Article

Quantitative Muscle Ultrasonography Using Textural Analysis in Amyotrophic Lateral Sclerosis

Ultrasonic Imaging
2017, Vol. 39(6) 357–368

DOI: 10.1177/0161734617711370
journals.sagepub.com/home/uix
SAGE

Jacinto Javier Martínez-Payá[1,*], José Ríos-Díaz[2,3,*], María Elena del Baño-Aledo[4,*], Jose Ignacio Tembl-Ferrairó[5], Juan Francisco Vazquez-Costa[5,6,7,*], and Francesc Medina-Mirapeix[4]

Abstract

The purpose of this study was to analyze differences in gray-level co-occurrence matrix (GLCM) parameters, as assessed by muscle ultrasound (MUS), between amyotrophic lateral sclerosis (ALS) patients and healthy controls, and to compare the diagnostic accuracy of these GLCM parameters with first-order MUS parameters (echointensity [EI], echovariation [EV], and muscle thickness [MTh]) in different muscle groups. Twenty-six patients with ALS and 26 healthy subjects underwent bilateral and transverse ultrasound of the biceps/brachialis, forearm flexor, quadriceps femoris, and tibialis anterior muscle groups. MTh was measured with electronic calipers, and EI, EV, and GLCM were obtained using Image J (v.1.48) software. Sensitivity, specificity, likelihood ratios, and area under the curve (AUC) were performed by logistic regression models and receiver operating characteristic curves. GLCM parameters showed reduced granularity in the muscles of ALS patients compared with the controls. Regarding the discrimination capacity, the best single diagnostic parameter in forearm flexors and quadriceps was GLCM and in biceps brachialis and tibialis anterior was EV. The respective combination of these two parameters with MTh resulted in the best AUC (over 90% in all muscle groups and close to the maximum combination model). The use of new textural parameters (EV and GLCM) combined with usual quantitative MUS variables is a promising biomarker in ALS.

[1]Faculty of Health Sciences, Universidad Católica de Murcia, Murcia, Spain
[2]Centro de Ciencias de la Salud San Rafael, Universidad Antonio de Nebrija, Madrid, Spain
[3]Fundación San Juan de Dios, Madrid, Spain
[4]Department of Physiotherapy, Facultad de Medicina, Universidad de Murcia, Espinardo, Murcia, Spain
[5]Department of Neurology, Hospital Universitario y Politécnico La Fe, Valencia, Spain
[6]Neuromuscular and Ataxias Research Unit, Instituto de Investigación Sanitaria la Fe (IIS La Fe), Valencia, Spain
[7]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Spain

[*]These authors contributed equally to the manuscript.

Corresponding Author:
Jacinto Javier Martínez-Payá, Faculty of Health Sciences, Universidad Católica de Murcia, Campus de los Jerónimos, Guadalupe, Murcia 30107, Spain.
Email: jmartinez@ucam.edu

Keywords
amyotrophic lateral sclerosis, biomarkers, area under the curve, sensitivity, specificity, texture analysis, muscle characterization, ultrasonography

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting both upper and lower motor neurons, which results in weakness and muscular atrophy. Despite the short median survival for these patients, there is a substantial diagnostic delay of about one year, mainly due to the lack of diagnostic biomarkers.[1] Currently, the diagnosis of ALS is based on the combined presence of clinical upper motor neuron signs and of clinical or neurophysiological lower motor neuron signs, for which electromyography (EMG) remains the gold standard.[2]

Muscle ultrasound (MUS) is a widely available, non-invasive and cost-effective tool, which rapidly allows the quantitative assessment of muscle characteristics (QMUS). The most frequently used first-order QMUS parameters are muscle thickness (MTh) and the mean echointensity (EI) of a region of interest (ROI). Echovariation (EV), determined by the relation between standard deviation (*SD*) and the mean pixel intensity, is also a first-order statistical parameter. EV can be interpreted as the uniformity of the ultrasonographic pattern and provides further information about the intensity range of the ROI.[3,4] However, both EI and EV are highly dependent on the ultrasound scanner settings[5] and neither provides information on wave energy scattering, that is, the distribution of the pixel intensities.[6,7]

The second-order statistical texture features based on the gray-level co-occurrence matrix (GLCM) investigate the relationship between neighboring pixel intensities[8] and provide information about gray-level patterns.[6] These parameters have been previously characterized in healthy individuals,[5] but studies in patients with neuromuscular disorders remain anecdotal.[6]

In ALS, MUS can detect fasciculations with more sensitivity than EMG, improving the diagnostic accuracy compared with EMG alone.[9] Moreover, we and others have found a diminished MTh, an increased EI, and a reduced EV in muscles of ALS patients.[10-13] However, to the best of our knowledge, GLCM parameters have not been previously assessed in ALS.

The purpose of this study was to assess differences in GLCM features in four muscle groups in ALS patients and age-matched controls. A second goal was to compare the diagnostic accuracy of all QMUS parameters.

Method

This cross-sectional study was performed according to the Standards for Reporting Diagnostic Accuracy (STARD criteria).[14]

Subjects

Patients were recruited from the Valencia ALS Association between September 2013 and April 2014. We included 26 patients diagnosed with ALS, according to the revised El Escorial criteria,[2] by an experienced neurologist (J.F.V.C.).

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

Standard Protocol Approval, Recruitment, and Patient Consent

This study was approved by the ethics committee of the Universidad Católica de Murcia (Spain). All participants provided written informed consent.

Recorded Variables

Demographical and clinical characteristics (sex, age, weight, height, body mass index [BMI], time of evolution from diagnosis) were recorded. Muscle strength was measured using the Medical Research Council (MRC) with a maximum value of 100, as described previously.[15] The global score of the revised ALS Functional Rating Scale (ALSFRS-R)[16] was assessed by the same investigator (J.M.-P.) on the same day that the MUS was performed.

Ultrasonography

MUS was performed in four muscle groups from each side in patients and controls by the same experienced examiner (J.M.-P.), with the participant sitting and completely relaxed. A phased array real-time scanner LOGIQe BT12 (General Electric Healthcare, China) and a 5- to 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), gray map, and focus (two focal points at 1.8 and 2.6 cm) were kept constant throughout the study.[13]

Applying the standardized protocol described by Arts,[10] bilateral transverse ultrasound images of the biceps/brachialis group (two-third distance acromion-antecubital crease, including biceps brachii and brachialis muscles), anterior forearm flexor group (two-fifth distance antecubital crease-distal end radius, including pronator teres, flexor carpi radialis, palmaris longus, flexor digitorum superficialis, flexor pollicis longus, and flexor digitorum profundus), quadriceps femoris group (one-half distance anterior superior iliac spine-superior aspect patella, including rectus femoris and vastus intermedius muscles), and tibialis anterior group (one-fourth distance inferior aspect patella-lateral malleolus) were obtained and measured (Figure 1). Three images were taken for each location to minimize variation in MTh and EI.[10]

The resulting images had a resolution of 820 × 614 pixels (with a scale of 99.5 px/cm for the tibialis anterior muscle and 83.5 px/cm for other muscles) with 256 gray levels and were stored as .TIFF files without compression or losses.[17]

Image Analysis

Quantitative MUS variables, including MTh, EI, EV, and GLCM, were obtained in each muscle group of patients and controls.

We have previously reported MTh, EI, and EV measurements[13] and, in this study, they are only used as reference standards for comparison purposes.

MTh was measured with electronic calipers of the ultrasound unit. 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.[13]

The image processing and analysis was performed by one researcher (J.R.-D.) using the Image J (v.1.48) software. This researcher, who was blind to the diagnosis, selected a ROI of 71 × 40 pixels for the tibialis anterior and 73 × 73 pixels for the other muscle groups on a 8-bit gray scale, using ROI Manager Application for Image J. The ROI was defined as the muscle region without bone and septum with the best reflection (Figure 1). The inter-rater reliability in the ROI selection has been reported in a previous study with the same data set.[13]

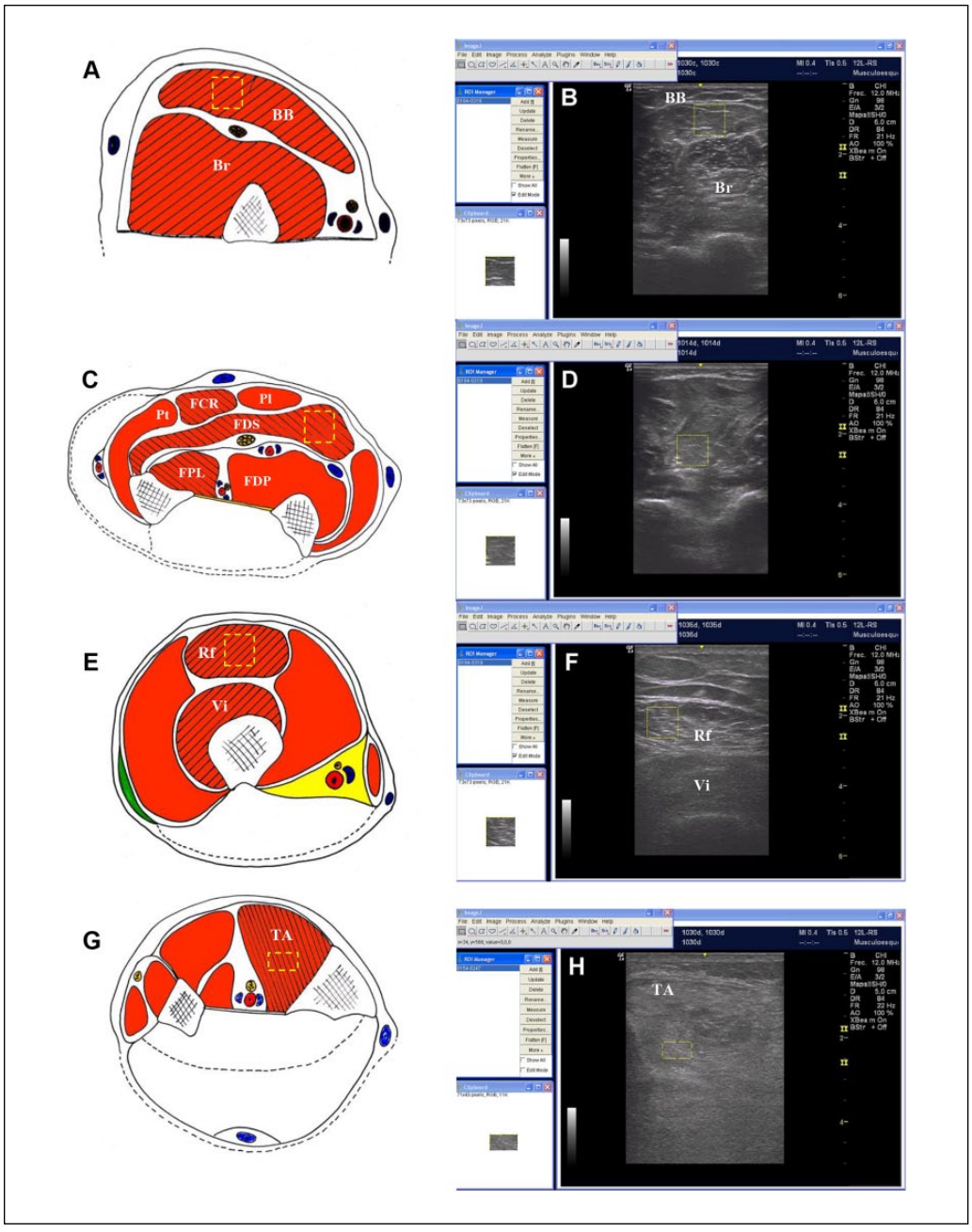

Figure 1. Ultrasonographic scans of the biceps/brachialis (a and b), forearm flexor (c and d), quadriceps (e and f), and tibialis anterior (g and h). The left panel depicts the different structures schematically. The selected ROI for EI, EV, and GLCM using the ImageJ (v.1.48) software is represented in both panels: BB = biceps brachii; Br = brachialis; Pt = pronator teres; FCR = flexor carpi radialis; PI = palmaris longus; FDS = flexor digitorum superficialis; FPL = flexor pollicis longus; FDP = flexor digitorum profundus; Rf = rectus femoris; Vi = vastus intermedius; TA = tibialis anterior; EI = echointensity; EV = echovariation; ROI = region of interest; GLCM = gray-level co-occurrence matrix.

The texture analysis based on a GLCM is derived from the angular relationship between neighboring pixels, as well as the distance between them, where *i* and *j* are the spatial adjacency gray tones, *n* is the number of gray levels (256 levels for an 8-bit image), and $p_{i,j}$ is the co-occurrence probability for distance δ and orientation θ (in this case, δ = 1 px and θ = average for 0° and 90°).[18]

The following textural parameters were selected:

- Energy or angular second moment (ASM). When the image is homogeneous, the ASM will have a high value [A]:

$$ASM = \sum_{i,j=0}^{n-1} (p_{i,j})^2.$$

- Homogeneity or inverse difference moment (IDM). This measures the local homogeneity of an image and is associated with pixel pairs. The result is a low IDM value for non-homogeneous images, and a higher value for homogeneous images [B]:

$$IDM = \sum_{i,j=0}^{n-1} \frac{p_{i,j}}{1+(i-j)^2}.$$

- Contrast (CON). The greater the variation in an image, the greater the contrast [C]:

$$CON = \sum_{i,j=0}^{n-1} p_{i,j} \left(i-j\right)^2.$$

- Textural correlation (TCOR). Higher values can be obtained for similar gray-level regions [D]:

$$TCOR = \sum_{i,j=0}^{n-1} p_{i,j} \left[\frac{(i-\mu_i)\cdot(j-\mu_j)}{\sqrt{\sigma_i^2 \cdot \sigma_j^2}} \right].$$

- Entropy (ENT). A homogeneous image will result in lower entropy than a non-homogenous one [E]:

$$ENT = \sum_{i,j=0}^{n-1} p_{i,j} \left[-Ln(p_{i,j}) \right].$$

Statistical Analysis

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

Variables were checked for normality and homoscedasticity.

Data were summarized by mean and *SD*s and 95% confidence intervals for continuous variables and absolute and relative frequencies for categorical variables.

Table 1. Baseline Characteristics.

Baseline Characteristics	ALS Patients (n = 26)	Controls (n = 26)	p Value
Females (n) (%)	8 (30.8%)	17 (65.4%)	<0.001
Age (year)	58.9 (12.02) [55.8, 62.0]	59.6 (6.41) [57.9, 61.4]	0.570
Weight (kg)	69.9 (17.42) [65.4, 74.4]	72.4 (17.19) [67.6, 77.2]	0.154
Height (m)	1.67 (0.086) [1.65, 1.69]	1.66 (0.08) [1.63, 1.68]	0.773
BMI (kg/m^2)	24.9 (5.13) [23.6, 26.3]	26.2 (4.87) [24.9, 27.6]	0.050
Disease onset diagnosis (months)	16.3 (9.89) [13.5, 19.1]		
ALSFRS-R (max 48)	26.2 (11.67) [22.9, 29.4]		
MRC (max 100)	58.5 (24.75) [51.7, 65.4]		

Data are presented as means (*SD*s) [95% CIs]. p values are for chi-square (sex) tests, and Student's t statistics are for independent samples. ALS = amyotrophic lateral sclerosis; BMI = body mass index; ALSFRS-R = ALS Functional Rating Scale–Revised; MRC = Medical Research Council; CI = confidence interval.

Independent-sample t tests were used to compare continuous variables, and a chi-square test was used to compare categorical variables at baseline between the ALS patients and controls.

Paired t tests were used to assess right–left differences in MTh, EI, EV and GLCM features.

QMUS variables in ALS patients and healthy controls. One-way analysis of covariance was used to compare QMUS variables of the patients and controls, controlling for the effects of clinical and demographical covariates.

Cohen's d statistic was calculated to evaluate the effect size ($d < 0.1$ small, around 0.3 medium and >0.5 large).

Diagnostic accuracy of QMUS. Simple logistic regression was performed for age, sex, and BMI by group. We introduced it in subsequent models if $p < 0.20$.

We investigated the sensitivity (Se), the specificity (Sp), and Jouden index (expressed as Se + Sp − 1), and positive and negative likelihood ratios (LRp and LRn) of all QMUS parameters and a combination of the GLCM parameters (designated GLCM).

All QMUS parameters were entered one by one and with all possible combinations (255 models) of MTh and texture parameters in logistic regression analyses, including a maximum combination logistic regression model containing all the parameters.

The studentized residuals, the leverages, and Cook's distances were determined to analyze outliers in the response variable, independent variables, and global data, respectively.[19]

Receiver operating characteristic curves and the Hosmer–Lemeshow goodness-of-fit test (where $p > 0.05$ indicates a good fit)[20] were calculated. An area under the curve (AUC) value close to 90%, and sensitivity and specificity values over 80% were considered acceptable.

Results

Characteristics of Subjects

Twenty-six ALS patients (8 women, mean age = 58.9 years, SD = 12.02) and 26 healthy controls (17 women; mean age = 59.6 years, SD = 6.41) were included in this study. Sex was unequally distributed in both groups and BMI was slightly different but no differences in age, height, and weight were noted between both groups (Table 1).

Table 2. Differences in Echotextural Parameters Between Groups.

	ALS (n = 52)		Control (n = 52)			
Ultrasonographic Parameters	*M* (*SD*)	95% CI	*M* (*SD*)	95% CI	*p* Value	Effect Size[a]
Biceps/brachialis						
Thickness (mm)	27.7 (6.34)	[26.4, 29.1]	33.7 (6.25)	[32.3, 35.0]	<0.001	0.90
Echointensity (EI)	93.5 (14.4)	[90.3, 96.8]	85.3 (8.78)	[82.1, 88.6]	0.001	0.67
Echovariation (EV)	22.48 (7.33)	[20.8, 24.2]	29.7 (4.24)	[28.1, 31.4]	<0.001	1.08
Energy (ASM)	18.5 (8.78)	[16.1, 21.0]	15.6 (5.33)	[14.1, 17.1]	0.040	0.40
Contrast (CON)	204.1 (103.48)	[175.3, 233.0]	184.7 (57.82)	[168.6, 200.8]	0.240	0.23
Textural correlation (TCOR)	18.1 (8.54)	[15.7, 20.4]	14.9 (4.62)	[13.6, 16.2]	0.021	0.45
Homogeneity (IDM)	25.5 (5.51)	[23.9, 27.0]	23.2 (4.09)	[22.0, 24.3]	0.017	0.47
Entropy (ENT)	6.98 (0.43)	[6.9, 7.1]	7.1 (0.28)	[7.0, 7.2]	0.060	0.37
Forearm flexors						
Thickness (mm)	28.97 (9.69)	[27.2, 30.8]	32.3 (5.96)	[30.5, 34.1]	0.016	0.42
Echointensity (EI)	93.2 (15.25)	[93.2, 107.2]	89.1 (15.07)	[84.9, 99.0]	<0.001	0.88
Echovariation (EV)	19.3 (4.55)	[18.1, 20.6]	25.5 (4.22)	[24.4, 26.7]	<0.001	1.16
Energy (ASM)	14.4 (6.03)	[12.7, 16.1]	12.2 (4.87)	[10.8, 13.5]	0.044	0.39
Contrast (CON)	223.9 (79.44)	[201.8, 246.0]	231.5 (79.06)	[209.5, 253.5]	0.625	0.10
Textural correlation (TCOR)	18.7 (8.14)	[16.5, 21.0]	15.3 (6.16)	[13.6, 17.1]	0.018	0.46
Homogeneity (IDM)	20.8 (3.83)	[19.7, 21.8]	19.5 (3.17)	[18.6, 20.4]	0.070	0.36
Entropy (ENT)	7.1 (0.38)	[7.0, 7.2]	7.3 (0.31)	[7.2, 7.4]	0.004	0.55
Quadriceps femoris						
Thickness (mm)	22.0 (8.97)	[19.9, 24.1]	30.3 (6.06)	[28.2, 32.4]	<0.001	1.00
Echointensity (EI)	100.6 (18.03)	[95.5, 105.6]	97.0 (12.77)	[93.4, 100.5]	0.245	0.23
Echovariation (EV)	18.9 (4.46)	[17.6, 20.1]	21.7 (5.66)	[20.2, 23.3]	0.005	0.55
Energy (ASM)	15.7 (7.72)	[13.5, 17.8]	14.9 (5.66)	[13.3, 16.5]	0.565	0.11
Contrast (CON)	244.0 (124.04)	[209.4, 278.5]	197.1 (72.23)	[177.0, 217.2]	0.020	0.45
Textural correlation (TCOR)	20.4 (10.12)	[17.6, 23.2]	13.5 (4.33)	[12.2, 14.7]	<0.001	0.82
Homogeneity (IDM)	21.6 (4.05)	[20.4, 22.8]	22.9 (4.67)	[21.7, 24.1]	0.121	0.31
Entropy (ENT)	7.1 (0.45)	[6.7, 7.2]	7.2 (0.32)	[7.1, 7.3]	0.165	0.27
Tibialis anterior						
Thickness (mm)	17.9 (5.59)	[16.7, 19.1]	22.9 (4.91)	[21.7, 24.0]	≤0.001	0.91
Echointensity (EI)	119.05 (16.36)	[115.3, 122.8]	102.1 (14.63)	[98.4, 105.8]	≤0.001	1.03
Echovariation (EV)	16.5 (4.31)	[15.3, 17.8]	25.0 (4.85)	[23.6, 26.3]	≤0.001	1.35
Energy (ASM)	16.3 (6.25)	[14.6, 18.1]	12.1 (4.48)	[10.8, 13.3]	≤0.001	0.74
Contrast (CON)	318.5 (131.06)	[279.6, 357.4]	296.0 (157.30)	[257.1, 334.9]	0.433	0.16
Textural correlation (TCOR)	15.8 (6.82)	[13.9, 17.7]	11.8 (5.18)	[10.3, 13.2]	0.001	0.64
Homogeneity (IDM)	21.8 (4.41)	[20.6, 23.1]	19.9 (4.26)	[18.7, 21.1]	0.026	0.43
Entropy (ENT)	6.9 (0.32)	[6.8, 7.0]	7.2 (0.29)	[7.1, 7.3]	≤0.001	0.87

CI = confidence interval. *p* values are for one-way analysis of variance. ALS = amyotrophic lateral sclerosis.
[a]Effect size was estimated with Cohen's *d*.

Ultrasound Variables

QMUS variables in patients and controls. QMUS variables for each muscle and group are shown in Table 2. There were no significant right–left differences for MTh, EI, EV, or GLCM in the four studied muscles groups. Therefore, a single sample of each right/left muscle group was selected for further analysis (52 ultrasonograms for each group).

Table 3. Diagnostic Validity for Ultrasonographic and Echotextural Parameters in the Biceps/Brachialis Group.

Variables[a]	AUC	Se [95% CI]	Sp [95% CI]	LRp [95% CI]	LRn* [95% CI]	P fit HL
Thickness	0.875	0.81 [0.73, 0.91]	0.75 [0.67, 0.83]	3.2 [2.2, 5.4]	3.9 [2.5, 9.0]	0.381
Echointensity (EI)	0.812	0.77 [0.69, 0.88]	0.69 [0.60, 0.78]	2.5 [1.7, 4.0]	3.0 [1.9, 6.3]	0.657
Echovariation (EV)	0.871	0.81 [0.73, 0.91]	0.83 [0.75, 0.9]	4.7 [3.0, 9.0]	4.3 [2.8, 9.7]	0.329
GLCM	0.849	0.81 [0.73, 0.91]	0.73 [0.65, 0.82]	3.0 [2.1, 4.9]	3.8 [2.4, 8.8]	0.832
Thickness + EI	0.884	0.81 [0.73, 0.91]	0.75 [0.67, 0.83]	3.2 [2.2, 5.4]	3.9 [2.5, 9.0]	0.611
Thickness + EV	0.926	0.88 [0.82, 0.97]	0.83 [0.75, 0.90]	5.1 [3.4, 9.6]	7.2 [4.3, 26.0]	0.759
Thickness + GLCM	0.913	0.87 [0.80, 0.95]	0.79 [0.71, 0.87]	4.1 [2.8, 7.2]	5.9 [3.6, 18.0]	0.186
Maximum model	0.949	0.92 [0.87, 0.99]	0.88 [0.82, 0.95]	8.0 [4.9, 18.5]	11.5 [6.4, 99.6]	0.744

AUC = area under the ROC curve; Se = sensibility; CI = confidence interval; Sp = specificity; LRp = positive likelihood ratio; LRn* = the inverse of negative likelihood ratio to allow for a direct comparison with LRp; P fit HL = Hosmer–Lemeshow goodness-of-fit test; GLCM = gray-level co-occurrence matrix; BMI = body mass index. *p* value > 0.05 indicates a good fit.
[a]Corrected by sex and BMI, 255 logistic regression models were analyzed.

Table 4. Diagnostic Validity for Ultrasonographic and Echotextural Parameters in Forearm Flexors.

Variables[a]	AUC	Se [95% CI]	Sp [95% CI]	LRp [95% CI]	LRn* [95% CI]	P Fit HL
Thickness	0.804	0.75 [0.67, 0.83]	0.77 [0.69, 0.85]	3.3 [2.1, 5.6]	3.1 [2.1, 5.1]	0.311
Echointensity (EI)	0.822	0.81 [0.73, 0.88]	0.73 [0.65, 0.82]	3.0 [2.1, 4.8]	3.8 [2.4, 7.0]	0.647
Echovariation (EV)	0.865	0.79 [0.71, 0.87]	0.75 [0.67, 0.83]	3.2 [2.1, 5.2]	3.6 [2.3, 6.3]	0.167
GLCM	0.874	0.79 [0.71, 0.87]	0.83 [0.75, 0.90]	4.6 [2.9, 8.6]	3.9 [2.6, 6.7]	0.548
Thickness + EI	0.828	0.79 [0.71, 0.87]	0.71 [0.62, 0.80]	2.7 [1.9, 4.3]	3.4 [2.2, 6.0]	0.546
Thickness + EV	0.876	0.81 [0.73, 0.88]	0.75 [0.67, 0.83]	3.2 [2.2, 5.3]	3.9 [2.5, 7.2]	0.217
Thickness + GLCM	0.905	0.81 [0.73, 0.88]	0.79 [0.71, 0.87]	3.8 [2.5, 6.6]	4.1 [2.7, 7.4]	0.478
Maximum model	0.913	0.79 [0.71, 0.87]	0.85 [0.78, 0.92]	5.1 [3.2, 10.3]	4.0 [2.7, 6.9]	0.879

AUC = area under the ROC curve; Se = sensibility; CI = confidence interval; Sp = specificity; LRp = positive likelihood ratio; LRn* = the inverse of negative likelihood ratio to allow for a direct comparison with LRp; P fit HL = Hosmer–Lemeshow goodness-of-fit test; GLCM = gray-level co-occurrence matrix; BMI = body mass index. *p* value > 0.05 indicates a good fit.
[a]Corrected by sex and BMI, 255 logistic regression models were analyzed.

As sex and BMI were unequally distributed in both groups, mean comparisons were made with the corresponding corrections in each case (for details, see footnotes in tables). As expected, GLCM parameters showed reduced granularity in the muscles of ALS patients compared with the controls. Effect sizes of GLCM varied significantly among muscle groups although the TCOR showed overall the best performance. However, EI and EV showed greater effect sizes in all muscle groups except in quadriceps. Furthermore, CON was the parameter with smallest effect size except, once again, in quadriceps.

Diagnostic accuracy of QMUS parameters. Tables 3 to 6 show the results of the best parameters and combinations of parameters differentiating patients from controls. GLMC was the best single diagnostic parameter in forearm flexors and quadriceps, whereas EV showed the best discrimination power in biceps brachialis and tibialis anterior. The respective combination of these two parameters with MTh resulted in the best AUC (over 90% in all muscle groups and close to the maximum combination model).

Table 5. Diagnostic Validity for Ultrasonographic and Echotextural Parameters in Quadriceps Femoris.

Variables[a]	AUC	Se [95% CI]	Sp [95% CI]	LRp [95% CI]	LRn* [95% CI]	P fit HL
Thickness	0.833	0.81 [0.73, 0.88]	0.71 [0.62, 0.80]	2.8 [2.0, 4.4]	3.7 [2.3, 6.9]	0.776
Echointensity (EI)	0.786	0.81 [0.73, 0.88]	0.75 [0.67, 0.83]	3.2 [2.2, 5.3]	3.9 [2.5, 7.2]	0.008
Echovariation (EV)	0.811	0.75 [0.67, 0.83]	0.81 [0.73, 0.88]	3.9 [2.5, 7.2]	3.2 [2.2, 5.3]	0.057
GLCM	0.977	0.94 [0.90, 0.99]	0.94 [0.90, 0.99]	16.3 [8.8, 76.6]	16.3 [8.8, 76.7]	0.298
Thickness + EI	0.835	0.81 [0.73, 0.88]	0.73 [0.65, 0.82]	3.0 [2.1, 4.8]	3.8 [2.4, 7.0]	0.817
Thickness + EV	0.888	0.79 [0.71, 0.87]	0.83 [0.75, 0.90]	4.6 [2.9, 8.6]	3.9 [2.6, 6.8]	0.765
Thickness + GLCM	0.983	0.94 [0.90, 0.99]	0.96 [0.92, 1.0]	24.5 [11.9, 657.5]	16.6 [9.0, 77.5]	0.954
Maximum model	0.985	0.94 [0.90, 0.99]	0.96 [0.92, 1.0]	24.5 [11.9, 657.5]	16.6 [9.0, 77.5]	0.972

AUC = area under the ROC curve; Se = sensibility; CI = confidence interval; Sp = specificity; LRp = positive likelihood ratio; LRn* = the inverse of negative likelihood ratio to allow for a direct comparison with LRp; P fit HL = Hosmer–Lemeshow goodness-of-fit test; GLCM = gray-level co-occurrence matrix; BMI = body mass index. *p* value > 0.05 indicates a good fit.
[a]Corrected by sex and BMI, 255 logistic regression models were analyzed.

Table 6. Diagnostic Validity for Ultrasonographic and Echotextural Parameters in Tibialis Anterior.

Variables[a]	AUC	Se [95% CI]	Sp [95% CI]	LRp [95% CI]	LRn* [95% CI]	P fit HL
Thickness	0.861	0.77 [0.69, 0.85]	0.79 [0.71, 0.87]	3.6 [2.4, 6.4]	3.4 [2.3, 5.8]	0.434
Echointensity (EI)	0.865	0.81 [0.73, 0.88]	0.77 [0.69, 0.85]	3.5 [2.4, 5.90]	4.0 [2.6, 7.3]	0.836
Echovariation (EV)	0.945	0.88 [0.82, 0.95]	0.88 [0.82, 0.95]	7.7 [4.7, 17.5]	7.7 [4.7, 17.5]	0.955
GLCM	0.934	0.85 [0.78, 0.92]	0.88 [0.82, 0.95]	7.3 [4.4, 17.0]	5.8 [3.7, 11.2]	0.034
Thickness + EI	0.906	0.79 [0.71, 0.87]	0.81 [0.73, 0.88]	4.1 [2.7, 7.4]	3.8 [2.5, 6.6]	0.278
Thickness + EV	0.953	0.85 [0.78, 0.92]	0.92 [0.87, 0.97]	11.0 [6.1, 35.6]	6.0 [3.9, 11.5]	0.489
Thickness + GLCM	0.948	0.85 [0.78, 0.92]	0.88 [0.82, 0.95]	7.3 [4.4, 17.0]	5.8 [3.7, 11.2]	0.132
Maximum model	0.975	0.92 [0.87, 0.97]	0.92 [0.87, 0.97]	12.0 [6.8, 37.9]	12.0 [6.8, 37.9]	0.907

AUC = area under the ROC curve; Se = sensibility; CI = confidence interval; Sp = specificity; LRp = positive likelihood ratio; LRn* = the inverse of negative likelihood ratio to allow for a direct comparison with LRp; P fit HL = Hosmer–Lemeshow goodness-of-fit test; GLCM = gray-level co-occurrence matrix; BMI = body mass index. *p* value > 0.05 indicates a good fit.
[a]Corrected by sex and BMI, 255 logistic regression models were analyzed.

Discussion

We found that EV and GLMC features differentiated ALS patients from the controls better than the previously reported EI or MTh. Moreover, combining EV and GLMC with MTh resulted in increased diagnostic accuracy.

Technical Issues

The quantitative analysis of muscle EI depends on the ROI selection. Previous studies included as much muscle area as possible, excluding bone or surrounding tissue.[10,15,21] By doing so, large muscle ROIs are evaluated, combining areas of maximum reflection with anisotropic areas, which results in a decrease in EI. Conversely, as suggested previously,[6] it is possible to select a small ROI of the most reflexive (echogenic) muscle segment, where the surrounding connective tissue has maximum brightness, avoiding the inclusion of anisotropic areas. We previously showed that by using this method, inter-rater reliability was found to be excellent for all QMUS parameters.[13]

GLMC Values in Patients versus Controls

As expected, GLCM parameters (especially TCOR) showed reduced granularity, in muscles of ALS patients. This implies a more homogeneous scattering pattern and greater gray-level correlation between pixels throughout the ROI, reflecting changes in the hierarchical organization of the muscle. Although effect sizes of each feature varied significantly among muscle groups, overall, EI and EV showed greater effect sizes than each separate GLMC parameter in all muscle groups, except in quadriceps. Variations in structure between muscle groups could account for these differences as, also in healthy individuals, different muscles show diverse GLMC properties.[5] Moreover, considering that ALS affects diverse muscle groups differently (typically sparing quadriceps), it could also mean that GLMC detects early but not late muscle changes. Further prospective longitudinal studies are warranted to address this issue.

Diagnostic Accuracy of QMUS in ALS Based on a Textural Analysis

All QMUS showed a moderately good diagnostic accuracy when considered independently. However, EV and a combination of GLMC parameters differentiate better than EI between patients from controls. Moreover, combining MTh with texture parameters (but not combining other texture parameters among themselves) increased the diagnostic accuracy. The diagnostic accuracy of EI in ALS has been reported previously,[10-12] but methodological differences in the study design and data analysis hinder a direct comparison of the results. One cross-sectional study suggested that visually assessed EI is more sensitive than EMG (90% vs. 88%) for ALS diagnosis, as it detected neurogenic changes in muscles where EMG did not.[11] However, data on specificity were not provided, so these may well have been false positive detections. Another prospective study in patients with suspected ALS found high sensitivity (96%) and more limited specificity (84%) of EI for diagnosing ALS, using El Escorial criteria as the gold standard.[12] However, the authors did not directly compare the diagnostic accuracy of EI with EMG for the detection of neurogenic changes, because in a previous pilot study, they found that EI did not improve the diagnosis of lower motor neurons impairment based only on fasciculation detection.[10] Furthermore, Gdynia et al.[6] compared GLMC parameters in subjects with inflammatory myopathies ($n = 7$), motor neuron diseases ($n = 9$, 6 subjects with ALS), dystrophic myopathies ($n = 12$), and controls, finding differences between healthy and affected musculature, but a comparison with EI or EV was not performed and the diagnostic accuracy of GLMC was not assessed.

QMUS as Biomarkers in ALS

In the absence of a specific marker, the diagnosis of ALS in clinical practice is currently based on clinical criteria with the support of compatible EMG findings.[2] However, there is an urgent need for new imaging biomarkers both for clinical trials and clinical practice, which will allow the long diagnostic delay to be reduced, and make it possible to monitor progression and predict prognosis.[22]

MUS is a widely available, non-invasive technique that detects fasciculations in ALS patients with a higher degree of sensitivity than EMG and clinical examination.[10] Fasciculations are characteristic of early ALS[23] but can also occur in healthy subjects. Therefore, fasciculations detected by MUS must be interpreted in the presence of chronic denervation on needle EMG.[2,9] Detecting neurogenic changes (QMUS) together with fasciculations in MUS could eventually replace EMG, and now we provide evidence that EV and GLMC parameters can differentiate ALS muscles from those of healthy individuals with higher specificity and sensitivity than the previously reported EI and MTh.[10-12]

Strengths and Limitations

To the best of our knowledge, our study, which used a highly reliable methodology, represents the most thorough analysis of muscle biomarkers in ALS performed to date. However, it has several limitations. First, patients were in a moderately advanced phase of the disease and the diagnostic accuracy of QMUS parameters in early disease may be lower. Second, we considered all studied muscle groups of ALS patients to be affected, because a correlation with EMG data was lacking. Consequently, data on sensitivity or specificity might be underestimated (e.g., a given muscle in an ALS patient may at some point be unaffected by the disease, but following the protocol it was considered as "ill") and should not be considered absolute measures differentiating neurogenic from non-neurogenic muscles. Third, QMUS parameters can vary considerably with age, sex, or muscle group, and currently, there is no range of normal values. Consequently, this study should be replicated in a prospective longitudinal study with a larger cohort of healthy individuals and patients with suspected ALS or early ALS, accounting for age, sex, and muscle group and considering EMG as gold standard. However, our main aim was to compare the diagnostic accuracy of several QMUS, and this comparison is still valid.

Conclusion

We propose that EV and GLCM can differentiate ALS patients from controls better than the previously reported EI and MTh. A combination of MTh with QMUS parameters renders the best diagnostic performance. Larger prospective longitudinal studies in clinical setting are warranted to replicate these findings and to evaluate the possible role of EV and GLCM as progression or predictive biomarkers.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Turner MR, Hardiman O, Benatar M, Brooks BR, Chio A, de Carvalho M, et al. Controversies and priorities in amyotrophic lateral sclerosis. Lancet Neurol. 2013;12:310-22.
2. Brooks BR, Miller RG, Swash M, Munsat TL; World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord. 2000;1:293-9.
3. Ríos-Díaz J, de Groot Ferrando A, Martínez-Payá JJ, del Baño Aledo ME. Reliability and reproducibility of a morpho-textural image analysis method over a patellar ligament ultrasonography. Reumatol Clin. 2010;6:278-84.
4. Ríos-Díaz J, Martínez-Payá JJ, del Baño-Aledo ME, de Groot-Ferrando A, Botía-Castillo P, Fernández-Rodríguez D. Sonoelastography of plantar fascia: reproducibility and pattern description in healthy subjects and symptomatic subjects. Ultrasound Med Biol. 2015;41:2605-13.
5. Molinari F, Caresio C, Acharya UR, Mookiah MR, Minetto MA. Advances in quantitative muscle ultrasonography using texture analysis of ultrasound images. Ultrasound Med Biol. 2015;41:2520-32.
6. 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;16:1151-8.

7. Aggarwal N, Agrawal RK. First and second order statistics features for classification of magnetic resonance brain images. JSIP. 2012;3:146-53.
8. Haralick RM, Shanmugam K, Dinstein I. Textural features for image classification. IEEE Trans Sys Man Cybern. 1973;3:610-21.
9. Misawa S, Noto Y, Shibuya K, Isose S, Sekiguchi Y, Nasu S, et al. Ultrasonographic detection of fasciculations markedly increases diagnostic sensitivity of ALS. Neurology. 2011;77:1532-37.
10. Arts IMP, van Rooij FG, Overeem S, Pillen S, Janssen HM, Schelhaas HJ, et al. Quantitative muscle ultrasonography in amyotrophic lateral sclerosis. Ultrasound Med Biol. 2008;34:354-61.
11. Grimm A, Prell T, Décard BF, Schumacher U, Witte OW, Axer H, et al. Muscle ultrasonography as an additional diagnostic tool for the diagnosis of amyotrophic lateral sclerosis. Clin Neurophysiol. 2015;126:820-7.
12. Arts IMP, Overeem S, Pillen S, Kleine BU, Boekestein WA, Zwarts MJ, et al. Muscle ultrasonography: a diagnostic tool for amyotrophic lateral sclerosis. Clin Neurophysiol. 2012;123:1662-7.
13. 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. 2017;43:1153-62.
14. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. BMJ. 2015;351:h5527.
15. Arts IMP, Overeem S, Pillen S, Schelhaas HJ, Zwarts MJ. Muscle changes in amyotrophic lateral sclerosis: a longitudinal ultrasonography study. Clin Neurophysiol. 2011;122:623-8.
16. Cartwright MS, Walker FO, Griffin LP, Caress JB. Peripheral nerve and muscle ultrasound in amyotrophic lateral sclerosis. Muscle Nerve. 2011;44:346-51.
17. Wiggins RH, Davidson HC, Harnsberger HR, Lauman JR, Goede P. Image file formats: past, present, and future. Radiogr. 2001;21:789-98.
18. 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.
19. Kleinbaum DG, Klein M. Logistic Regression: A Self-Learning Test. New York, NY: Springer; 2010.
20. Hosmer DW, Lemeshow S. Applied Logistic Regression. 2nd ed. New York, NY: John Wiley & Sons; 2004.
21. Arts IMP, Overeem S, Pillen S, Schelhaas HJ, Zwarts MJ. Muscle ultrasonography to predict survival in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2011;82:552-4.
22. Turner MR, Agosta F, Bede P, Govind V, Lulé D, Verstraete E. Neuroimaging in amyotrophic lateral sclerosis. Biomark Med. 2012;6:319-37.
23. de Carvalho M, Swash M. Fasciculation potentials and earliest changes in motor unit physiology in ALS. J Neurol Neurosurg Psychiatry. 2013;84:963-8.