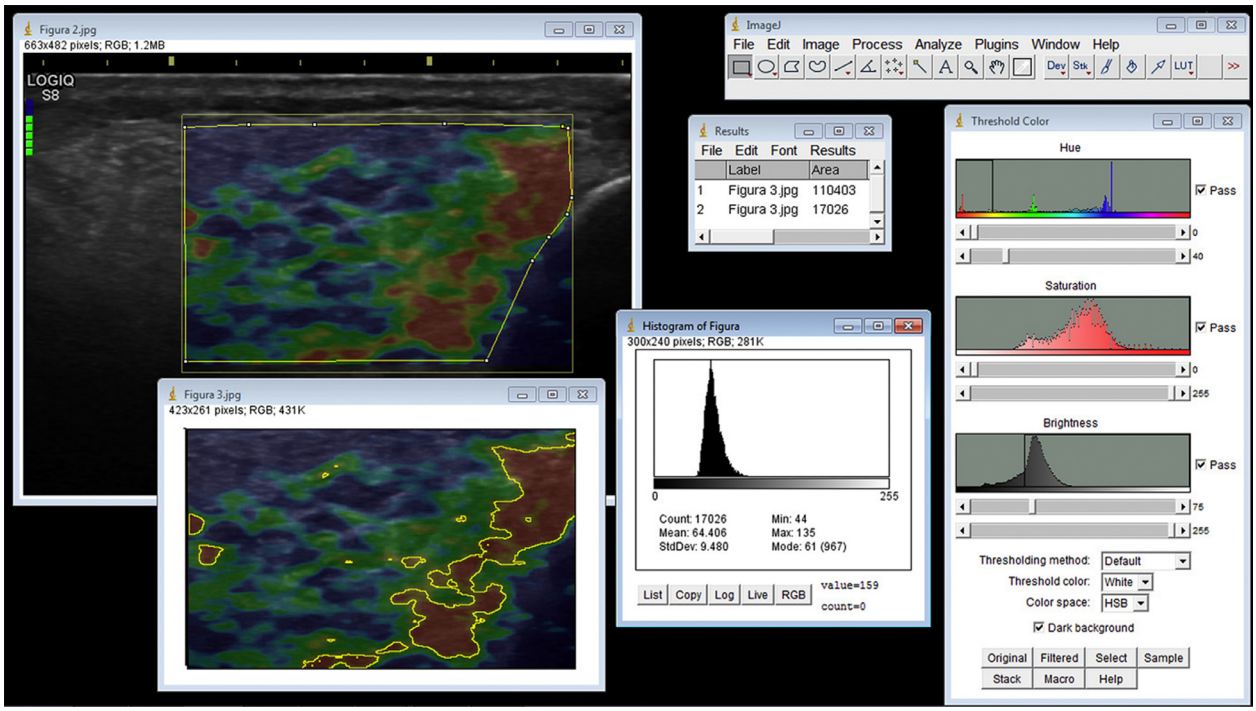

Fig. 1. Quantitative analysis of FRs of RGB channels with ImageJ (Version 1.48) software in elastography of tibialis anterior muscle affected by amyotrophic lateral sclerosis. FRs = fraction ratios.

Data were normally distributed in each group, as confirmed by $Q-Q$ plots, kurtosis and skewness coefficients and the Kolmogorov–Smirnov test. These were summarized as means and standard deviations (SD) and ranges for continuous variables and as absolute and relative frequencies for categorical variables.

One-way analysis of variance (ANOVA) was used to compare age, weight, height and BMI at baseline between ALS patients and controls. Analyses of covariance (ANCOVAs) adjusted for sex, age and BMI were used to compare thickness, EI and RGB FR between patients and controls. Levene's test was used to check the homogeneity of variances. Repeated ANOVAs were carried out segmented for the group to compare RGB channel FRs within and between muscles. The sphericity of variances was checked with Mauchly' test; in the absence of sphericity, degrees of freedom were corrected with Greenhouse's method. For multiple comparisons, the level of significance was corrected with the Bonferroni–Dunn method. For all comparisons, mean differences (Δ) and confidence intervals, the control group was taken as reference.

An adjusted t-test was used to calculate Hedges' g statistic, which is less biased than Cohen's d statistic for effect sizes. A Hedges' g statistic <0.12 corresponds to a small effect size, a $g \sim 0.3$ to a medium effect size and a $g > 0.5$ a large size effect. Additionally, relative change was calculated as 100(FR_ALS/FR_Control) *100.

To assess the association of the FR (using the green channel as a reference) with the predictive variables age, sex, BMI, MTh and MRC score, a beta regression model was performed for each muscle. Owing to the nature of the study, the variable "side of the body" nested to "patient" was included as a random effect factor to correct for the non-independent data. Given that the MRC score would affect the FR distribution only in ALS patients (all controls were considered to have an MRC score of 5), the interaction between diagnosis, MRC score and colors was considered for this item.

A p value < 0.05 was considered to indicate statistical significance.

RESULTS

Patients

Fourteen ALS patients (mean age: 59.3 y, SD: 10.09) and 20 healthy controls (mean age: 55.8 y, SD: 6.14) were included in this study. Patients had a lower BMI than controls, whereas there were no differences in

sex, age or height between groups. Demographic and clinical characteristics are summarized in Table 1.

There were no significant right−left differences in MTh and FR in the four muscle groups studied, so only one sample of each right/left muscle group was selected for further analysis (40 ultrasonograms for healthy controls and 28 ultrasonograms for ALS patients).

Muscle strength and thickness

Both muscle strength (on the MRC scale) and thickness were lower in ALS patients than in the controls, but differences were not statistically significant for all muscles (Table 2). Overall, quadriceps was the muscle group with the greatest atrophy, whereas the tibialis anterior was the weakest muscle.

Table 1. Characteristics of the study sample, clinical variables and fasciculations

Baseline characteristics	Healthy Controls (n=20)	ALS Patients (n=14)	p-value
Women (n) (%)	11 (55%)	6 (42.9%)	0.324
Age (yr)	59.3 (10.09); 55.4 to 63.2	55.8 (6.14); 53.9 to 57.8	0.085
Weight (kg)	75.1 (11.43); 70.7 to 79.6	69.7 (9.78); 66.6 to 72.8	0.039
Height (m)	1.67 (0.123); 1.62 to 1.71	1.68 (0.087); 1.65 to 1.71	0.525
BMI (kg/m^2)	27.2 (4.41); 25.5 to 28.9	24.6 (2.23); 23.9 to 25.3	0.002
Time from diagnosis (months)		6.5 (3.52); 5.1 to 7.9	
Disease onset (n) (%)			
Lower Limbs		9 (64.3%)	
Upper Limbs		3 (21.4%)	
Bulbar		2 (14.3%)	
Fasciculations both sides (n) (%)		11 out of 14 (78.6%)	
− Biceps brachialis (n=28)		20 (71.4%)	
− Forearm flexors (n=28)		17 (60.7%)	
− Quadriceps femoris (n=28)		16 (57.1%)	
− Tibialis anterior (n=28)		12 (42.9%)	
ALFSFR-r (max 48)		36.6 (5.84); 34.4 to 38.9	
MRC (max 130)		111.5 (16.04); 105.3 to 117.7	

Table 2. Muscle strength (MRC), muscle thickness (MTh) and EL fraction ratio (FR) in healthy and ALS patients group. FR, fraction ratio; S.D., standard deviation. Effect size was estimated with Hedges' *g* Statistic. All data were adjusted for sex, age and, body mass index

Ultrasound parameters	Healthy Controls (n=40) Mean (SD)	Range	ALS Patients (n=28) Mean (SD)	Range	Mean Difference Delta (95%CI)	Relative change Patients/Controls (%)	p-value	Effect size
Biceps brachialis								
MRC (elbow flexion)	5.0 (0.31)	–	4.7 (0.31)	3.7 to 5.0	−0.36 (−0.52 to -0.2)	−7.8%	<0.001	0.96
MTh (mm)	24.8 (4.0)	15.5 to 34.9	24.5 (4.06)	12.5 to 37.9	−0.35 (−2.41 to 1.72)	−1.4%	0.738	0.07
Red FR (%)	23.1 (4.82)	14.6 to 34.7	22.1 (4.90)	11.9 to 36.1	−0.92 (−3.41 to 1.57)	−4.2%	0.463	0.20
Green FR (%)	41.6 (5.71)	33.9 to 55.6	43.4 (5.79)	32.2 to 52.2	1.88 (−1.06 to 4.83)	4.3%	0.206	0.31
Blue FR (%)	35.4 (7.41)	21.8 to 48.8	34.6 (7.52)	17.7 to 50.4	−0.8 (−4.62 to 3.03	−2.3%	0.679	0.11
Forearm flexors group								
MRC (palmar flexion wrist)	5.1 (0.49)	–	4.4 (0.50)	3.0 to 5.0	−0.69 (−0.95 to −0.44)	−15.8%	<0.001	1.40
MTh (mm)	29.5 (4.82)	21.1 to 39.6	27.2 (4.89)	17.2 to 39.6	−2.27 (−4.76 to 0.22)	−8.3%	0.073	0.72
Red FR (%)	17.6 (2.33)	12.3 to 21.5	18.3 (2.36)	7.6 to 21.5	0.76 (−0.45 to 1.96)	4.1%	0.213	0.30
Green FR (%)	32.4 (3.76)	26.3 to 39.8	33.6 (3.82)	26.4 to 41.2	1.18 (−0.76 to 3.12)	3.5%	0.230	0.31
Blue FR (%)	50.1 (5.05)	40.3 to 61.4	48.2 (5.12)	38.0 to 62.6	−1.90 (−4.5 to 0.71)	−3.9%	0.150	0.37
Quadriceps femoris								
MRC (knee extension)	5.0 (0.67)	-	4.6 (0.68)	2.0 to 5.0	−0.38 (−0.72 to −0.03)	−8.2%	0.034	0.59
MTh (mm)	26.9 (5.1)	19.6 to 38.7	22.9 (5.17)	6.9 to 37.7	−3.92 (−6.55 to −1.29)	−17.1%	0.004	0.77
Red FR (%)	19.3 (3.49)	13.2 to 26.2	19.7 (3.54)	15.1 to 34.8	0.48 (−1.32 to 2.28)	2.4%	0.599	0.11
Green FR (%)	32.9 (4.94)	26.2 to 44	35.4 (5.01)	21.6 to 49.7	2.55 (0 to 5.1)	7.2%	0.050	0.50
Blue FR (%)	48.0 (7.21)	35 to 60.3	45.0 (7.32)	15.5 to 55.6	−2.99 (−6.71 to 0.73)	−6.7%	0.113	0.41
Tibialis anterior								
MRC (dorsal flexion ankle)	5.0 (1.11)	–	3.8 (1.12)	0 to 5.0	−1.2 (−1.77 to −0.63)	−31.9%	<0.001	1.06
MTh (mm)	22.4 (3.40)	13.3 to 29.3	21.6 (3.45)	6.9 to 26.9	−0.87 (−2.62 to 0.89)	−4.0%	0.328	0.23
Red FR (%)	11.2 (7.24)	0.07 to 27.7	5.7 (7.35)	0.2 to 17.7	−5.52 (−9.26 to −1.78)	−96.6%	0.004	0.75
Green FR (%)	29.2 (8.52)	12.9 to 53	21.6 (8.65)	7.3 to 38.5	−7.63 (−12.03 to −3.23)	−35.4%	0.001	0.88
Blue FR (%)	59.7 (13.12)	35.2 to 87	72.9 (13.31)	53.5 to 91	13.2 (6.42 to 19.97)	18.1%	<0.001	0.99

Muscle sonoelastographic characteristics in healthy controls and ALS patients

Two main patterns of muscle EL could be distinguished in healthy controls. First, in the forearm flexor group, quadriceps femoris and tibialis anterior, there was a significant predominance of the blue channel, followed by the green and red channels. These differences were greatest for the tibialis anterior. The second pattern was much more heterogeneous, with a slight but significant predominance of the green channel in the biceps brachialis (Fig. 2).

The same two muscle patterns were found in ALS patients, although a much more homogeneous pattern with significant predominance of the blue channel was found in the tibialis anterior compared with the quadriceps and forearm flexor (Fig. 2).

EL FR differences between ALS patients and controls

In the tibialis anterior, ALS patients had significantly lower FRs in the red and green channels and significantly higher FRs in the blue channel. These differences had higher effect sizes than those of MTh (Table 2). In the quadriceps femoris, ALS patients exhibited an increase in the green channel. However, in the upper limb muscles, there were no statistically significant differences in FRs (Table 2).

Association of demographic and clinical variables with EL characteristics

The multivariable models exhibited no association between age, sex, BMI and MTh, on the one hand, and FR in the green channel, on the other hand, in any muscle in either patients or controls (Table 3). However, the blue channel tended to decrease as weakness increased in the biceps brachialis and tibialis anterior of ALS patients (Table 3 and Fig. 3). Conversely, the green channel increased with increasing muscle weakness, although this association was statistically significant only in the biceps brachialis (Table 3).

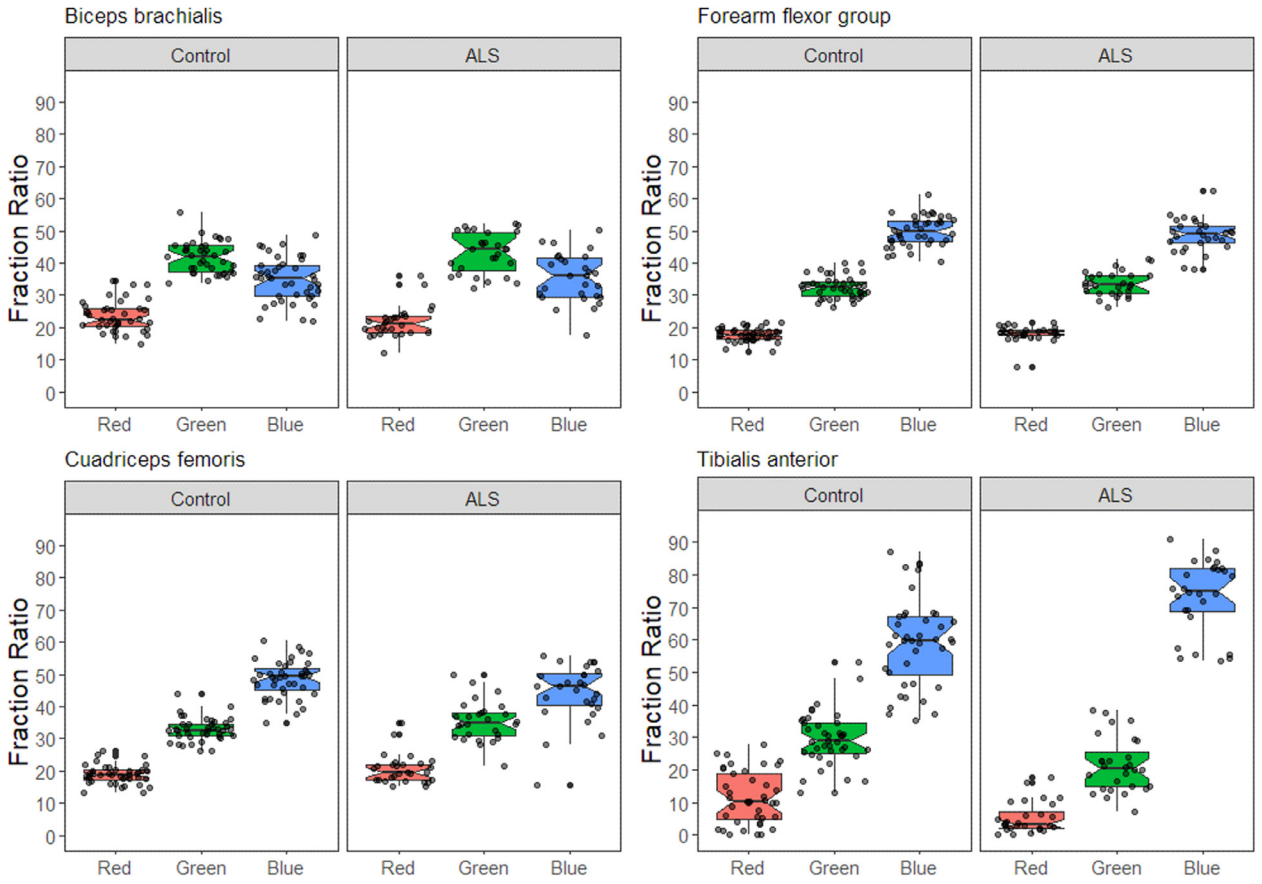

Fig. 2. Plot representing the distribution of FRs of RGB channels in each muscle group. The *box* represents the median and first and third quartiles. If the notches of two plots do not overlap, this is strong evidence that the two medians differ. The *dots* represent the individual values. The scales of the graphs are the same to improve comparisons. ALS = amyotrophic lateral sclerosis; FRs = fraction ratios.

Table 3. Associations between the FR (using FR green as a reference) and demographic and clinical variables as per muscle group. Statistically significant differences are highlighted in bold. BMI, body mass index; E, exponential estimate; FR, fraction ratio; MTh, muscle thickness; MRC_{ALS}:FR, interaction between muscle strength in ALS patients (as measured with the MRC scale) and the FR (green or blue)

	Biceps brachialis				Forearm flexors group				Quadriceps femoris				Tibialis anterior			
	E	**Lower 95%**	**Upper 95%**	**p**	**E**	**Lower 95%**	**Upper 95%**	**p**	**E**	**Lower 95%**	**Upper 95%**	**p**	**E**	**Lower 95%**	**Upper 95%**	**p**
FR blue	**0.742**	**0.661**	**0.832**	**<0.001**	**2.057**	**1.904**	**2.222**	**<0.001**	**1.908**	**1.706**	**2.134**	**<0.001**	**3.531**	**2.869**	**4.346**	**<0.001**
Control	**2.752**	**1.047**	**7.237**	**0.04**	1.328	0.882	2	0.175	1.261	0.776	2.048	0.349	0.877	0.534	1.44	0.603
Age	1.002	0.995	1.008	0.601	1	0.996	1.004	0.999	1.003	0.997	1.01	0.328	0.999	0.987	1.011	0.833
Sex (male)	1.013	0.883	1.163	0.849	0.984	0.918	1.055	0.659	0.933	0.844	1.031	0.173	1.076	0.892	1.298	0.443
BMI	1.002	0.985	1.018	0.845	1.004	0.994	1.014	0.418	0.991	0.977	1.005	0.215	1.001	0.971	1.033	0.94
MTh	1.005	0.992	1.018	0.474	1.002	0.995	1.009	0.624	1.008	0.999	1.018	0.086	0.985	0.959	1.011	0.25
MRC_{ALS}:FR green	**0.812**	**0.661**	**0.997**	**0.046**	0.946	0.864	1.035	0.224	0.986	0.892	1.089	0.777	0.919	0.818	1.032	0.151
MRC_{ALS}:FR blue	**1.385**	**1.055**	**1.817**	**0.019**	1.081	0.966	1.211	0.176	1.039	0.908	1.188	0.577	**1.213**	**1.036**	**1.42**	**0.016**

DISCUSSION

Amyotrophic lateral sclerosis is a fatal neurodegenerative disease, for which there is an urgent need for new diagnostic and progression biomarkers for use in both clinical practice and clinical trials. In ALS, because of muscle denervation, the muscles progressively atrophy. Nevertheless, MTh is neither sensitive nor specific as a diagnostic biomarker (Arts et al. 2012), and its usefulness as a progression biomarker is also limited (Martínez-Payá et al. 2017c).

Several first- and second-order muscle US biomarkers have been described to date (Arts et al. 2011a; Martínez-Payá et al. 2017a, 2017b, 2017c). Although their exact significance remains unknown, because they reflect different properties of the muscle, it is likely that each plays an important role in different stages of the disease or for different purposes (Arts et al. 2011a; Martínez-Payá et al. 2017a, 2017c).

Elastography can measure the mechanical properties of soft tissue both qualitatively and quantitatively. Qualitative studies include the use of equations, scoring systems or gradation (De Zordo et al. 2010; Ríos-Díaz et al. 2015; Sconfienza et al. 2013). Other semiquantitave measurements use the built-in software (strain ratio), where the average strain of a ROI within the lesion is compared with that of surrounding fat tissue (Aşkın et al. 2017; Mutala et al. 2016). Finally, the use of post-processing software packages (Paluch et al. 2016) has allowed quantitative analysis of the RGB channel values included in each pixel of a given ROI (Botar-Jid et al. 2010; Ríos-Díaz et al. 2015). Each pixel within a ROI was classified into red, green and blue color channels to compute the number of pixels of each color channel with respect to the whole ROI. This analysis results in a quantitative percentage value for each channel, which is easier to interpret clinically and is possibly more reproducible than other qualitative methods or quantitative analysis of the mean echo-intensity for each color (Magarelli et al. 2014; Ríos-Díaz et al. 2015).

Elastograms in healthy controls reveal that normal muscle at rest is heterogeneous (a mosaic of colors) and characterized by intermediate or somewhat increased stiffness (predominance of green or blue channel, respectively) with scattered softer and harder areas, especially at the periphery near boundaries (Paluch et al. 2016). The FR distribution in each muscle can provide information on the tissue composition of this muscle but can also reflect the elasticity. In our study, the biceps brachialis was the muscle with the greatest elasticity (higher proportions of green and red channels) in both patients and controls, whereas the other muscles exhibited a clear predominance of the blue channel, the tibialis anterior being the least elastic. Previous studies have studied different muscles and used a variety of experimental setups and methods of data processing

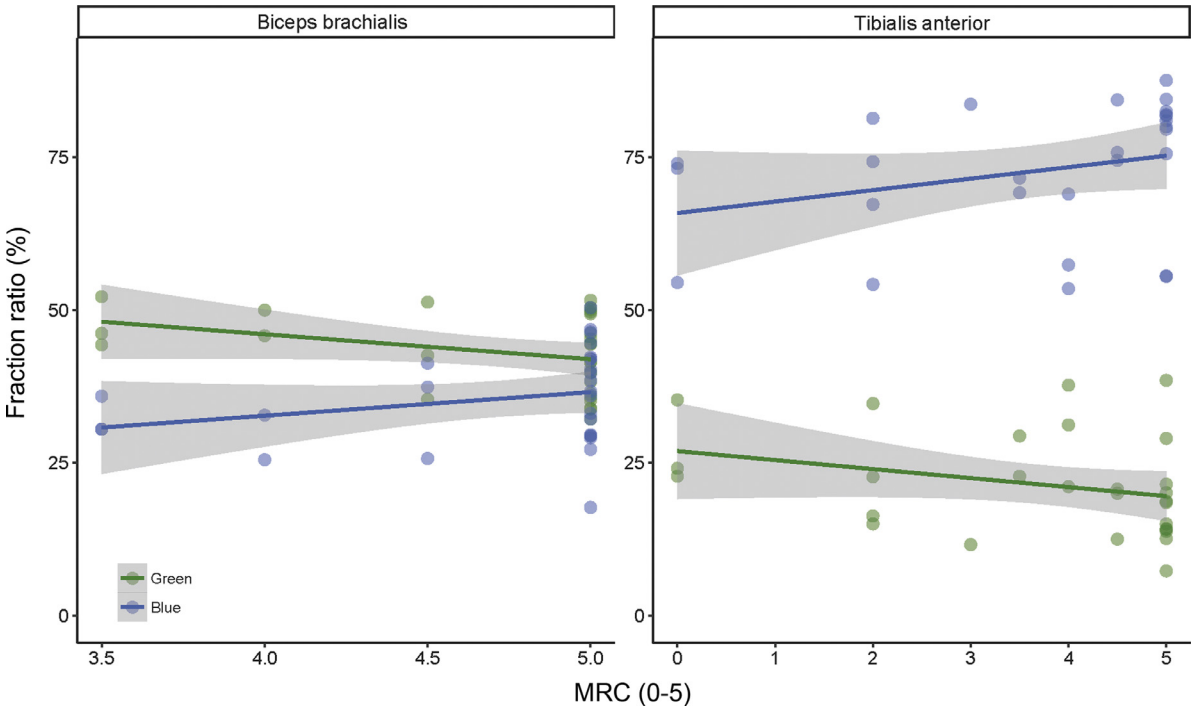

Fig. 3. Representation of the changes in the FRs of green and blue channels with decreasing muscle weakness in ALS patients. ALS = amyotrophic lateral sclerosis; FRs = fraction ratios; MRC = Medical Research Council score.

(Chakouch et al. 2016), which hinders any direct comparison of the results.

Although EL has been used infrequently to study neuromuscular diseases (Klauser et al. 2014), changes in muscle composition with the disease, such as fibrotic and adipose tissue infiltration, may lead to changes in muscle stiffness.

Muscle changes in ALS are a result of muscle denervation. Acute denervation results in edema-like changes and atrophy, which are followed by fibrous and fatty tissue infiltration (Martínez-Payá et al. 2017c). This infiltration could therefore affect the rigidity of muscles: Rigidity increases by fibrous tissue infiltration and decreases by fatty tissue infiltration. We therefore hypothesized that EL would detect differences in ALS patients and healthy control muscles. Such differences were observed for the quadriceps femoris and tibialis anterior, which is not surprising given that the disease started in the lower limbs in most patients. Interestingly, whereas the tibialis anterior exhibited an increase in hard areas (blue channel), there was an increase in intermediate stiffness areas (green channel) in the quadriceps femoris. The former finding is probably the result of advanced denervation, accompanied by fibrous tissue infiltration, because the tibialis anterior exhibited both atrophy and reduced muscle strength. The latter finding could be the result of an early-moderate denervation stage, in which a reduction in muscle fibers is already evident but fibrous replacement is still incipient, as reflected by the reduced MTh but almost normal strength of this muscle in ALS patients. Unlike EL, which reveals greater changes in muscles in more advanced clinical stages (based on the degree of muscle strength impairment), MTh does not allow this differentiation and even reveals greater effect sizes in quadriceps femoris than in tibialis anterior, despite the former being affected at an earlier clinical stage. Based on these results, we think that EL could be a better biomarker of progression than MTh in muscles in a moderate to advanced disease stage. However, overall, muscle strength indicated higher effect sizes than EL when ALS patients were compared with controls.

No associations between age, sex, BMI and MTh and the FR distribution were detected in patients and controls. However, loss of muscle strength was associated with a decrease in the blue channel in biceps brachialis and tibialis anterior in ALS patients. This result is intriguing, because patients had a higher blue channel score than controls for the tibialis anterior. Overall, this suggests that although EL can monitor muscle elasticity changes as the disease progresses, such changes are not lineal throughout the disease course, as previously suggested by other US biomarkers (Martínez-Payá et al. 2017c).

Strengths and limitations

There are several limitations to the study. First, EL is considered operator dependent, requires a specific level of learning and poses technical problems with the reproduction of images because of the instability of the pressure applied using the freehand technique. To decrease the effect of this problem, the same experienced examiner performed all EL examinations. We also used a quality factor $\geq$60 to indicate the optimal compression force. Second, the number of patients involved was relatively small, and there was relative heterogeneity concerning the region of disease onset, which could result in muscles being in different denervation stages. However, patients were quite homogeneous with respect to disease stage as represented by degree of disability and time from diagnosis. Third, quantitative EL requires post-processing, which cannot be directly implemented during clinical practice. However, the analysis is easy to perform and provides a more accurate measurement of the color distribution than visual grading, improving its performance as a progression biomarker for clinical trials.

CONCLUSIONS

Our pilot study confirms the occurrence of changes in muscle stiffness in ALS patients caused by denervation and suggests that EL could serve as a progression biomarker to monitor changes in moderate to advanced muscle denervation stages. Larger longitudinal studies are needed to confirm this hypothesis.

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