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Quantitative neuromuscular ultrasound analysis as biomarkers in amyotrophic lateral sclerosis.

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Abstract:	Objectives: To assess the differences in morphological and texture parameters of median nerve (MN) and abductor pollicis brevis (APB) between ALS patients and controls. Methods: The cross sectional area (CSA) of the MN and the thickness (MTh) of APB were measured bilaterally in 59 ALS recently diagnosed patients and 20 matched healthy controls. Echointensity (EI), echovariation (EV) and grey level co-occurrence matrix (GLCM) texture features of both structures were also analysed. Correlations between these parameters and clinical variables (muscle strength and disability) were analysed. Results: The CSA of MN was significantly lower in ALS patients (MD:-1.83 mm2; [95% I.C:2.89; -0.77 mm2]; p=0.01). ALS patients showed a significantly lower MTh (-2.23 mm [3.16;-1.30 mm]; p<0.001) and EV (-7.40; [11.5; -3.33]; p=0.004), and higher EI (21.2; [11.9; 30.6; p<0.001) in the APB muscle. No relevant differences were detected in GLCM features for this muscle. The model including all parameters (CSA for MN and MTh, EI and EV for APB) showed an AUC of 82% (Se 87%; Sp 42%). Muscle strength and disability correlated with APB muscle ultrasound parameters but not with those of the MN. Conclusions: APB muscle ultrasound biomarkers (especially MTh and EI) showed better discrimination capacity and correlation with clinical variables than MN biomarkers. However, the combination of both biomarkers increased their ability to detect lower motor neuron impairment, suggesting that both biomarkers could be used in a complementary manner for the diagnosis and progressions monitoring in ALS.
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Author Comments:	Dear Editor, We wish to submit an original research article entitled,

	"Quantitative neuromuscular ultrasound analysis as biomarkers in ALS" José Ríos-Díaz (PhD), María E. del Baño-Aledo (PhD), José I. Tembl-Ferrairó (MD), María J Chumillas (MD), Juan F. Vázquez-Costa (MD), Jacinto J. Martínez-Payá (PhD). for consideration by European Radiology, as part of the research topic "Imaging Biomarkers for the Diagnosis and Prognosis of Neurodegenerative Diseases". This study has been approved by the Ethics Committee for Biomedical Research of the La Fe Hospital (Valencia) and the manuscript has not been submitted for publication elsewhere. This study does not include prior published studies as well as work currently undergoing review or in press at a journal where subjects overlap with the current manuscript. The "split hand" phenomenon is a characteristic deformity of ALS that refers to a particular pattern of atrophy of the hand, which involves the thenar muscles. In this paper, we studied the ability for discrimination of several quantitative (morphological and textural) neuromuscular ultrasound biomarkers between a large sample of recently diagnosed ALS patients and healthy controls for abductor pollicis brevis muscle, characteristically involved in this phenomenon, and its corresponding median nerve. The main finding of this study is that although only 27% of recently diagnosed ALS patients started their symptoms in the upper limbs, the cross sectional area of median nerve and the muscle thickness, echointensity and echovariation of abductor pollicis brevis showed significant differences between ALS patients and healthy controls. This finding suggests that these ultrasound parameters may be involved early in the disease, even in those without clinical deterioration. We believe that this manuscript is appropriate for publication by European Radiology because we provided a new reliable, short and easy to carry out biomarker in ALS. We feel that findings from this study will be of special interest to the readers of European Radiology. We await your response and the comments of reviewers. Yours sincerely, Jacinto J. Martínez-Payá Health Sciences Department. Universidad Católica de Murcia. Campus de los Jerónimos s/n 30107 Guadalupe (Murcia). E-mail: jmartinez@ucam.edu

We have responded point-by-point to all the comments made by the reviewers pointing out where reviewers will be able to find those changes with "track changes" in Microsoft Word. We have added a copy of the rewritten manuscript "Quantitative neuromuscular ultrasound analysis as biomarkers in amyotrophic lateral sclerosis". Manuscript Number: EURA-D-18-01913.

Reviewers Comments:

- You should slightly re-organize the discussion, as recommended in the instructions to authors: The first § should summarize and interpret the results in a simple manner. The second § is a comparison with the literature, background and any useful comment. The third § is dedicated to biases and limitations. The fourth § is a short and straightforward conclusion. As not is , the first § of the discussion is only the continuation of the introduction, which is not what the readers expect at this place.

- You are right. We have modified the discussion section.

- As mentioned in my previous decision letter, you should delete all geographical information about commercial products, and also check the correct name of the company. For your information, Toshiba does not exist any more and is replaced by Canon Medical Systems.

- Thanks for the information. We made the changes in the main document.

- As mentioned also, the format of the bibliography is not correct. Please check, especially the number of cited authors. Find examples in the instructions to authors.

- We agree. We made the changes in the main document.

Other changes:

- We upload a new Title Page because the author Jacinto J. Martínez-Payá has changed his affiliation.

TITLE: Quantitative neuromuscular ultrasound analysis as biomarkers in amyotrophic lateral sclerosis.

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TITLE: Quantitative neuromuscular ultrasound analysis as biomarkers in amyotrophic lateral sclerosis.

ABSTRACT.

Objectives: To assess the differences in morphological and texture parameters of median nerve (MN) and abductor pollicis brevis (APB) between ALS patients and controls. Methods: The cross sectional area (CSA) of the MN and the thickness (MTh) of APB were measured bilaterally in 59 ALS recently diagnosed patients and 20 matched healthy controls. Echointensity (EI), echovariation (EV) and grey level co-occurrence matrix (GLCM) texture features of both structures were also analysed. Correlations between these parameters and clinical variables (muscle strength and disability) were analysed.

Results: The CSA of MN was significantly lower in ALS patients (MD:-1.83 mm^2; [95% I.C:2.89; -0.77 mm^2]; p=0.01). ALS patients showed a significantly lower MTh (-2.23 mm [3.16;-1.30 mm]; p<0.001) and EV (-7.40; [11.5; -3.33]; p=0.004), and higher EI (21.2; [11.9; 30.6; p<0.001) in the APB muscle. No relevant differences were detected in GLCM features for this muscle. The model including all parameters (CSA for MN and MTh, EI and EV for APB) showed an AUC of 82% (Se 87%; Sp 42%). Muscle strength and disability correlated with APB muscle ultrasound parameters but not with those of the MN.

Conclusions: APB muscle ultrasound biomarkers (especially MTh and EI) showed better discrimination capacity and correlation with clinical variables than MN biomarkers. However, the combination of both biomarkers increased their ability to detect LMN impairment, suggesting that both biomarkers could be used in a complementary manner for the diagnosis and progressions monitoring in ALS.

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Keywords: Amyotrophic lateral sclerosis; Biomarkers; Ultrasonography.

Key points: 1. Abductor pollicis brevis muscle and median nerve impairment are detectable by ultrasound in amyotrophic lateral sclerosis patients, even in those without clinical impairment; 2. Muscle ultrasound biomarkers show better discrimination capacity than nerve biomarkers in amyotrophic lateral sclerosis; 3. Quantitative neuromuscular ultrasound biomarkers could be usefulin a general amyotrophic lateral sclerosis population early on the disease.

Abbreviations and acronyms:

ALS. Amyotrophic lateral sclerosis.

UMN. Upper motor neuron.

LMN. Lower motor neuron.

APB. Abductor pollicis brevis.

FDI. First dorsal interosseous.

QNUS. Quantitative neuromuscular ultrasound.

CMAP. Compound muscle action potential.

MTh. Muscle thickness.

EI. Echointensity.

EV. Echovariation.

GLCM. Grey-level co-occurrence matrix.

INTRODUCTION.

Amyotrophic lateral sclerosis (ALS) is characterized by a progressive upper and lower motor neuron (UMN and LMN) degeneration that leads to muscle weakness and wasting. In ALS, certain muscle groups in the upper limbs are preferentially involved, giving rise to the "split hand" phenomenon. This refers to a particular pattern of hand atrophy, involving both the abductor pollicis brevis (APB) and the first dorsal interosseous (FDI) muscles, with relative sparing of the hypothenar muscles [1].

In the absence of specific diagnostic biomarkers, ALS diagnosis remains clinical, based on the presence of clinical UMN and LMN signs with the support of electrophysiological LMN findings [2]. Moreover, reliable progression and prognostic biomarkers are lacking.

Recently, quantitative neuromuscular ultrasound (QNUS) has been proposed as a source of LMN impairment biomarkers. More specifically, muscular ultrasound has been found useful for the diagnosis and prognosis stratification of ALS [3–6]. Most studies have found differences between ALS patients and controls in several ultrasound parameters such as thickness (MTh), echointensity (EI), echovariation (EV) and grey-level co-occurrence matrix (GLCM) texture parameters [3–7]. However, and although the split hand phenomenon is characteristic of ALS, ultrasound studies analysing changes in the involved muscle groups are scarce [8, 9].

Alternatively, the application of nerve ultrasound has identified a reduction in the cross sectional area (CSA) of some peripheral nerves in ALS patients compared with that of healthy controls [10]. This parameter has been shown to differentiate within ALS phenotypes [11] and between ALS patients and multifocal motor neuropathy [12, 13]. Moreover, longitudinal changes in CSA have been found in ALS patients, highlighting the potential of such changes as biomarkers of progressions [14]. However, results in

nerve ultrasound are controversial in ALS, probably as a result of methodological differences. Indeed, some studies reported greater changes in MN [15] others in ulnar nerve [14] and others failed to find reductions in the CSA of some nerves [11, 12, 15]. Furthermore, most studies included small samples and neither the intensity nor the texture of nerves has been analysed.

Finally, the most useful QNUS biomarkers for detecting and monitoring changes in ALS are not known, since no study has made a comparative analysis of nerve and muscle biomarkers.

Here, we aimed to study the ultrasonographic characteristics of a muscle group and its corresponding nerve involved in the split hand phenomenon in ALS. We chose the APB as representative of the split hand phenomenon because of two reasons: 1) its better accessibility to ultrasound examination and 2) there is neurophysiological evidence that changes in the APB compound muscle action potential (CMAP) are more sensitive and specific than those in FDI for the diagnosis of ALS [16]. Consequently, we assessed and compared the discriminatory capacity of several QNUS biomarkers, on both the APB muscle and in the MN, in a cohort of ALS patients and controls. We also studied the correlations of these parameters with the corresponding clinical variables.

METHODS.

This cross-sectional study was performed according to the STARD criteria for reporting diagnostic accuracy studies.

Patient selection.

Between January 2017 and February 2018, the study prospectively enrolled patients recently diagnosed (3.5 [6.27] months since diagnosis) with possible, probable or definitive ALS, according to current diagnostic criteria [2]. The number of fasciculations were also recorded, and the results, in a subgroup of these patients, have been published elsewhere [17]. All patients were diagnosed, recruited and examined by the same experienced neurologist (*BLINDED*) in the *BLINDED HOSPITAL*(*BLINDED COUNTRY*).

Twenty healthy volunteers matched for age, body mass index (BMI) and sex, without neurological conditions were recruited as controls.

Standard protocol approval, recruitment, and patient consent.

This study was approved by the ethics committee of the *BLINDED HOSPITAL*(*BLINDED COUNTRY*). All participants provided written informed consent.

Recorded clinical and neurophysiological variables.

Demographic and clinical characteristics (sex, age, weight, height, BMI, time of evolution from diagnosis) were recorded. Patients were examined by *BLINDED* on the same day that the ultrasound was performed. The disability was assessed with the ALSFRS-r scale (0-48). The upper limb subscore (UL-ALSFRS-r) corresponding to items 4-6 (0-12) was also recorded [18]. The muscle strength was measured bilaterally with the modified Medical Research Council (MRC)[19] rating scale (ranging from 0 to 5 and including grades 4- and 4+) in the wrist flexor muscles and the APB muscles,

which are both median nerve innervated. Motor nerve conduction studies in the median nerves and electromyography in the APB muscles were performed using Dantec Keypoint equipment in those patients showing no clinical LMN signs (weakness and atrophy) in APB muscles. The APB muscles were considered to be involved whenever clinical LMN signs or electrophysiological signs of chronic or acute denervation were detected.

Ultrasonography.

An experienced neuroradiologist (*BLINDED INITIALS), blinded to clinical details, performed ultrasound examinations with a Canon Medical Systems ~~Toshiba~~ Aplio XG (~~Tokyo, Japan~~ 2008) equipped with a 7-13 MHz phased-array transducer. All system-setting parameters, such as gain (80 dB), time gain compensation (in neutral position), depth, frequency (13 MHz), compression and focus were kept constant throughout the study. Participants were assessed in supine position with the arm supinated and abducted beside the body [10, 15]. To avoid oblique scanning angles the position of the transducer was adjusted until the best EI was obtained in each image [5].

Bilateral transverse ultrasound images of MN were obtained and measured at midpoint of the arm between the medial epicondyle and the axilla (Figure 1) [10]. Previous studies have assessed the MN at different levels, all favouring the study of more proximal ones [11–13]. This proximal level has also the advantage of having a greater number of axons and of avoiding other nerve lesions or entrapments that usually occur more distally. For the APB assessment, the transducer was placed along the line connecting the midpoint of the volar aspect of the first metacarpophalangeal joint and the volar prominence of the scaphoid bone (Figure 2) [20]. Three images were taken of each structure in order to minimize variation in parameters [5, 21].

The resulting bitmaps had a resolution of 716 x 537 pixels with 256 grey levels, and were stored as .TIFF files without compression or losses [22].

Image analysis.

The CSA for MN and the MTh of APB in all three images of each structure were measured using an electronic calliper equipped with an ultrasound device by an experienced ultrasonographer (*BLINDED INITIALS*) (Figures 1 and 2). The mean of the three values was used for the corresponding analysis. The CSA (mm^2) for MN was measured by tracing the nerve just inside the hyperechoic rim, corresponding to the epineurium (Figure 1) [15]. In addition, EI (0-255), EV (0-100) and GLCM texture features were analysed in both structures.

The analysis was performed by one researcher (*BLINDED*), blind to the diagnosis, using ImageJ (v.1.50) software as previously reported [5, 6]. The region of interest (ROI) was selected with the ROI Manager application for Image J. The ROI was defined as the nerve region inside the hyperechoic rim for MN (size of 18x11 pixels; 76 ppp) (Figure 1); and as the muscle region without bone and fascia with the best reflection for APB muscle (size of 71x40 pixels; 320 ppp) (Figure 2).

A set of 20 images for APB muscle and MN were re-analysed by another researcher (*BLINDED*), who was blinded to the previous results.

Statistical analysis.

Data were analysed using IBM SPSS Statistics for Windows 19.0 (IBM Company, 2010) and R software (version 3.5.0) Mean, standard deviation, range and 95% confidence intervals were calculated for continuous variables and absolute and relative frequencies for categorical variables. The significance level was fixed at 0.05 for all the statistical tests.

Baseline assessment.

Independent-sample t-tests were used to assess weight, height, BMI and age differences between groups, and the chi-square test was used to compare inter gender differences. The inter-observer reliability, was measured with the intraclass correlation coefficient (ICC).

Comparisons between groups.

Linear mixed models were used to compare all quantitative ultrasound parameters adjusted for age, sex and BMI. To account for right-left side differences between individuals, these models were extended with the variable "Side" nested to "Patient" to take into account dependency among observations. The effect size was estimated with Hedges' *g* statistic.

Diagnostic accuracy of QNUS parameters.

The ability to detect LMN impairment from/based on QNUS parameters was assessed using the presence of clinical or electrophysiological LMN signs in APB as gold standard of LMN impairment. The sensitivity (Se), specificity (Sp) and the likelihood ratios for the diagnosis of LMN impairment were calculated for all ultrasound parameters and different combinations of them with logistic regressions, adjusting for age, sex and BMI. Subsequently, we constructed receiver operating characteristic (ROC) curves. The best Se and Sp was determined from the area under the curve (AUC) and the Akaike criterion information (AIC); the Hosmer-Lemeshow goodness-of-fit test and the maximum likelihood logarithm were also used.

Clinical – QNUS variables correlations.

Multiple linear regressions were used to detect correlations between muscle and nerve ultrasound parameters and between QNUS parameters and clinical variables (MRC of wrist flexors and APB muscles, ALSFRS-r and UL-ALSFRS-r). All models were adjusted for age, sex and BMI. Data are presented as B coefficients and 95% CI. The

relation between variables was studied with a partial correlation coefficient that adjusted the linear relation between the dependent and independent variables. In addition, the goodness of fit was calculated with the partial determination coefficient (R^2 in %).

RESULTS.

Patient characteristics.

Fifty-nine recently diagnosed (3.5 [6.27] months) ALS patients and 20 healthy controls were included in this study. No differences in age, sex, weight, height and BMI were noted between them. The symptoms started in the upper limbs in 27% of the patients (Table 1).

Between groups differences.

The ICC was >0.95 in all the studied parameters for APB muscle and MN, which indicates very good inter-observer reliability.

The QNUS parameters of the APB muscle and the MN are shown in Table 2. Overall, the APB muscle showed a significantly lower MTh and EV, and higher EI (Figure 3). However, only slight differences were observed in GLCM texture features for this muscle. The CSA of MN was also significantly lower in ALS patients (Figure 3), but significant differences were not observed for EI, EV and GLCM texture features.

The magnitude of changes was greater for some APB ultrasound parameters such as MTh (25.8%), EV (16.5%) and EI (35.5%) than for the CSA (16.4%) or clinical variables (18% for MRC of APB muscle and 3.1% for the UL-ALSFRS-r scale).

Diagnostic accuracy of QNUS variables.

Table 3 show the results of the diagnostic validity for the best discriminatory QNUS variables and combinations of these variables. MTh was the single parameter with the best discriminatory potential, although the combination of several APB muscle (MTh, EI and EV) and MN (CSA) parameters provided the greatest AUC=82% (Se 87%; Sp 42%).

QNUS correlations

The CSA of the MN showed a correlation moderate with the MTh of the APB muscle (r = 0.252; r^2= 6.35%; p =0.006~~; df = 115~~) in ALS patients. However, no other significant correlations between nerve and muscle parameters were found.

Moreover, several ultrasound parameters of the APB muscle showed moderate correlations with the clinical variables (Table 4). The strongest correlations were found with the UL-ALSFRS-r. Overall, first order parameters (MTh, EV and EI) showed better correlations than second order parameters. Conversely, ultrasound parameters of the MN only correlated marginally with clinical variables (Supplementary Table 1).

DISCUSSION.

~~ALS is characterized by a progressive UMN and LMN loss. When the LMN loss begins, it is initially compensated by collateral sprouting. Therefore, although changes in reinnervation (chronic denervation) are visible by electromyography, no reduction in the CMAP is detected by electroneurography (23). When more than a third of the LMN have degenerated, mild muscle weakness and atrophy may become evident (23,24). Despite several efforts, the ability to quantify LMN loss through electrophysiological techniques is limited (23). Moreover, it is an invasive and painful technique. For these reasons, several muscular ultrasound biomarkers (measuring MTh, EI and texture) have been studied in recent years, and they have demonstrated the ability to differentiate ALS patients from healthy or disease controls (4–7). However, very few longitudinal muscle studies have been performed, with heterogeneous results (7,24). Consequently, their role as a progression biomarker has not been established. Interestingly, nerve CSA has been found to steadily decrease with disease duration in a longitudinal study in ALS and might therefore be considered a good biomarker to monitor disease progression (14). Nevertheless, texture biomarkers have not been studied in the nerves (9–14). . Moreover, a direct comparison of the performance of nerve and muscle biomarkers is lacking.~~

In this study, we aimed to address the comparison of the performance of nerve and muscle biomarkers, ~~In this study, we aimed to address is comparison,~~ by focusing on one muscle (APB), which is typically affected in ALS but which has been only scarcely studied by ultrasound, and its innervating median nerve. As has been previously observed in other muscle groups [5, 6], a decrease in MTh and EV, and an increase in EI were found. However, unlike in a previous report [6], few changes in the second order texture biomarkers were found. Differences in the disease duration and the studied

muscle groups [6, 7] could explain these discrepancies. Overall, MTh and EI showed the greatest effect sizes, and, interestingly, the magnitude of changes were higher than those found for clinical variables (UL-ALSFRS-r and MRC).

CSA values of the MN were also significantly decreased in ALS patients although changes were modest compared with the MTh and EI of the APB muscle. Our results replicate previous findings [10]. Intriguingly, other authors failed to find differences in the MN CSA in ALS patients *vs*. controls [11, 12, 15]. However, some methodological pitfalls, such as the lack of statistical power or of appropriate controls and variations in the location of the probe, could account for these differences. Finally, no significant differences in intensity or in texture parameters were found in the MN of ALS patients *vs.* controls.

~~*Diagnostic accuracy*~~

When interpreting the diagnostic accuracies, it should be considered that only 27% of the patients in our study showed upper limbs onset and that patients were in a relatively early disease stage. Thus, in a previous study with a similar proportion of upper limbs onset patients, the sensitivity of a neurophysiologic index to detect split hand changes was only 40% [16]. Interestingly, a recent study confirmed a better diagnostic accuracy for a split hand EI index (76%) than for a neurophysiologic index, highlighting the role of ultrasound for the study of the split hand phenomenon [8].

The diagnostic accuracy of the ultrasound APB muscle parameters was limited (AUC < 76%) and lower than that found in a previous study for biceps/brachialis, forearm flexors, quadriceps femoris and tibialis anterior muscle group (AUC>90%) [6]. Differences in the disease duration (3.5 *vs.*16.3 months since diagnosis) of both study populations could account for these discrepancies.

The diagnostic accuracy of the MN CSA was also limited (AUC = 72.6%), as previously reported [15]. Other authors found better diagnostic accuracy for this parameter compared with multifocal motor neuropathy, a disease which causes an enlargement of peripheral nerves [12, 13, 15].

Our results suggest that muscle biomarkers could be more sensitive for detecting LMN impairment than nerve biomarkers. Although the LMN is primarily impaired in ALS, the degeneration begins in both the neuronal soma and distal axon. Therefore, although the muscle becomes denervated relatively early, the largest portion of the axon would only later be affected by a dying-back or dying-forward process. Indeed, a previous study suggested that changes are more pronounced in cervical roots, which are more proximal [15], than in the nerves. Moreover, nerves carry both sensitive and motor fibres and sensitive fibres are usually spared in ALS. All this could result in a better diagnostic performance of muscle *vs* nerve biomarkers. Conversely, peripheral nerve biomarkers could be more useful to monitor changes in moderate to advanced stages of LMN impairment.

Despite the superiority of muscle biomarkers ~~of muscle biomarkers~~, the combination of several muscle (MTh, EI and EV) and nerve (CSA) ultrasound parameters increased the diagnostic performance up to 81.6%, beating the previously described split hand EI index [8].

~~*Clinical correlations*~~

A moderate correlation between the CSA of the MN and the MTh of the APB muscle ($r= 0.252$; $r^2= 6.35\%$; $p= 0.006$~~; df= 115~~) was found, suggesting that both parameters measure the same pathophysiological process (nerve and muscle atrophy). However, other muscle ultrasound parameters failed to correlate with the CSA.

Overall, APB muscle parameters correlated well with clinical variables of LMN impairment (APB muscle strength, global disability and upper limb disability). Conversely, as previously reported [15, 23], no relevant correlations with the MN ultrasound parameters were found. The above mentioned limitations of using ultrasound nerve parameters as biomarkers of LMN impairment could account for this lack of clinical correlation.

~~STRENGTHS AND LIMITATIONS.~~

This study analyses and compares, for the first time, several muscle and nerve morphological and texture biomarkers in ALS patients. Moreover, the focus was on a muscle group (APB) that, although typically impaired in ALS, has not been studied before. Apart from this, the main strength of this study is its cautious methodology, which follows STARD criteria. Patients, who were mostly in an early stage of the disease, were thoroughly examined, the cohort representing one of the largest studied to date. Furthermore, controls were carefully selected to match patients.

One limitation of this study is that only the upper limbs were assessed, while ALS can start in four different body regions (bulbar, cervical, dorsal and lumbar). However, although less than one third of our patients had upper limbs onset (as usually happens in ALS), we were able to identify differences between ALS patients and controls. Moreover, the magnitude of biomarker changes was greater than clinical ones. This suggests that impairment of the APB muscle and the MN nerve is a relatively early phenomenon in ALS, and both are measurable even in the absence of clinical impairment.

Another limitation is that, while the split hand phenomenon involves several muscle groups (APB, opponens pollicis, FDI, hypothenar muscles) and two nerves (median and ulnar), in our study only one muscle (APB) and one nerve (FDI) was studied. However,

our diagnostic accuracy combining the APB muscle biomarkers and CSA of the MN was superior to an EI index which combined of several muscle groups in the thenar and hypothenar eminences (81.6% *vs* 76%).

In our study the weight, height and BMI were matched for patients and controls. However, other anthropometric measures such as the size of the hand could also be considered in future studies.

Finally, specific transducers with a higher frequency and therefore with a higher surface resolution could provide better results.

~~**CONCLUSIONS.**~~

As conclusions, APB muscle ultrasound biomarkers (especially MTh and EI) showed better discrimination capacity and correlation with clinical variables than MN biomarkers in a cohort of recently diagnosed ALS patients with different regions of onset. However, the combination of both biomarkers increased their ability to detect LMN impairment, suggesting that both biomarkers could be used in a complementary manner for the diagnosis and progressions monitoring in ALS irrespectively of the region of onset. Multicentric large, longitudinal studies are warranted to confirm its utility in the clinical practice and clinical trials.

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Table 1

Baseline characteristics	ALS Patients (n=59)	Healthy controls (n=20)	p-value
Males (n) (%)	35 (59.3 %)	10 (50 %)	0.467
Age (years)	63.5 (10.89); 38.1 to 82.2	60.2 (9.97); 40.2 to 70.9	0.242
Weight (kg)	70.6 (11.57); 45 to 101	70.2 (12.24); 49 to 92	0.895
Height (m)	1.64 (0.109); 1.42 to 1.92	1.62 (0.092); 1.48 to 1.77	0.349
BMI (kg/m^2)	26.2 (4.01); 16.6 to 34.5	26.6 (3.16); 22.1 to 33.8	0.595
Time from diagnosis (months)	3.5 (6.25); 2.3 to 4.6		
Disease onset (n) (%)			
- Upper Limb	16 (27.1%)		
- Lower Limb	28 (47.5%)		
- Bulbar	15 (25.4%)		
ALSFRS-r (max. 48)	38.5 (5.44); 25 to 46		
UL-ALSFRS-r (max. 12)	8.9 (2.67); 1 to 12		
MRC wrist flexor muscles (max. 5)	4.6 (0.69); 2 to 5		
MRC APB muscle (max. 5)	4.1 (0.97); 1 to 5		

Data are presented as mean (Standard Deviation); Range. P-value for Chi-Square (Sex), and T-Student for independent samples. ALSFRS-r: Amyotrophic Lateral Sclerosis Rating Scale revised. UL-ALSFRS-r: Upper limb subscore of the ALSFRS-r. MRC: Medical Research Council. APB: abductor pollicis brevis muscle.

Table 2

QNUS parameters	ALS patients (n=59)		Healthy controls (n=20)		p-value	Effect size*
	Mean (SD)	95% CI	Mean (SD)	95% CI		
			Median nerve.			
Cross sectional area	9.2 (2.87)	8.7 to 9.7	11.0 (2.91)	10.1 to 12.0	**0.010**	0.62
Echointensity	83.7 (22.83)	79.6 to 87.9	90.0 (23.1)	82.7 to 97.2	0.309	0.27
Echovariation	34.7 (10.84)	32.7 to 36.7	32.1 (10.97)	28.6 to 35.5	0.507	0.24
GLCM textural features						
Energy	31.54 (3.533)	30.9 to 32.19	30.6 (3.575)	29.48 to 31.72	0.124	0.26
Contrast	909 (461.2)	824.8 to 993	1039 (466.7)	893 to 1185	0.335	0.28
Textural correlation	62.1 (29.29)	56.8 to 67.4	58.0 (29.64)	48.7 to 67.2	0.814	0.14
Homogeneity	1.05 (0.258)	1.0 to 1.1	1.05 (0.261)	0.96 to 1.13	0.965	0.02
Entropy	5.83 (0.164)	5.8 to 5.86	5.88 (0.166)	5.83 to 5.93	0.068	0.30
			Abductor pollicis brevis.			
Thickness	6.6 (2.52)	6.2 to 7.1	8.9 (2.55)	8.1 to 9.7	**<0.001**	0.91
Echointensity	81.3 (25.38)	76.6 to 85.9	60.0 (25.68)	52.0 to 68.1	**<0.001**	0.83
Echovariation	37.4 (11.05)	35.4 to 39.4	44.8 (11.18)	41.3 to 48.3	**0.004**	0.66
GLCM textural features						
Energy	6.24 (1.791)	5.92 to 6.57	7.02 (1.812)	6.46 to 7.59	0.119	0.43
Contrast	345 (123.3)	323 to 368	279 (124.8)	240 to 318	**0.036**	0.53
Textural correlation	50.9 (20.79)	47.1 to 54.7	58.9 (21.03)	52.3 to 65.5	0.107	0.38
Homogeneity	1.36 (0.205)	1.32 to 1.40	1.39 (0.207)	1.32 to 1.45	0.797	0.15
Entropy	7.70 (0.217)	7.66 to 7.74	7.61 (0.219)	7.54 to 7.68	0.113	0.41

SD: standard deviation. 95% CI: 95% confidence interval. GLCM: grey-level co-occurrence matrix. QNUS: quantitative neuromuscular ultrasound. * Hedge's *g.*

Table 3

QNUS variables*	AUC	Se (95% CI)	Sp (95% CI)	LLR+ (95% CI)	LLR-** (95% CI)	P fit HL
CSA (MN)	0.726	0.92 (0.87 to 0.99)	0.27 (0.18 to 0.35)	1.27 (0.45 to 3.57)	3.57 (13.52 to 0.94)	0.808
MTh (APB)	0.754	0.85 (0.73 to 0.95)	0.33 (0.25 to 0.4)	1.26 (0.5 to 3.16)	2.17 (1.43 to 3.29)	0.257
EI (APB)	0.727	0.95 (0.87 to 1.01)	0.19 (0.13 to 0.25)	1.18 (0.32 to 4.25)	3.78 (0.57 to 24.95)	0.153
EV (APB)	0.655	0.91 (0.81 to 0.99)	0.08 (0.04 to 0.12)	0.98 (0.11 to 8.62)	0.82 (0.27 to 2.49)	0.045
CSA (MN). + MTh (APB)	0.790	0.89 (0.83 to 0.97)	0.38 (0.29 to 0.48)	1.44 (0.64 to 3.23)	3.4 (1.56 to 7.42)	0.561
CSA (MN) + MTh-EI-EV (APB)	0.816	0.87 (0.8 to 0.95)	0.42 (0.33 to 0.52)	1.5 (0.71 to 3.19)	3.2 (1.89 to 5.44)	0.554

*1024 models were analyzed. AUC: area under the ROC curve. Se: sensibility. Sp: specificity. LR: Likelihood ratio. LR-** is the inverse of LR- for better interpretation. P fit HL: Hosmer-Lemeshow goodness-of fit test. p-value >0.05 indicates a good fit. MN: median nerve. APB: abductor pollicis brevis. CSA: cross sectional area. MTh: Thickness. EI: echointensity. EV: echovariation.

Table 4

QNUS variables	B coefficient (SE)	95% CI for B	p-value	R (R_p^2 %)
	MRC APB muscle			
MTh	0.122 (0.032)	0.059 to 0.19	**<0.001**	0.333 (11.1%)
EI*	-0.009 (0.003)	-0.02 to -0.003	**0.005**	-0.258 (6.6%)
EV*	0.021 (0.008)	0.005 to 0.04	**0.009**	0.240 (5.7%)
GLCM textural features				
Energy*	0.121 (0.051)	0.02 to 0.22	**0.019**	0.217 (4.7%)
Contrast*	-0.002 (0.001)	-0.003 to 0	**0.012**	-0.231 (5.3%)
Textural correlation*	0.007 (0.004)	-0.001 to 0.02	0.103	0.152 (2.3%)
Homogeneity*	1.429 (0.426)	0.584 to 2.27	**0.001**	0.298 (8.9%)
Entropy*	-0.99 (0.409)	-1.799 to -0.18	**0.017**	-0.22 (4.9%)
	ALSFRS-r			
MTh*	0.808 (0.175)	0.461 to 1.16	**<0.001**	0.395 (15.6%)
EI	-0.006 (0.002)	-0.01 to 0	**0.006**	-0.252 (6.4%)
EV**	0.094 (0.045)	0.004 to 0.18	**0.040**	0.19 (3.6%)
GLCM textural features				
Energy**	0.66 (0.279)	0.108 to 1.21	**0.020**	0.216 (4.7%)
Contrast**	-0.009 (0.004)	-0.016 to -0.002	**0.012**	-0.231 (5.4%)
Textural correlation**	0.041 (0.022)	-0.003 to 0.08	0.067	0.17 (2.9%)
Homogeneity**	7.259 (2.403)	2.5 to 12.02	**0.003**	0.271 (7.4%)
Entropy**	-4.926 (2.251)	-9.386 to -0.47	**0.031**	-0.2 (4.0%)
	UL-ALSFRS-r			
MTh	0.347 (0.088)	0.172 to 0.52	**<0.001**	0.342 (11.71%)
EI	-0.037 (0.008)	-0.053 to -0.02	**<0.001**	-0.388 (15.08%)
EV	0.092 (0.021)	0.05 to 0.13	**<0.001**	0.376 (14.15%)
GLCM textural features				
Energy	0.445 (0.137)	0.174 to 0.72	**0.002**	0.289 (8.35%)
Contrast	-0.006 (0.002)	-0.01 to 0	**<0.001**	-0.32 (10.23%)
Textural correlation	0.025 (0.011)	0.003 to 0.05	**0.026**	0.205 (4.2%)
Homogeneity	5.617 (1.131)	3.378 to 7.86	**<0.001**	0.419 (17.55%)
Entropy	-3.228 (1.11)	-5.425 to -1.03	**0.004**	-0.261 (6.8%)

SE=standard error. 95% CI=95% confidence interval. The dependent variables were the MRC for hand flexion, MRC for abduction, ALSFRS-r and UP-ALSFRs-r (upper limbs) subscale. Rp = partial correlation coefficient. Rp^2= partial determination coefficient in %. *Adjusted by age. **Adjusted by sex. Mth: muscular thickness. EI: echointensity. EV: echovariation. GLCM: grey-level co-occurrence matrix.

Figure 1

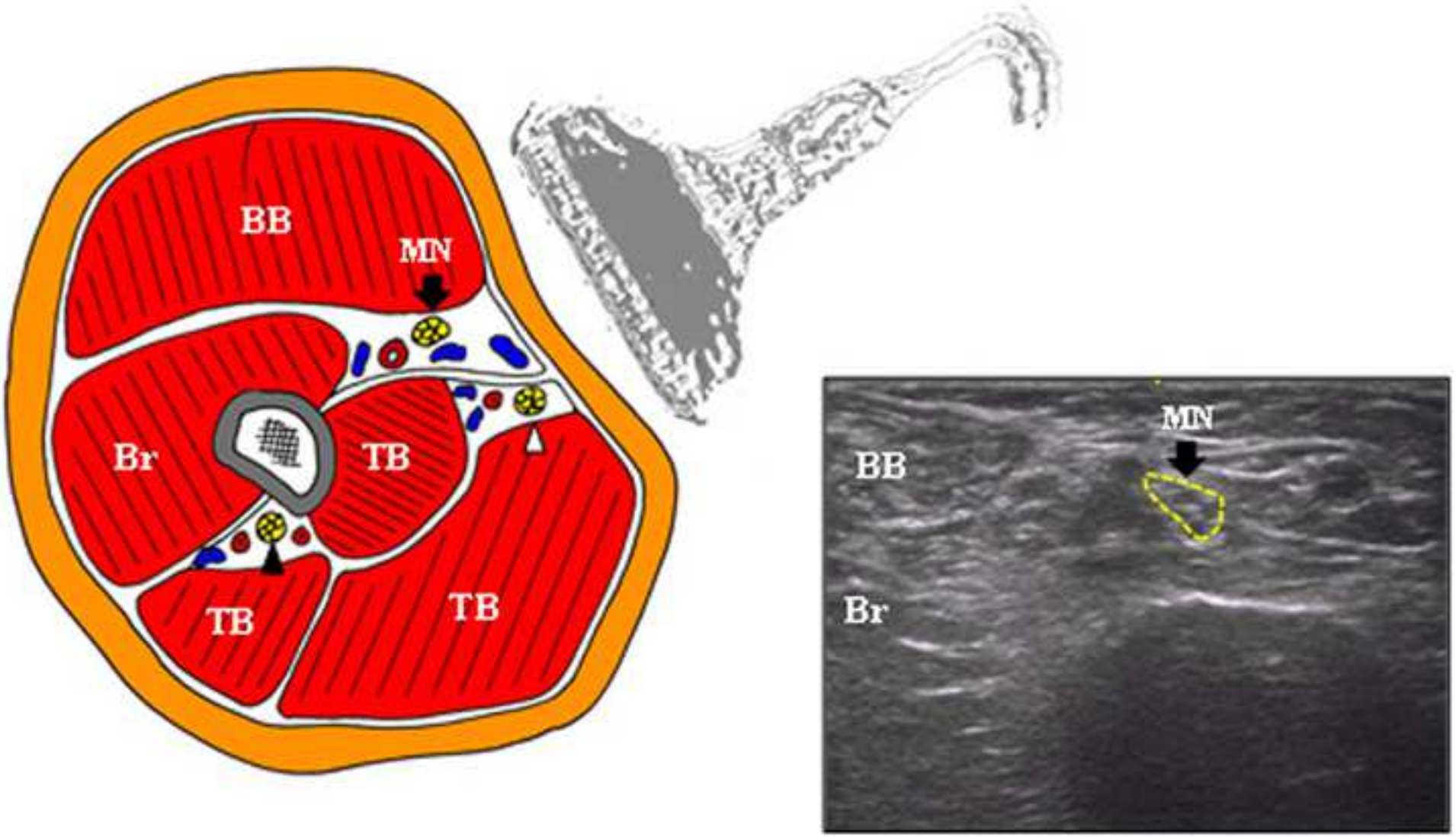

Figure 2

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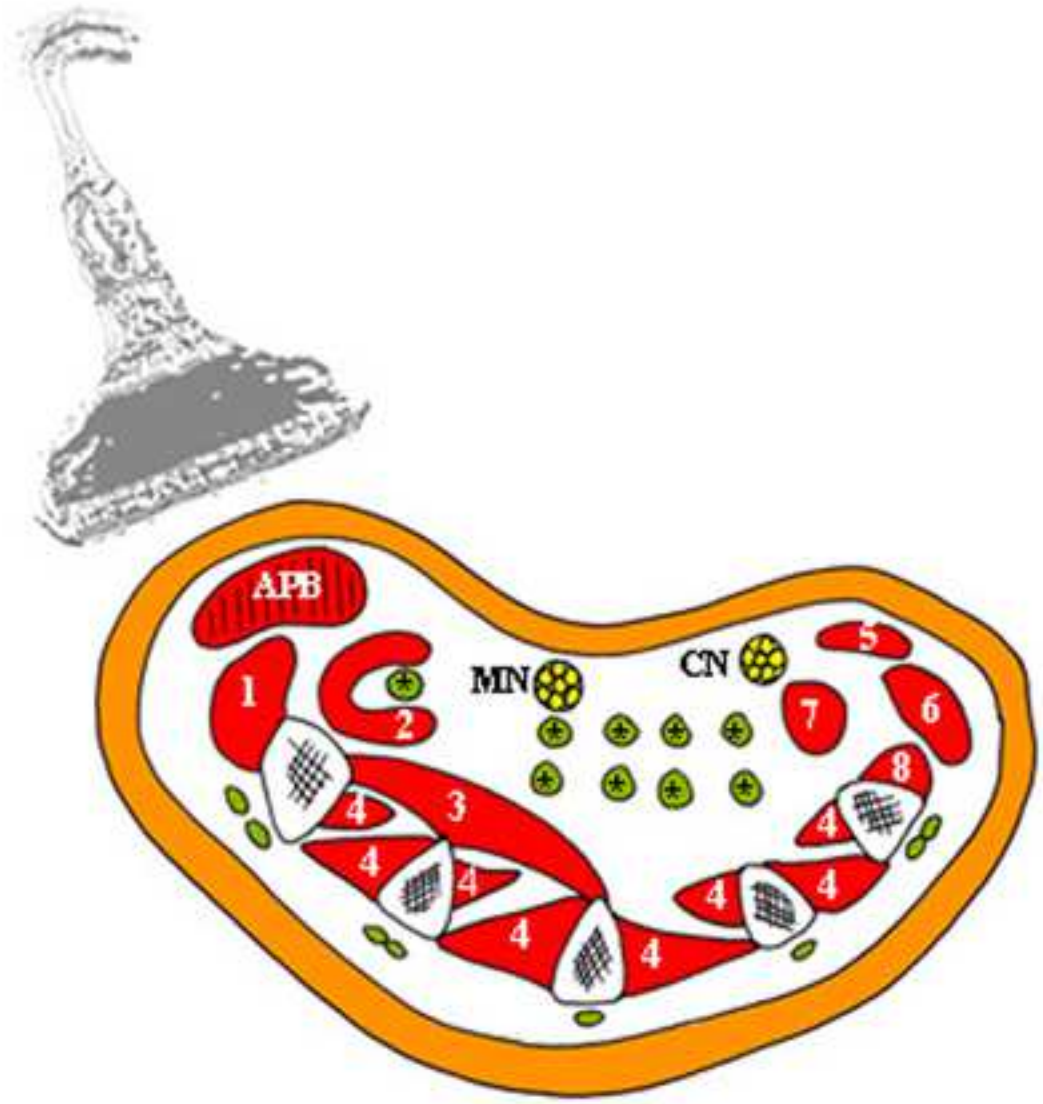

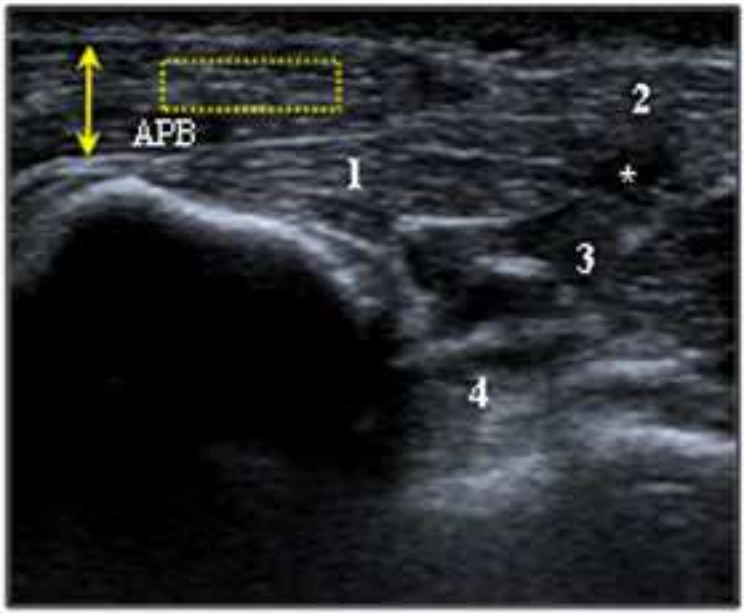

Figure 3

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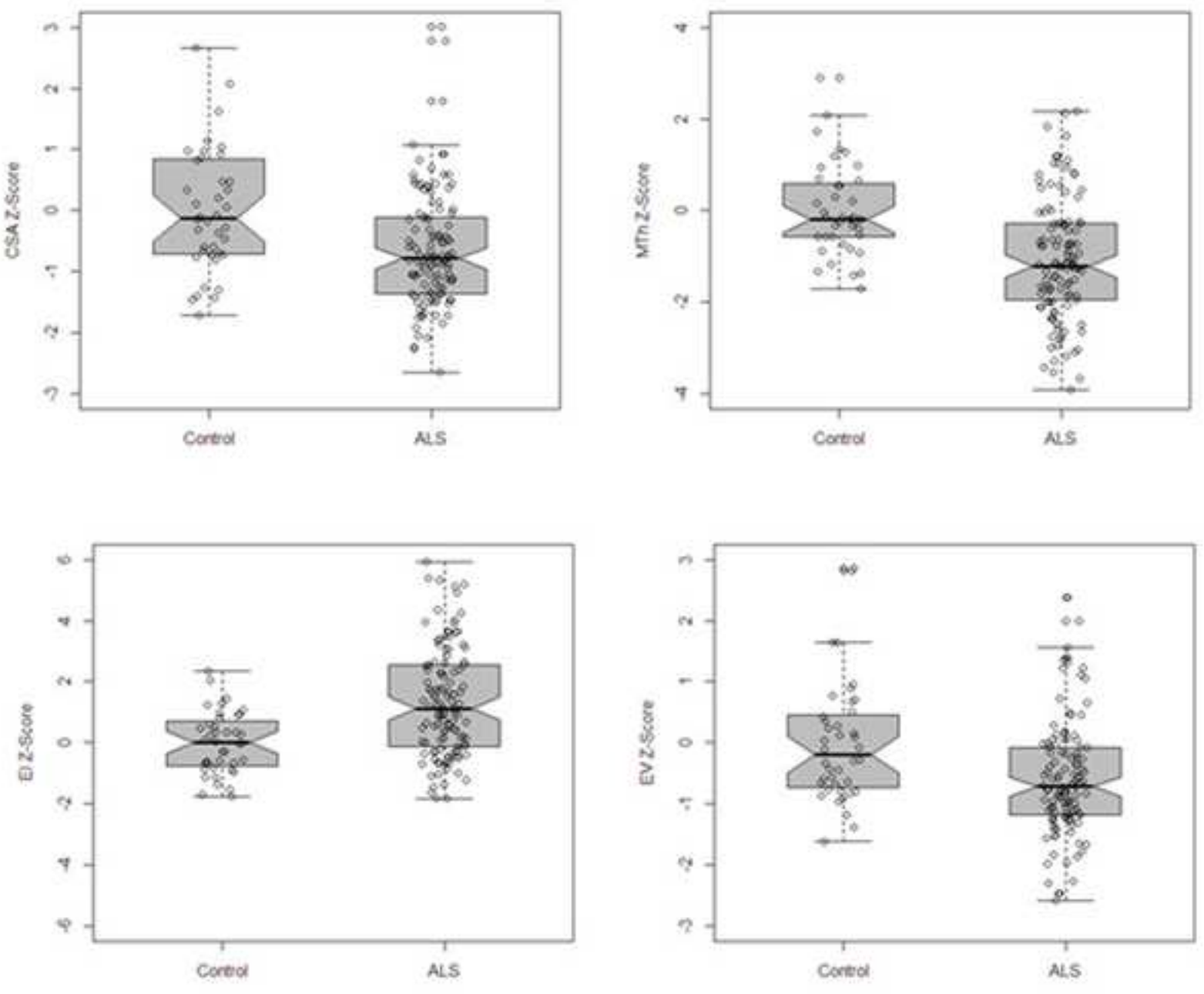

Compliance with ethical standards:
This study was approved by the ethics committee of the Hospital La Fe of Valencia (Spain) and performed following the Helsinki Declaration principles.

Guarantor:
The scientific guarantor of this publication is Department of Neurology (Hospital Universitario y Politécnico La Fe (Valencia). Spain.

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- Prospective.
- Case-control study.
- Multicentre study.