Ultrasonic Imaging

Quantitative muscle ultrasonography using textural analysis in amyotrophic lateral sclerosis

Journal:	*Ultrasonic Imaging*
Manuscript ID	UIX-17-0006.R2
Manuscript Type:	Technical Article
Date Submitted by the Author:	28-Apr-2017
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Keywords:	Amyotrophic lateral sclerosis, biomarkers, area under the curve, sensitivity, specificity, texture analysis, muscle characterization, ultrasonography

Abstract:

Background:
To analyze differences in grey level co-ocurrence matrix (GLCM) parameters, as assessed by muscle ultrasound (MUS), between amyotrophic lateral sclerosis (ALS) patients and healthy controls. 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.
Methods:
Twenty-six patients with ALS and twenty-six 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 callipers 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 ROC-curves.
Results:
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). Conclusions: The use of new textural parameters (EV and GLCM) combined with usual quantitative MUS variables are a promising biomarker in ALS.

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 neurons signs and of clinical or neurophysiological lower motor neurons 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 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 , i.e. the distribution of the pixel intensities [6,7].

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

In amyotrophic lateral sclerosis, MUS can detect fasciculations with more sensitivity than EMG, improving the diagnostic accuracy compared to 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.

METHODS.

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 (JFVC).

Twenty-six healthy volunteers without a history of hereditary neuromuscular disease were recruited as 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, time of evolution from diagnosis) were recorded. Muscle strength was measured using the Medical Research Council rating scale (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 (JM-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 (JM-P), with the participant sitting and completely relaxed. An phased array real-time scanner General Electric Healthcare LOGIQe BT12 and a 5–13 MHz linear array transducer (12L–RS) was used for MUS. All system-setting parameters, such as gain (98dB), time gain compensation (in neutral position),

depth (5cm for tibialis anterior and 6 cm for the other muscle groups), frequency (12MHz), 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 (2/3 distance acromion-antecubital crease, including biceps brachii and brachialis muscles), anterior forearm flexor group (2/5th 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 (1/2 distance anterior superior iliac spine-superior aspect patella, including rectus femoris and vastus intermedius muscles) and tibialis anterior (1/4th distance inferior aspect patella-lateral malleolus) were obtained and measured (Figure 1). Three images were taken for each location in order to minimize variation in MTh and EI [10].

The resulting images had a resolution of 820 x 614 pixels (with a scale of 99.5px/cm for tibialis anterior muscle and 83.5px/cm for other muscles) with 256 grey 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 standard for comparison purposes.

Muscle thickness 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 (JR-D) using the ImageJ (v.1.48) software. This researcher, who was blind to the diagnosis, selected a ROI of 71x40 pixels for the tibialis anterior and 73x73 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].

The texture analysis based on a GLCM is derived from the angular relationship between neighbouring pixels, as well as the distance between them, where i and j are the spatial adjacency grey tones, n is the number of grey 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 0º and 90º) [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), which 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].

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

- Textural Correlation (TCOR). Higher values can be obtained for similar grey-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 standard deviations (SD) and 95% confidence intervals (CI) for continuous variables and absolute and relative frequencies for categorical variables.

Independent-sample t-tests were used to compare continuous variables and a chi-square test 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 ANCOVA 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 muscle thickness 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 (ROC) 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).

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).

Since 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).

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 interrater reliability is excellent for all QMUS parameters [13].

GLMC values in patients vs controls

As expected, GLCM parameters (especially TCOR) showed reduced granularity, in muscles of ALS patients. This implies a more homogenous scattering pattern and greater grey 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 since, 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, since 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 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 (2009) 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 [6], 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.

Conclusions

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.

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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 (yr)	58.9 (12.02); 55.8 to 62.0	59.6 (6.41); 57.9 to 61.4	0.570
Weight (kg)	69.9 (17.42); 65.4 to 74.4	72.4 (17.19); 67.6 to 77.2	0.154
Height (m)	1.67 (0.086); 1.65 to 1.69	1.66 (0.08); 1.63 to 1.68	0.773
BMI (kg/m^2)	24.9 (5.13); 23.6 to 26.3	26.2 (4.87); 24.9 to 27.6	0.050
Disease onset-diagnosis (months)	16.3 (9.89); 13.5 to 19.1		
ALFSFR-r (max 48)	26.2 (11.67); 22.9 to 29.4		
MRC (max 100)	58.5 (24.75); 51.7 to 65.4		

Data are presented as mean (SD); C.I. 95%. P-value for Chi-Square (Sex), and T-Student for independent samples

Table 2. Differences in echotextural parameters between groups.

Ultrasonographic parameters	ALS (n=52)		Control (n=52)		p-value	Effect Size*
	Mean (SD)	95% C.I.	Mean (SD)	95% C.I.		
			Biceps/Brachialis			
Thickness (mm) †	27.7 (6.34)	26.4 to 29.1	33.7 (6.25)	32.3 to 35.0	<0.000	0.90
Echointensity (EI) §	93.5 (14.4)	90.3 to 96.8	85.3 (8.78)	82.1 to 88.6	0.001	0.67
Echovariation (EV) §	22.48 (7.33)	20.82 to 24.15	29.7 (4.24)	28.06 to 31.4	<0.000	1.08
Energy (ASM)	18.5 (8.78)	16.1 to 21	15.6 (5.33)	14.1 to 17.1	0.040	0.40
Contrast (CON)	204.1 (103.48)	175.3 to 233	184.7 (57.82)	168.6 to 200.8	0.240	0.23
Textural Correlation (TCOR)	18.1 (8.54)	15.7 to 20.4	14.9 (4.62)	13.6 to 16.2	0.021	0.45
Homogeneity (IDM)	25.5 (5.51)	23.9 to 27	23.2 (4.09)	22.02 to 24.3	0.017	0.47
Entropy (ENT)	6.98 (0.43)	6.9 to 7.1	7.1 (0.28)	7.03 to 7.2	0.060	0.37
			Forearm Flexors			
Thickness (mm)	28.97 (9.69)	27.2 to 30.8	32.3 (5.96)	30.5 to 34.14	0.016	0.42
Echointensity (EI)§	93.2 (15.25)	93.2 to 107.2	89.1 (15.07)	84.9 to 99.0	<0.000	0.88
Echovariation (EV)	19.3 (4.55)	18.06 to 20.6	25.5 (4.22)	24.4 to 26.7	<0.000	1.16
Energy (ASM)	14.4 (6.03)	12.7 to 16.1	12.2 (4.87)	10.8 to 13.5	0.044	0.39
Contrast (CON)	223.9 (79.44)	201.8 to 246.03	231.5 (79.06)	209.5 to 253.5	0.625	0.10
Textural Correlation (TCOR)	18.7 (8.14)	16.5 to 20.99	15.3 (6.16)	13.6 to 17.05	0.018	0.46
Homogeneity (IDM)	20.8 (3.83)	19.7 to 21.8	19.5 (3.17)	18.64 to 20.4	0.070	0.36
Entropy (ENT)	7.1 (0.38)	7.02 to 7.2	7.3 (0.31)	7.24 to 7.4	0.004	0.55
			Quadriceps Femoris			
Thickness (mm) §	22.0 (8.97)	19.9 to 24.1	30.3 (6.06)	28.2 to 32.4	<0.000	1.00
Echointensity (EI)	100.6 (18.03)	95.5 to 105.6	96.98 (12.77)	93.4 to 100.5	0.245	0.23
Echovariation (EV)	18.9 (4.46)	17.6 to 20.1	21.7 (5.66)	20.2 to 23.3	0.005	0.55
Energy (ASM)	15.7 (7.72)	13.5 to 17.8	14.9 (5.66)	13.3 to 16.5	0.565	0.11
Contrast (CON)	244.0 (124.04)	209.4 to 278.5	197.1 (72.23)	177.0 to 217.2	0.020	0.45
Textural Correlation (TCOR)	20.4 (10.12)	17.6 to 23.2	13.5 (4.33)	12.2 to 14.7	<0.000	0.82
Homogeneity (IDM)‡	21.6 (4.05)	20.4 to 22.8	22.9 (4.67)	21.7 to 24.12	0.121	0.31
Entropy (ENT)	7.1 (0.45)	6.96 to 7.2	7.2 (0.32)	7.1 to 7.3	0.165	0.27
			Tibialis Anterior			
Thickness (mm) §	17.9 (5.59)	16.7 to 19.1	22.9 (4.91)	21.7 to 24.04	0.000	0.91
Echointensity (EI)§	119.05 (16.36)	115.3 to 122.8	102.1 (14.63)	98.4 to 105.8	0.000	1.03
Echovariation (EV)	16.5 (4.31)	15.3 to 17.75	24.95 (4.85)	23.6 to 26.3	0.000	1.35
Energy (ASM)	16.3 (6.25)	14.6 to 18.1	12.05 (4.48)	10.8 to 13.3	0.000	0.74
Contrast (CON)	318.5 (131.06)	279.6 to 357.4	296 (157.3)	257.1 to 334.9	0.433	0.16
Textural Correlation (TCOR)	15.8 (6.82)	13.9 to 17.7	11.8 (5.18)	10.3 to 13.2	0.001	0.64
Homogeneity (IDM)	21.8 (4.41)	20.6 to 23.1	19.9 (4.26)	18.73 to 21.1	0.026	0.43
Entropy (ENT)	6.9 (0.32)	6.8 to 7	7.2 (0.29)	7.1 to 7.3	0.000	0.87

S.D.: standard deviation. C.I. 95%.: Confidence Interval. P-value for one-way ANOVA. * Effect size was estimated with Cohen's d.

Table 3. Diagnostic validity for ultrasonographic and echotextural parameters in biceps/brachialis group.

Variables*	AUC	Se (95% CI)	Sp (95% CI)	LRp (95% CI)	LRn* (95% CI)	P fit HL
Thickness	0.875	0.81 (0.73 to 0.91)	0.75 (0.67 to 0.83)	3.23 (2.2 to 5.44)	3.9 (2.49 to 9)	0.381
Echointensity (EI)	0.812	0.77 (0.69 to 0.88)	0.69 (0.6 to 0.78)	2.5 (1.74 to 4)	3 (1.94 to 6.29)	0.657
Echovariation (EV)	0.871	0.81 (0.73 to 0.91)	0.83 (0.75 to 0.9)	4.67 (2.98 to 9.04)	4.3 (2.81 to 9.72)	0.329
GLCM	0.849	0.81 (0.73 to 0.91)	0.73 (0.65 to 0.82)	3 (2.06 to 4.93)	3.8 (2.41 to 8.81)	0.832
Thickness+EI	0.884	0.81 (0.73 to 0.91)	0.75 (0.67 to 0.83)	3.23 (2.2 to 5.44)	3.9 (2.49 to 9)	0.611
Thickness+EV	0.926	0.88 (0.82 to 0.97)	0.83 (0.75 to 0.90)	5.11 (3.35 to 9.62)	7.17 (4.27 to 26.04)	0.759
Thickness+GLCM	0.913	0.87 (0.8 to 0.95)	0.79 (0.71 to 0.87)	4.09 (2.76 to 7.15)	5.86 (3.55 to 17.97)	0.186
Maximum model	0.949	0.92 (0.87 to 0.99)	0.88 (0.82 to 0.95)	8 (4.93 to 18.35)	11.5 (6.42 to 99.61)	0.744

Corrected by Sex and BMI 255 logistic regression models were analyzed. AUC: area under the ROC curve. Se: sensibility. Sp: specificity. LRp: positive likelihood ratio. LRn is the inverse of negative likelihood ratio to allow for direct comparison with LRp. P fit HL: Hosmer-Lemeshow goodness-of fit test. p-value >0.05 indicates good fit.

Table 4. Diagnostic validity for ultrasonographic and echotextural parameters in forearm flexors.

Variables*	AUC	Se (95% CI)	Sp (95% CI)	LRp (95% CI)	LRn* (95% CI)	P fit HL
Thickness	0.804	0.75 (0.67 to 0.83)	0.77 (0.69 to 0.85)	3.25 (2.14 to 5.56)	3.08 (2.07 to 5.1)	0.311
Echointensity (EI)	0.822	0.81 (0.73 to 0.88)	0.73 (0.65 to 0.82)	3 (2.06 to 4.8)	3.8 (2.41 to 7)	0.647
Echovariation (EV)	0.865	0.79 (0.71 to 0.87)	0.75 (0.67 to 0.83)	3.15 (2.13 to 5.2)	3.55 (2.3 to 6.26)	0.167
GLCM	0.874	0.79 (0.71 to 0.87)	0.83 (0.75 to 0.9)	4.56 (2.89 to 8.64)	3.91 (2.6 to 6.76)	0.548
Thickness+EI	0.828	0.79 (0.71 to 0.87)	0.71 (0.62 to 0.8)	2.73 (1.89 to 4.3)	3.36 (2.15 to 6)	0.546
Thickness+EV	0.876	0.81 (0.73 to 0.88)	0.75 (0.67 to 0.83)	3.23 (2.2 to 5.3)	3.9 (2.49 to 7.15)	0.217
Thickness+GLCM	0.905	0.81 (0.73 to 0.88)	0.79 (0.71 to 0.87)	3.82 (2.52 to 6.64)	4.1 (2.65 to 7.44)	0.478
Maximum model	0.913	0.79 (0.71 to 0.87)	0.85 (0.78 to 0.92)	5.13 (3.18 to 10.26)	4 (2.68 to 6.88)	0.879

Corrected by Sex and BMI 255 logistic regression models were analyzed. AUC: area under the ROC curve. Se: sensibility. Sp: specificity. LRp: positive likelihood ratio. LRn is the inverse of negative likelihood ratio to allow for direct comparison with LRp. P fit HL: Hosmer-Lemeshow goodness-of fit test. p-value >0.05 indicates good fit.

Table 5. Diagnostic validity for ultrasonographic and echotextural parameters in quadriceps femoris.

Variables*	AUC	Se (95% CI)	Sp (95% CI)	LRp (95% CI)	LRn* (95% CI)	P fit HL
Thickness	0.833	0.81 (0.73 to 0.88)	0.71 (0.62 to 0.8)	2.8 (1.95 to 4.39)	3.7 (2.33 to 6.85)	0.776
Echointensity (EI)	0.786	0.81 (0.73 to 0.88)	0.75 (0.67 to 0.83)	3.23 (2.2 to 5.3)	3.9 (2.49 to 7.15)	0.008
Echovariation (EV)	0.811	0.75 (0.67 to 0.83)	0.81 (0.73 to 0.88)	3.9 (2.49 to 7.15)	3.23 (2.2 to 5.3)	0.057
GLCM	0.977	0.94 (0.9 to 0.99)	0.94 (0.9 to 0.99)	16.33 (8.76 to 76.64)	16.33 (8.76 to 76.64)	0.298
Thickness+EI	0.835	0.81 (0.73 to 0.88)	0.73 (0.65 to 0.82)	3 (2.06 to 4.8)	3.8 (2.41 to 7)	0.817
Thickness+EV	0.888	0.79 (0.71 to 0.87)	0.83 (0.75 to 0.9)	4.56 (2.89 to 8.64)	3.91 (2.6 to 6.76)	0.765
Thickness+GLCM.	0.983	0.94 (0.9 to 0.99)	0.96 (0.92 to 1)	24.5 (11.9 to 657.45)	16.67 (9.02 to 77.52)	0.954
Maximum model	0.985	0.94 (0.9 to 0.99)	0.96 (0.92 to 1)	24.5 (11.9 to 657.45)	16.67 (9.02 to 77.52)	0.972

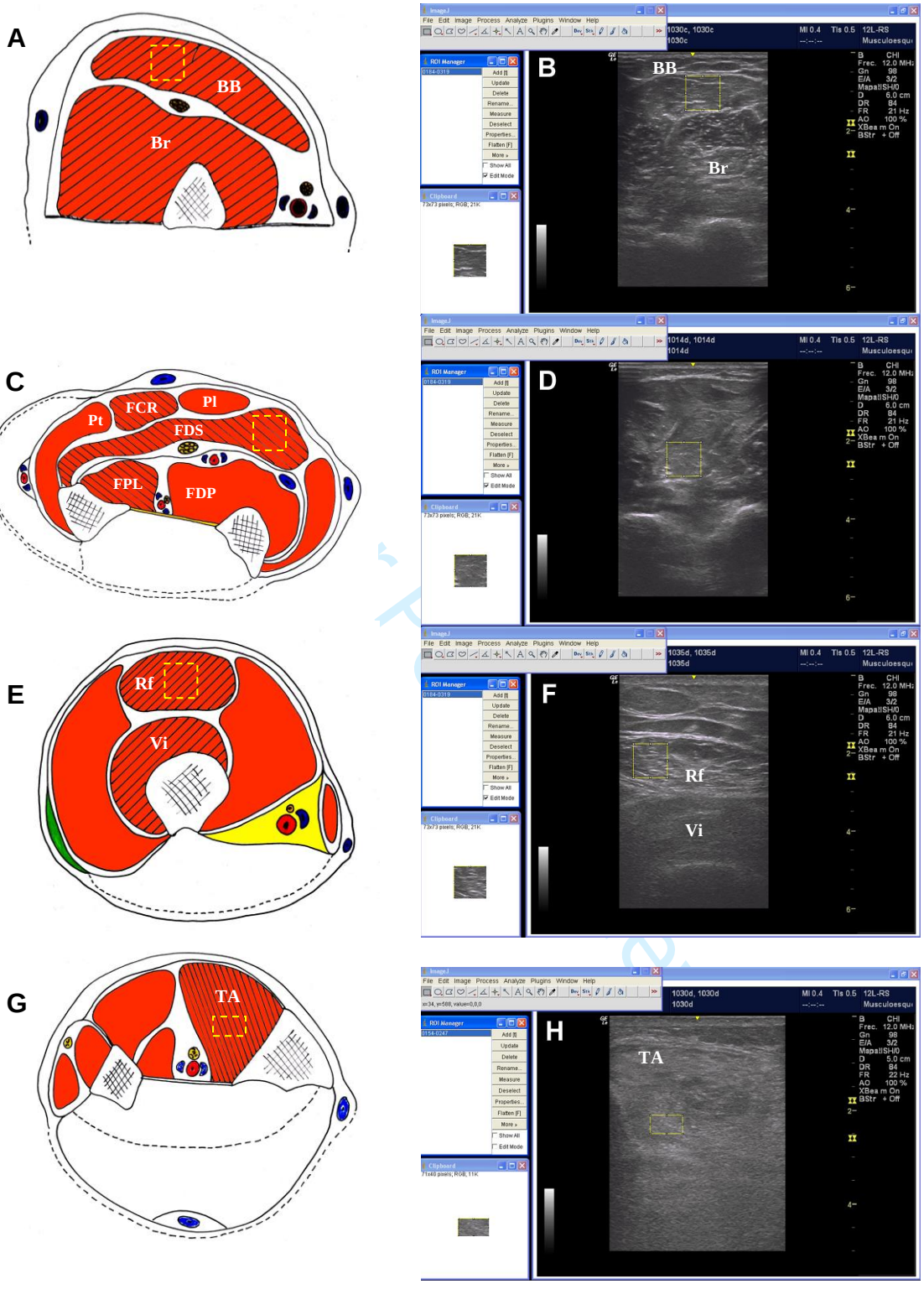
Corrected by Sex and BMI 255 logistic regression models were analyzed. AUC: area under the ROC curve. Se: sensibility. Sp: specificity. LRp: positive likelihood ratio. LRn is the inverse of negative likelihood ratio to allow for direct comparison with LRp n. P fit HL: Hosmer-Lemeshow goodness-of fit test. p-value >0.05 indicates good fit.

Table 6. Diagnostic validity for ultrasonographic and echotextural parameters in tibialis anterior.

Variables*	AUC	Se (95% CI)	Sp (95% CI)	LRp (95% CI)	LRn* (95% CI)	P fit HL
Thickness	0.861	0.77 (0.69 to 0.85)	0.79 (0.71 to 0.87)	3.64 (2.37 to 6.39)	3.42 (2.28 to 5.79)	0.434
Echointensity (EI)	0.865	0.81 (0.73 to 0.88)	0.77 (0.69 to 0.85)	3.5 (2.35 to 5.9)	4 (2.57 to 7.29)	0.836
Echovariation (EV)	0.945	0.88 (0.82 to 0.95)	0.88 (0.82 to 0.95)	7.67 (4.66 to 17.52)	7.67 (4.66 to 17.52)	0.955
GLCM	0.934	0.85 (0.78 to 0.92)	0.88 (0.82 to 0.95)	7.33 (4.39 to 16.96)	5.75 (3.69 to 11.2)	0.034
Thickness+EI	0.906	0.79 (0.71 to 0.87)	0.81 (0.73 to 0.88)	4.1 (2.65 to 7.44)	3.82 (2.52 to 6.64)	0.278
Thickness+EV	0.953	0.85 (0.78 to 0.92)	0.92 (0.87 to 0.97)	11 (6.06 to 35.61)	6 (3.91 to 11.53)	0.489
Thickness+GLCM	0.948	0.85 (0.78 to 0.92)	0.88 (0.82 to 0.95)	7.33 (4.39 to 16.96)	5.75 (3.69 to 11.2)	0.132
Maximum model	0.975	0.92 (0.87 to 0.97)	0.92 (0.87 to 0.97)	12 (6.8 to 37.9)	12 (6.8 to 37.9)	0.907

Corrected by Sex and BMI 255 logistic regression models were analyzed. AUC: area under the ROC curve. Se: sensibility. Sp: specificity. LRp: positive likelihood ratio. LRn is the inverse of negative likelihood ratio to allow for direct comparison with LRp. P fit HL: Hosmer-Lemeshow goodness-of fit test. p-value >0.05 indicates good fit.

Figure 1. Ultrasonographic scans of the biceps/brachialis (A-B), forearm flexor group (C-D), quadriceps (E-F), and tibialis anterior (G-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; FCR. Flexor carpi radialis; FDS. Flexor digitorum superficialis; Pl. Palmaris longus; FDP. Flexor digitorum profundus; FPL. Flexor pollicis longus Pt. Pronator teres Rf. Rectus femoris; TA. Tibialis anterior.Vi. Vastus intermedius.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