European Radiology (2019) 29:4266–4275
https://doi.org/10.1007/s00330-018-5943-8

ULTRASOUND

Quantitative neuromuscular ultrasound analysis as biomarkers in amyotrophic lateral sclerosis

José Ríos-Díaz[1,2] · María Elena del Baño-Aledo[3] · José I. Tembl-Ferrairó[4] · Maria J. Chumillas[4,5,6] · Juan F. Vázquez-Costa[5,6,7] · Jacinto J. Martínez-Payá[3]

Received: 30 July 2018 / Revised: 9 November 2018 / Accepted: 30 November 2018 / Published online: 21 January 2019

Abstract

Objectives To assess the differences in morphological and texture parameters of median nerve (MN) and abductor pollicis brevis (APB) between amyotrophic lateral sclerosis (ALS) patients and controls.

Methods The cross-sectional area (CSA) of the MN and the muscle thickness (MTh) of APB were measured bilaterally in 59 recently diagnosed ALS 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% CI = 2.89; − 0.77 mm^2]; $p = 0.01$). ALS patients showed 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% (sensitivity 87%; specificity 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 progression monitoring in ALS.

Key Points

- *Abductor pollicis brevis muscle and median nerve impairment is detectable by ultrasound in amyotrophic lateral sclerosis patients, even in those without clinical impairment.*
- *Muscle ultrasound biomarkers show better discrimination capacity than nerve biomarkers in amyotrophic lateral sclerosis.*
- *Quantitative neuromuscular ultrasound biomarkers could be useful in a general amyotrophic lateral sclerosis population early on the disease.*

Keywords Amyotrophic lateral sclerosis · Biomarkers · Ultrasonography

Juan F. Vázquez-Costa and Jacinto J. Martínez-Payá contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00330-018-5943-8) contains supplementary material, which is available to authorized users.

✉ María Elena del Baño-Aledo
mbano@um.es

[1] Centro de Ciencias de la Salud San Rafael, Universidad Antonio de Nebrija, Paseo de la Habana 70 bis, 28036 Madrid, Spain

[2] Fundación San Juan de Dios, Madrid, Spain

[3] Physiotherapy Department, Facultad de Medicina, Universidad de Murcia, Campus de Espinardo, 30100 Murcia, Spain

[4] Department of Neurophysiology, Hospital Universitario y Politécnico La Fe, Valencia, Spain

[5] Neuromuscular and Ataxias Research Unit, Instituto de Investigación Sanitaria La Fe (IIS La Fe), Valencia, Spain

[6] Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Valencia, Spain

[7] Department of Neurology, Hospital Universitario y Politécnico La Fe, Valencia, Spain

Abbreviations

ALS	Amyotrophic lateral sclerosis
APB	Abductor pollicis brevis
CMAP	Compound muscle action potential
EI	Echointensity
EV	Echovariation
FDI	First dorsal interosseous
GLCM	Grey-level co-occurrence matrix
LMN	Lower motor neuron
MTh	Muscle thickness
QNUS	Quantitative neuromuscular ultrasound
UMN	Upper motor neuron

Introduction

Amyotrophic lateral sclerosis (ALS) is characterised by a progressive upper motor neuron (UMN) and lower motor neuron (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 muscle 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 median nerve (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, both on 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 (Standards for Reporting of Diagnostic Accuracy Studies) 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 was 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 (JFV-C) in the ALS Unit of Hospital La Fe (Valencia, Spain).

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 Hospital La Fe of Valencia (Spain). 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 JFV-C on the same day that the ultrasound was performed. The disability was assessed with the Amyotrophic Lateral Sclerosis Functional Rating Scale revised (ALSFRS-r) scale (0–48). The upper limb subscore of the ALSFRS-r (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 (JIT-F), blinded to clinical details, performed ultrasound examinations with the Canon Medical Systems Aplio XG (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 the midpoint of the arm between the medial epicondyle and the axilla (Fig. 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 (Fig. 2) [20]. Three images of each structure were taken in order to minimise variation in parameters [5, 21].

The resulting bitmaps had a resolution of 716 × 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 (JJM-P) (Figs. 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 (Fig. 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 (JR-D), 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 ImageJ. The ROI was defined as the nerve region inside the hyperechoic rim for MN (size of 18 × 11 pixels; 76 ppp) (Fig. 1) and as the muscle

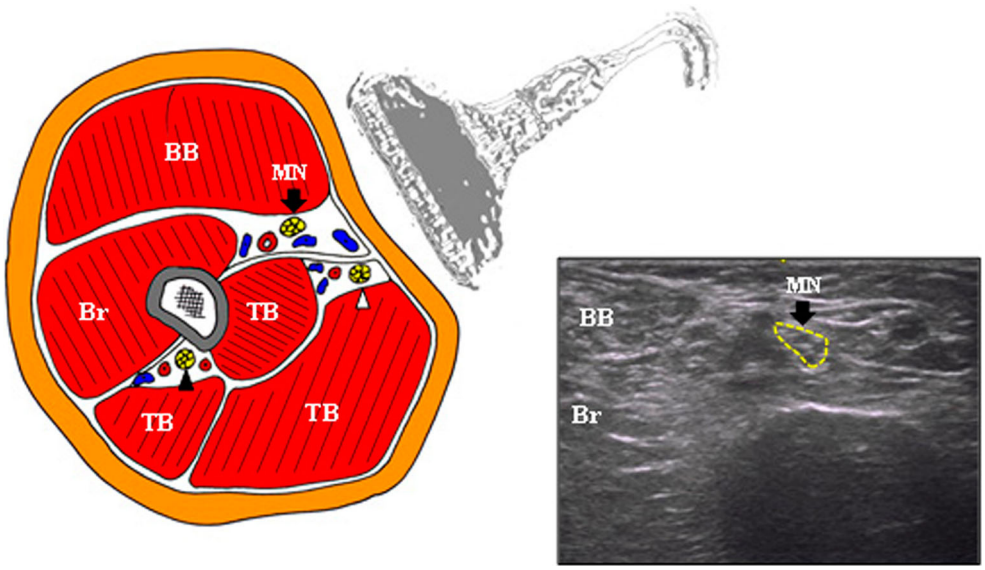

Fig. 1 Ultrasonographic scan of the MN. The CSA of the MN is delimited with a dotted yellow line. BB, biceps brachialis muscle; Br, brachialis muscle; MN, median nerve; TB, triceps brachialis muscle; black arrowhead, radial nerve; white arrowhead, cubital nerve

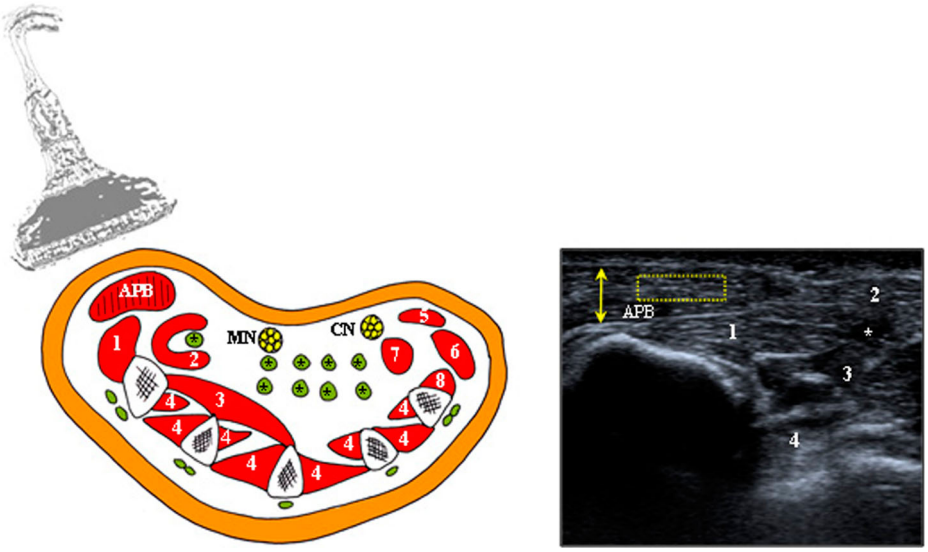

Fig. 2 Ultrasonographic scan of the APB muscle. The arrow measures the muscle thickness, whereas the ROI is represented with a rectangle. CN, cubital nerve; MN, median nerve; 1, opponens pollicis muscle; 2, flexor pollicis brevis muscle; 3, adductor pollicis muscle; 4, interosseus muscles; 5, short palmar muscle; 6, abductor digiti minimi muscle; 7, flexor digiti minimi brevis muscle; 8, opponens digiti minimi muscle; asterisk, flexor tendons

region without bone and fascia with the best reflection for APB muscle (size of 71 × 40 pixels; 320 ppp) (Fig. 2).

A set of 20 images for APB muscle and MN were re-analysed by another researcher (MEDB-A), 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 intergender 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 the gold standard of LMN impairment. The sensitivity (Se), the 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 were 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.

Correlations between QNUS and clinical variables

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 interobserver 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 (Fig. 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 (Fig. 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 shows 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 equal to 82% (Se 87%; Sp 42%).

QNUS correlations

The CSA of the MN showed a moderate correlation with the MTh of the APB muscle ($r = 0.252$; $r^2 = 6.35\%$; $p = 0.006$) 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

In this study, we aimed to address the comparison of the performance of nerve and muscle biomarkers, 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 that found for clinical variables (UL-ALSFRS-r and MRC).

Table 1 Baseline characteristics

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 Student's t test for independent samples

ALSFRS-r Amyotrophic Lateral Sclerosis Functional Rating Scale revised, *UL-ALSFRS-r* upper limb subscore of the ALSFRS-r, *MRC* Medical Research Council, *APB* abductor pollicis brevis muscle

Table 2 Differences in QNUS parameters between ALS patients and healthy control groups for the MN and the APB muscle

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*

Italics are used in significative *p* values

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.

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 the biceps/brachialis, forearm flexors, quadriceps femoris and tibialis anterior muscle group (AUC > 90%) [6]. Differences in the disease duration (3.5 months 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.

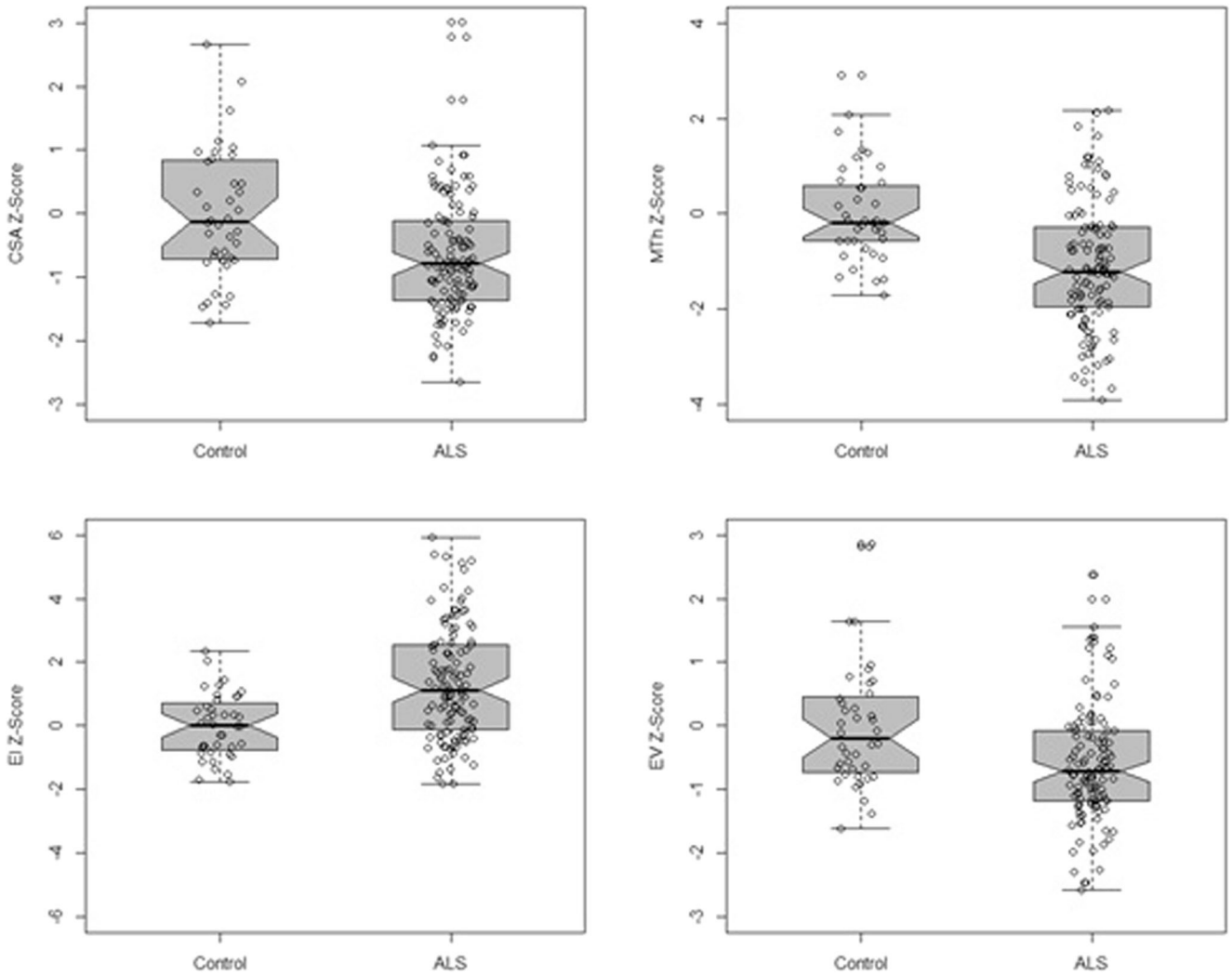

Fig. 3 Boxplot differences in the cross-sectional area (CSA) of the MN and the muscle thickness (MTh), echointensity (EI) and echovariation (EV) of the APB muscle between healthy control groups and ALS patients

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.

Table 3 Diagnostic accuracy of the QNUS parameters

QNUS variables*	AUC	Se (95% CI)	Sp (95% CI)	LR+ (95% CI)	LR−** (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

p value > 0.05 indicates a good fit

AUC area under the ROC curve, *Se* sensitivity, *Sp* specificity, *LR* likelihood ratio, *p fit HL* Hosmer-Lemeshow goodness-of fit test, *MN* median nerve, *APB* abductor pollicis brevis, *CSA* cross-sectional area, *MTh* muscle thickness, *EI* echointensity, *EV* echovariation

*One thousand twenty-four models were analysed

**The inverse of LR− for better interpretation

Table 4 Correlations between the ultrasound parameters of the APB muscle and clinical variables

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)

The dependent variables were the MRC for hand flexion, MRC for abduction, as well as ALSFRS-r and UP-ALSFRs-r (upper limbs) subscale. R_p is the partial correlation coefficient, and R_p^2 is the partial determination coefficient in %

SE standard error, *95% CI* 95% confidence interval, *MTh* muscle thickness, *EI* echointensity, *EV* echovariation, *GLCM* grey-level co-occurrence matrix

Italics are used in significative *p* values

*Adjusted by age

**Adjusted by sex

Despite the superiority 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].

A moderate correlation between the CSA of the MN and the MTh of the APB muscle ($r = 0.252$; $r^2 = 6.35\%$; $p = 0.006$) 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.

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) were studied. However, our diagnostic accuracy combining the APB muscle biomarkers and CSA of the MN was superior to an EI index which combined 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.

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 progression monitoring in ALS irrespective of the region of onset. Multicentric large, longitudinal studies are warranted to confirm its utility in the clinical practice and clinical trials.

Acknowledgments This study was approved by the ethics committee of the Hospital La Fe of Valencia (Spain) and performed following the Helsinki Declaration principles.

Compliance with ethical standards

Guarantor The scientific guarantor of this publication is Juan F. Vázquez-Costa, MD.

Conflict of interest The authors declare that they have no competing interests.

Statistics and biometry One of the authors has significant statistical expertise.

Informed consent Written informed consent was obtained from all subjects (patients) in this study.

Ethical approval Institutional Review Board approval was obtained.

Methodology
- Prospective
- Case-control study
- Multicentre study

References

1. Eisen A, Kuwabara S (2012) The split hand syndrome in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 83(4):399–403
2. Brooks BR, Miller RG, Swash M, Munsat TL (2000) El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 1(5):293–299
3. Arts IM, Overeem S, Pillen S, Schelhaas HJ, Zwarts MJ (2011) Muscle ultrasonography to predict survival in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 82(5):552–554
4. Arts IM, Overeem S, Pillen S et al (2012) Muscle ultrasonography: a diagnostic tool for amyotrophic lateral sclerosis. Clin Neurophysiol 123(8):1662–1667
5. Martínez-Payá JJ, del Baño-Aledo ME, Ríos-Díaz J, Tembl-Ferrairó JI, Vázquez-Costa JF, Medina-Mirapeix F (2017) Muscular echovariation: a new biomarker in amyotrophic lateral sclerosis. Ultrasound Med Biol 43(6):1153–1162
6. Martínez-Payá JJ, Ríos-Díaz J, Del Baño-Aledo ME, Tembl-Ferrairó JI, Vazquez-Costa JF, Medina-Mirapeix F (2017) Quantitative muscle ultrasonography using textural analysis in amyotrophic lateral sclerosis. Ultrason Imaging 39(6):357–368
7. Martínez-Payá JJ, Ríos-Díaz J, Medina-Mirapeix F, Vázquez-Costa JF, del Baño-Aledo ME (2018) Monitoring progression of amyotrophic lateral sclerosis using ultrasound morpho-textural muscle biomarkers: a pilot study. Ultrasound Med Biol 44(1):102–109
8. Seok HY, Park J, Kim YH, Oh KW, Kim SH, Kim BJ (2018) Split hand muscle echo intensity index as a reliable imaging marker for differential diagnosis of amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 89(9):943–948
9. Grimm A, Prell T, Décard BF et al (2015) Muscle ultrasonography as an additional diagnostic tool for the diagnosis of amyotrophic lateral sclerosis. Clin Neurophysiol 126(4):820–827
10. Cartwright MS, Walker FO, Griffin LP, Caress JB (2011) Peripheral nerve and muscle ultrasound in amyotrophic lateral sclerosis. Muscle Nerve 44(3):346–351
11. Schreiber S, Abdulla S, Debska-Vielhaber G et al (2015) Peripheral nerve ultrasound in amyotrophic lateral sclerosis phenotypes. Muscle Nerve 51(5):669–675
12. Grimm A, Décard BF, Athanasopoulou I, Schweikert K, Sinnreich M, Axer H (2015) Nerve ultrasound for differentiation between

amyotrophic lateral sclerosis and multifocal motor neuropathy. J Neurol 262(4):870–880
13. Loewenbrück KF, Liesenberg J, Dittrich M et al (2016) Nerve ultrasound in the differentiation of multifocal motor neuropathy (MMN) and amyotrophic lateral sclerosis with predominant lower motor neuron disease (ALS/LMND). J Neurol 263(1):35–44
14. Schreiber S, Dannhardt-Stieger V, Henkel D et al (2016) Quantifying disease progression in ALS using peripheral nerve sonography. Muscle Nerve 54(3):391–397
15. Nodera H, Takamatsu N, Shimatani Y et al (2014) Thinning of cervical nerve roots and peripheral nerves in ALS as measured by sonography. Clin Neurophysiol 125(9):1906–1911
16. Kuwabara S, Sonoo M, Komori T et al (2008) Dissociated small hand muscle atrophy in amyotrophic lateral sclerosis: frequency, extent, and specificity. Muscle Nerve 37(4):426–430
17. Vázquez-Costa JF, Campins-Romeu M, Martínez-Payá JJ et al (2018) New insights into the pathophysiology of fasciculations in amyotrophic lateral sclerosis: an ultrasound study. Clin Neurophysiol. https://doi.org/10.1016/j.clinph.2018.09.014
18. Cedarbaum JM, Stambler N, Malta E et al (1999) The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J Neurol Sci 169(1–2):13–21
19. Florence JM, Pandya S, King WM et al (1992) Intrarater reliability of manual muscle test (Medical Research Council scale) grades in Duchenne's muscular dystrophy. Phys Ther 72(2):115–122
20. Hokkoku K, Matsukura K, Uchida Y et al (2017) Quantitative muscle ultrasound is useful for evaluating secondary axonal degeneration in chronic inflammatory demyelinating polyneuropathy. Brain Behav 7(10):e00812
21. Arts IM, van Rooij FG, Overeem S et al (2008) Quantitative muscle ultrasonography in amyotrophic lateral sclerosis. Ultrasound Med Biol 34(3):354–361
22. Wiggins RH 3rd, Davidson HC, Harnsberger HR, Lauman JR, Goede PA (2001) Image file formats: past, present, and future. Radiographics 21(3):789–798
23. Cartwright MS, Shin HW, Passmore LV, Walker FO (2009) Ultrasonographic reference values for assessing the normal median nerve in adults. J Neuroimaging 19(1):47–51