Clinical Neurophysiology 129 (2018) 2650–2657

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

New insights into the pathophysiology of fasciculations in amyotrophic lateral sclerosis: An ultrasound study

J.F. Vázquez-Costa [a,b,c,*], M. Campins-Romeu [b], J.J. Martínez-Payá [d], J.I. Tembl [e], M.E. del Baño-Aledo [f], J. Ríos-Díaz [g,h], V. Fornés-Ferrer [i], M.J. Chumillas [a,c,j], T Sevilla [a,b,c,k]

[a] Neuromuscular Research Unit, Instituto de Investigación Sanitaria la Fe (IIS La Fe), Valencia, Spain
[b] ALS Unit, Department of Neurology, Hospital Universitario y Politécnico La Fe, Valencia, Spain
[c] Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Valencia, Spain
[d] ECOFISTEM Research Group, Health Sciences Department, Facultad de Ciencias de la Salud, Universidad Católica de Murcia, Campus de los Jerónimos s/n, 30107 Guadalupe (Murcia), Spain
[e] Neurosonology Laboratory, Department of Neurology, Hospital Universitario y Politécnico La Fe, Valencia, Spain
[f] Physiotherapy Department, Facultad de Medicina, Campus de Espinardo 30100, Universidad de Murcia, Spain
[g] Centro de Ciencias de la Salud San Rafael, Universidad Antonio de Nebrija, Madrid, Paseo de la Habana 70 bis, 28036 Madrid, Spain
[h] Fundación San Juan de Dios, Madrid, Spain
[i] Biostatistics Unit, Instituto de Investigación Sanitaria la Fe (IIS La Fe), Valencia, Spain
[j] ALS Unit, Department of Neurophisiology, Hospital Universitario y Politécnico La Fe, Valencia, Spain
[k] Department of Medicine, University of Valencia, Valencia, Spain

See Editorial, pages 2638–2639

ARTICLE INFO

Article history:
Accepted 16 September 2018
Available online 24 September 2018

Keywords:
Amyotrophic lateral sclerosis
Fasciculations
Ultrasound
Hyperexcitability
Pathophysiology

HIGHLIGHTS

- Fasciculations are more frequent in proximal and cervical muscles and in upper limb onset patients.
- Fasciculations associate directly with BMI loss and upper motor neuron impairment and inversely with disability.
- Fasciculations increase with the initial lower motor neuron impairment but they decrease when it progresses.

ABSTRACT

Objective: To describe the fasciculation pattern in ALS and to analyse its clinical and pathophysiological significance.
Methods: Ultrasound of 19 muscles was performed in 44 patients with a recent diagnosis (<90 days) of ALS. The number of fasciculations was recorded in each muscle and the muscle thickness and strength were additionally measured in limb muscles. A subgroup of patients were electromyographically assessed.
Results: US was performed in 835 muscles and EMG was available in 263 muscles. US detected fasciculations more frequently than EMG. Fasciculations were widespread, especially in upper limbs onset patients and in the cervical region. Fasciculations' number inversely associated with ALSFR-R and body mass index (BMI) and directly with BMI loss and upper motor neuron (UMN) impairment. Our statistical model suggest that fasciculations increase with the initial lower motor neuron (LMN) degeneration, reach their peak when the muscle became mildly to moderately weak, decreasing afterwards with increasing muscle weakness and atrophy.

Abbreviations: LL, lower limbs; LMN, lower motor neuron; MRC, muscle strength based on the medical research council scale; MTh, muscle thickness; UL, upper limbs; UMN, upper motor neuron; US, ultrasound.

* Corresponding author at: Department of Neurology, Hospital Universitario y Politécnico La Fe, Avenida Fernando Abril Martorell 106, 46026 Valencia, Spain.
E-mail address: vazquez_juacos@gva.es (J.F. Vázquez-Costa).

https://doi.org/10.1016/j.clinph.2018.09.014

Conclusions: Our study suggests that both UMN and LMN degeneration trigger fasciculations causing BMI loss. The degree of LMN impairment could account for differences in fasciculations' rates within and between muscles.
Significance: In ALS, fasciculations could explain the link between hyperexcitability and BMI loss.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects the upper (UMN) and lower motor neurons (LMN). Fasciculations are involuntary twitches due to spontaneous contraction of muscle fascicles, originating from motor unit depolarizations as recorded by electromyography (EMG) (de Carvalho et al., 2017). Widespread fasciculations are characteristic of ALS and, therefore, have been incorporated to the diagnostic criteria (de Carvalho et al., 2008).

Ultrasound (US) imaging, which allows the examination of a wide muscle area and several muscle fascicles simultaneously, has been proven more sensitive than EMG for fasciculations' detection in ALS (Walker et al., 1990; Misawa et al., 2011). Moreover, US is a painless technique and can be more suitable than EMG for a widespread examination.

Previous studies have studied the origin of fasciculations based on their electrophysiological characteristics (de Carvalho et al., 2017). However, the clinical factors associated with them have been scarcely studied and consequently its clinical meaning remains largely unknown. Unravelling these associations may lead to a better understanding of their pathophysiology.

The aim of this study is to describe the ultrasonographic pattern of fasciculations in several muscle groups in patients recently diagnosed with ALS and to analyse the relationship between these patterns and several demographic, clinical, analytical and neurophysiological variables.

2. Material and methods

2.1. Study population

This observational study prospectively enrolled patients with a recent (<90 days) clinical diagnosis of ALS. Patients were classified into three phenotypes according to the degree of UMN and LMN impairment at diagnosis: LMN predominant ALS (LMN-ALS) patients showed none or equivocal UMN signs upon recruitment; UMN predominant ALS (UMN-ALS) patients showed no clinical or neurophysiological signs of LMN impairment; and classical ALS patients showed both signs. All patients were diagnosed, recruited and examined by the same experienced neurologist (JFVC) in the ALS Unit of Hospital La Fe. Patients not meeting Awaji criteria at recruitment, were followed up until death or for at least 12 months and the emergence of new LMN and UMN signs was monitored. At the end of the study, the ALS diagnosis was confirmed in all the included patients, based either on Awaji criteria (de Carvalho et al., 2008) or in a compatible clinical course (progressive involvement of several regions including either bulbar or respiratory impairment) in those patients who did not developed UMN signs.

2.2. Clinical and analytical variables

Age, sex, dates of symptoms onset and of diagnosis, site of symptoms onset, disability (ALSFRS-R) (Cedarbaum et al., 1999), and degree of UMN impairment (UMN score) were recorded for all the patients upon recruitment. The UMN score measures the degree of UMN impairment ranging from 0 to 16 (Vázquez-Costa et al., 2018). It was calculated by summing up the number of pathologically brisk reflexes (brisk facial and jaw jerks; biceps, supinator, triceps, Hoffmann, knee and ankle reflexes; and extensor plantar responses). Premorbid body mass index (BMI) was estimated by systematically asking patients about their usual weight the year before symptoms onset. Moreover, medical records were reviewed to confirm this datum, although it was not available for all the patients. Only mild discrepancies between the weight reported by the patient and the one registered in medical records were found ($\leq \pm 1$ kg). In those cases, the latter was chosen for this study. The percentage of BMI loss was calculated as follows: (premorbid BMI − BMI at recruitment) * 100/premorbid BMI. The muscle strength, measured with the modified Medical Research Council (MRC) rating scale (ranging from 0 to 5 and including grades 4− and 4+), was assessed in all US examined limb muscles (see below).

Serum creatinine levels at diagnosis were recorded and the results are reported in mg/dL.

2.3. Ultrasound study

An experienced neuroradiologist (JIT), blind to clinical details, performed US examinations at recruitment with a Toshiba Aplio XG (Tokyo, Japan 2008) equipped with a 7.2–14 MHz linear-array transducer. All system-setting parameters, such as global gain (80 dB), time gain compensation (in neutral position), depth (30 mm for cricothyroid and ABP muscles and 50 mm for the remaining muscles), width (38 mm), frequency (13 MHz), the position of the transducer, compression and focus were kept constant throughout the study. Ten muscle groups representing the 4 anatomical regions were examined in the transverse plane in a supine relaxed patient as previously reported (Martínez-Payá et al., 2017a, 2017b, 2018). In the bulbar region we studied the tongue and cricothyroid; in the cervical region, the biceps brachialis, triceps brachialis and the abductor pollicis brevis (APB) muscle; in the thoracic region the rectus abdominis; and in the lumbosacral region the rectus femoris, tibialis anterior and medial gastrocnemius. Further information about the probe placement in each muscle group can be found as supplementary information (see Supplementary Methodology). Trapezius was also examined but it was not ascribed to any region since it receives both bulbar and cervical innervation (Pu et al., 2008). The exploration was performed bilaterally in all muscles except the tongue; therefore, altogether, 19 muscles were studied in each patient. Each muscle was observed for 30 seconds and fasciculations, defined as elsewhere (Misawa et al., 2011), were categorized as focal or multifocal. Moreover, the number of fasciculations in each muscle group was counted and the total number of fasciculations was calculated in each individual. A maximum value of 30 fasciculations was assigned to those muscles showing uncountable continuous multifocal fasciculations.

Images of all the 527 limbs muscles were acquired and their muscle thickness was measured with electronic callipers. The thickness of the biceps brachialis, rectus femoris, and tibialis anterior was measured as previously described (Martínez-Payá et al., 2017a). The thickness of the triceps brachialis was measured between the uppermost part of the bone echo of the humerus

and the superficial fascia of the triceps. Finally, for the APB and medial gastrocnemius muscles, the thickness was measured including both the superficial and deep epimysiums.

2.4. Electromyography

In 36 patients an EMG study performed with a Dantec Keypoint equipment was accessible in a period not exceeding 30 days (median time 0.16 months [0, 1]). The presence of fasciculations, as well as acute and chronic denervation in each studied muscle were recorded, but this study was performed for diagnostic purposes following consensus criteria for ALS diagnosis (de Carvalho et al., 2008). Briefly, the presence of fasciculations and acute (fibrillations or positive sharp waves, fib-sw) and chronic denervation was assessed by inserting the EMG needle in at least 5 different points and for at least 90 seconds in each muscle group. Acute denervation was considered to be present in a muscle group when fib-sw, persisting at least 2 seconds after the needle insertion, were registered in at least two insertion points. Chronic denervation was considered to be present in a muscle group when increases in the duration, amplitude and phases in at least 3 motor unit potentials together with a decreased interference pattern were found. Most muscles were studied unilaterally and not all muscles were studied in all patients. A total of 263 muscles were studied in the whole cohort (Supplementary Table 1).

2.5. Statistical analysis

Data were summarized by mean, standard deviation, median, and first and third quartiles for the continuous variables, and by relative and absolute frequencies for the categorical variables. A heat map was used to describe the effect of the symptoms' onset site on the fasciculations pattern. An exploratory analysis was performed by Spearman's correlations to evaluate potential associations of the demographical, clinical and analytical variables with the total number of fasciculations.

Since, both BMI ($r = -0.441$, $p = 0.003$) and BMI loss ($r = 0.424$, $p = 0.004$), but not the premorbid BMI ($r = -0.208$, $p = 0.17$), correlated with the number of fasciculations, two multivariable mixed negative binomial regression model were carried out to assess their respective association with the fasciculations' number. In the first model, age, sex, BMI loss, region of onset, disability (ALSFRS-R score) and the degree of UMN (UMN score) and LMN impairment (MRC and muscle thickness) were analysed. We hypothesized that the association of the muscle strength with the fasciculations' number would be non-linear, since previous EMG studies have shown that the fasciculations' rate and frequency increase with the initial LMN impairment (Mills, 2010; Krarup, 2011), but they decrease in severely denervated and weak muscle (Krarup, 2011; de Carvalho and Swash, 2016). Consequently, a quadratic term was adjusted for MRC. Finally, the effect of muscle thickness on fasciculations was thought to depend on the muscle strength so an interaction between both variables was considered in the model. The estimated effects resulting from this model equation were used to predict the estimated number of fasciculations as well as their 95% credibility intervals using the package brms (version 2.2.0).

In the second model, BMI and creatinine (an indirect measure of muscle mass (van Eijk et al., 2018)) were introduced as covariables and BMI loss, MRC and muscle thickness were excluded to avoid collinearity issues between BMI and BMI loss and creatinine with MRC and muscle thickness. The following are additional specifications that were introduced in both models to better fit our assumptions:

1. Given that a maximum value of 30 fasciculations was assigned to those muscles showing uncountable continuous multifocal fasciculations, we included an upper bound set at 30 for censored data, in the model.
2. Since we did not anticipate there was any consistent right-left side difference between individuals, these models were extended with the variable "Side" nested to "Patient" as a random effect with random intercept in order to take dependency among observations into account. Likewise, because the observations from the same muscle are more likely to have a similar number of fasciculations than those from other muscles, and these were considered a sample of the human body muscles, another random effect for "Muscle" was introduced as a random intercept. In the first model, the variance in fasciculations number between the selected muscles was 0.87 CI95% [0.44, 1.77] and the variance between patients was 0.74 CI95% [0.55, 0.94].
3. Weak informative priors for the coefficients of the fixed effects were set in both models: N(0,10), as well as a Cauchy (0,2) was set for the standard deviation of random effects. A sensitivity analysis using less informative priors (N(0,20) and Cauchy (0,5)) was performed to assess the influence of the chosen priors in the final estimates of the models.
4. Given the degree of complexity required to carry out the models, a more flexible framework was needed and, therefore, a bayesian approach was used.

95% Confidence Intervals (95% CI) are provided for all estimates and a marginal effect plot was performed to ease the interpretation of the interaction between muscle thickness and strength.

The number of muscles showing fasciculations as per US and EMG was compared with a X^2 test. For this comparison, the same muscle groups on the same side were selected in both EMG and US. The differences in the fasciculations' number in each muscle depending on the presence or absence of acute or chronic denervation was assessed with a mixed negative binomial regression model, accounting for the aleatory effects of muscle and individual.

All statistical analyses were performed by an experienced biostatistician (VFF) using R software (version 3.4.3) and clickR (version 0.3.35) packages.

2.6. Ethical approval

The study was approved by the Ethics Committee for Biomedical Research of the La Fe Hospital (Valencia). All the participants gave written informed consent.

3. Results

3.1. Study population

The study included 44 patients and their demographic and clinical characteristics are summarized in Table 1. Eleven patients were classified as LMN-ALS and three as UMN-ALS.

3.2. Fasciculations' characteristics

US was performed in 835 muscles, instead of the expected 836, because one patient had his left hand amputated previously and this APB could not be studied.

Fasciculations were found in at least one region in all patients (although other LMN signs were lacking in 3 patients), and in at least 3 regions in 73% of patients (Table 1). The mean number of fasciculations was similar in cALS patients (125 [84]), LMN-ALS patients (121 [91]) and UMN-ALS patients (115 [122]).

Table 2 summarizes the frequency and number of fasciculations per muscle and region. Fasciculations were detected most frequently in muscles of the limbs, especially in the cervical region,

Table 1
Demographic, clinical and analytical characteristics of ALS patients at recruitment.

Variable	n (%)	Mean (SD)	Median (1st, 3rd Q)
Age (years)	–	65.37 (10.49)	67.99 (57.52, 72.41)
Male sex	24 (54.5%)	–	–
BMI	–	26.10 (3.92)	25.71 (23.57, 28.26)
Premorbid BMI	–	28.19 (4.11)	28.17 (24.95, 30.1)
BMI loss (%)	–	7.13 (7.93)	4.86 (0.57, 11.95)
Time from disease onset (months)		11.65 (7.82)	8.52 (5.77, 14.31)
Time from diagnosis (months)	–	0.63 (0.92)	0.47 (0, 1.32)
ALSFRS-R Score	–	39.84 (4.8)	42 (36, 43)
UMN Score	–	4.48 (5.04)	2 (0, 8.25)
Region of onset			
Bulbar	12 (27.3%)	–	–
Upper limbs	14 (31.8%)	–	–
Lower limbs	18 (40.9%)	–	–
Creatinine (mg/dL)	–	0.73 (0.16)	0.74 (0.61, 0.81)
Regions with LMN impairment[a]			
0	3 (6.8%)		
1	3 (6.8%)		
2	13 (29.5%)		
3	18 (40.9%)		
4	7 (15.9%)		
Regions with fasciculations (as per US)			
1	1 (2.3%)	–	–
2	10 (22.7%)	–	–
3	15(34.1%)	–	–
4	18 (40.9%)	–	–

BMI: body mass index; LMN: lower motor neuron; UMN: upper motor neuron; US: ultrasound.
[a] Clinical and/or neurophysiological LMN impairment as per Awaji criteria.

Table 2
Muscle characteristics as per ultrasound.

Region and muscle	MRC	Muscle thickness	Muscles with fasciculations[a]	Type of fasciculations		Number of fasciculations
	Mean (SD)	Mean (SD)	n (%)	Focal (%)	Multifocal (%)	Mean (SD)
Bulbar	–	–	**35 (26%)**	**28%**	**72%**	–
Tongue	–	–	28 (64%)	21%	79%	18.3 (12.36)
Cricothyroid	–	–	7 (8%)	86%	14%	1.71 (0.76)
Trapezius	–	–	**58 (66%)**	**45%**	**55%**	**11.4 (12.30)**
Cervical	–	–	**220 (83%)**	**21%**	**79%**	–
Biceps brachialis	4.8 (0.62)	23.2 (6.19)	81 (92%)	12%	88%	17.2 (11.43)
Triceps brachialis	4.8 (0.39)	28.6 (6.01)	71 (81%)	23%	77%	12.6 (10.62)
APB	4.2(0.89)	7.2 (2.53)	69 (79%)	31%	69%	9 (8.84)
Thoracic (rectus abdominis)	–	–	**49 (56%)**	**37%**	**63%**	**10.8 (12.69)**
Lumbosacral	–	–	**157 (59%)**	**37%**	**63%**	–
Rectus femoris	4.8 (0.46)	20.1 (5.23)	69 (78.41%)	36%	64%	10.2 (10.18)
Tibialis anterior	4.3 (1.41)	22.4 (3.85)	70 (79.55%)	40%	60%	6.7 (7.76)
Gastrocnemius	4.8 (0.67)	13.6 (2.53)	57 (65%)	35%	65%	7.1 (7.58)

Bold values correspond to the total number and percentage of muscles showing fasciculations in each region.
ABP: abductor pollicis brevis.
[a] The US was performed bilaterally in all muscles except the tongue.

Table 3
Percentage of regions showing fasciculations according to the region of onset.

Anatomical region	Region of onset		
	Bulbar (n = 12)	UL (n = 14)	LL (n = 18)
Bulbar, n (%)	10 (83.3%)	10 (71.4%)	8 (44.4%)
Cervical, n (%)	12 (100%)	14 (100%)	18 (100%)
Lumbar, n (%)	12 (100%)	13 (92.9%)	17 (94.4%)

All patients showed fasciculations on the cervical region irrespective of the site of onset. Conversely, two bulbar onset patients did not show fasciculations in the bulbar region. Of those, one patient, who had been classified as pseudobulbar palsy, showed fasciculations in US (without acute or chronic denervation in EMG) in the cervical and lumbar region but not in trapezius. The other patient showed widespread acute and chronic denervation as well as fasciculations in both US and EMG, including in trapezius. Finally, only one lower limb onset patient did not show fasciculations in the lumbar region, but did it in the bulbar and cervical regions. LL: lower limbs; UL: upper limbs; EMG: electromyography; US: ultrasound.

and biceps brachialis was, by far, the muscle with the greatest number and frequency of fasciculations. Fasciculations were not detected in the bulbar region in 16 patients (2 of bulbar onset), but were visible in trapezius in 10 of them (one of bulbar onset). Conversely, fasciculations were present in the tongue in 4 out of the 10 patients not showing fasciculations in trapezius.

Table 3 and Fig. 1 represent the frequency and number of fasciculations in each muscle according to the region of onset and show that fasciculations are more frequent in cervical muscles independently of the region of onset. Moreover, fasciculations were overall more frequent and widespread in upper limb (UL) onset patients (Figs. 1 and 2). Conversely, fasciculations were equally frequent in the ipsilateral and contralateral side of onset (OR 1.08 [0.87, 1.35], p = 0.46).

Of the 263 muscles studied with EMG, acute or chronic denervation was found in 183 (82.9%). Overall, US detected fasciculations more frequently than EMG (79.6% vs 51.9%, p < 0.001) (Supplementary Table 2).

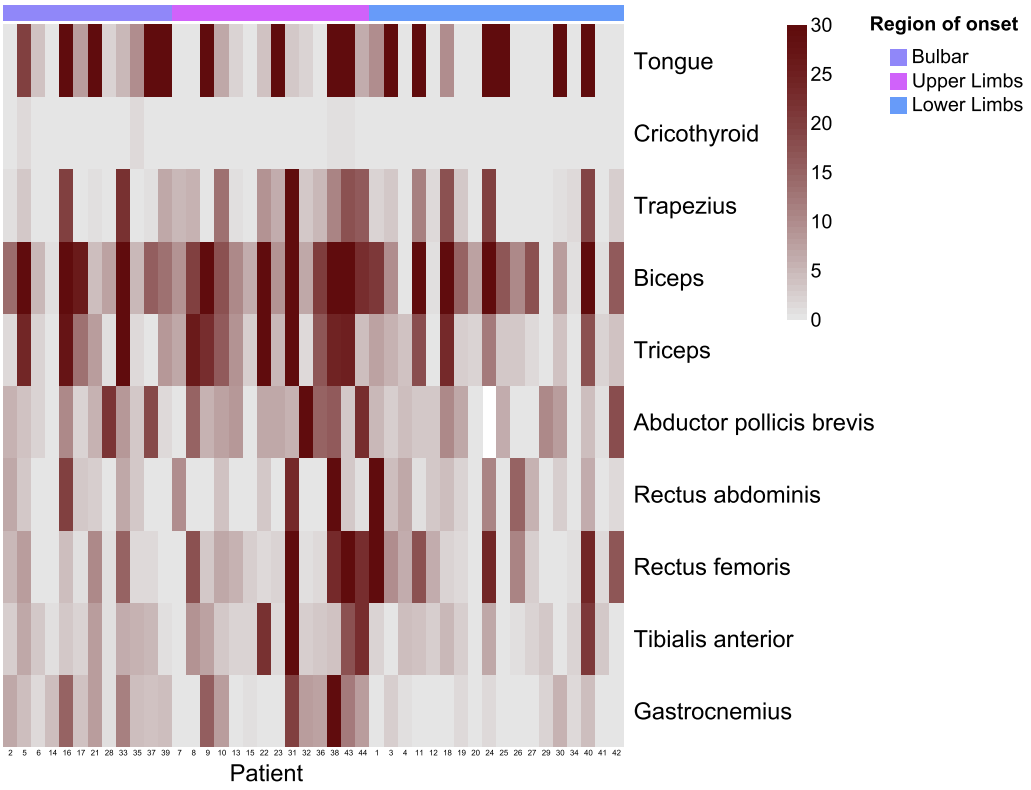

Fig. 1. Heat map representing the number of fasciculations in each muscle according to the region of onset. In bulbar onset patients, fasciculations are less frequent in the thoracic and lumbar region. Patients with onset in the upper limbs show widespread fasciculations, although predominating in the cervical region. Intriguingly, in lower limbs onset patients, fasciculations are less frequent in the distal muscles of the lumbar region. Fasciculations in the tongue seem to be more frequent in bulbar onset patients. Conversely, in trapezius, fasciculations are more frequent in UL onset patients. Fasciculations in the cervical and thoracic regions show little variation regardless of the disease onset, whereas fasciculations in tibialis anterior and especially in gastrocnemius appear to be less frequent in lower limb onset patients. LL: lower limbs; UL: upper limbs.

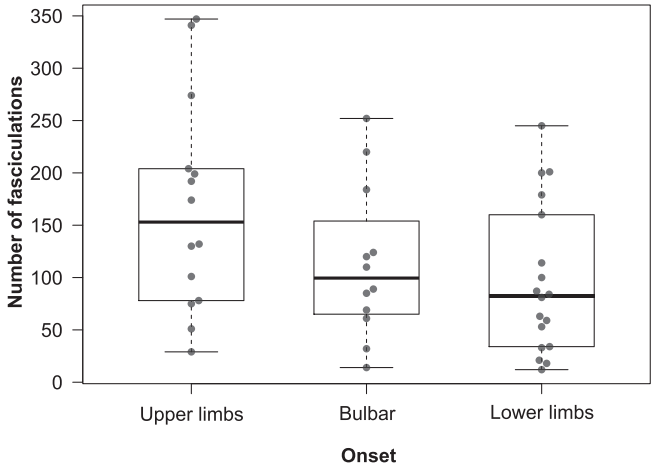

Fig. 2. Number of fasciculations per region of onset. UL onset patients show greater fasciculations than bulbar onset patients and lower limb onset patients.

Table 4
Model analysing the association between fasciculations and demographic and clinical variables.

	exp(Estimate)	Credibility interval 95%	
Age	0.982	0.958	1.006
Male gender	0.748	0.464	1.202
BMI loss	1.074	1.043	1.106
ALSFRS-R	1.074	0.995	1.158
UMN score	1.095	1.036	1.160
Bulbar onset	0.650	0.38	1.104
LL onset	0.420	0.254	0.699
MRC	8.584	2.995	24.882
MRC^2	0.805	0.712	0.908
MTh	1.203	1.077	1.352
MRC:MTh	0.960	0.937	0.982

BMI loss, ALSFRS-R and UMN score associate directly with fasciculations, whereas LL onset associates with less fasciculations. Moreover, the model confirms the existence of a non-lineal association between fasciculations and muscle strength and of an interaction between muscle strength and thickness (see also Fig. 4). In bold are highlighted those variables showing an independent effect on the fasciculations' number. BMI: body mass index; LL: lower limbs; MRC: muscle strength based on the medical research council scale; MRC^2: quadratic adjustment of MRC; MTh: muscle thickness; UMN: upper motor neuron.

3.3. Determinants of fasciculations

In the first model (Table 4), the multivariable analysis confirms that UL onset patients have more fasciculations than LL onset patients. Moreover it shows that cervical and proximal muscles fasciculate more frequently than lumbar and distal ones respectively, independently of other covariables (Fig. 3). The model also showed a direct association of fasciculations with the BMI loss, UMN impairment and muscle thickness as well as an inverse association with disability (ALSFRS-R). Finally, a non-linear association with muscle strength and an interaction between muscle strength and thickness was found (Fig. 4).

To further analyse the relationship between fasciculations and the LMN impairment, we assessed the differences in the fasciculations' number in denervated vs non-denervated muscles. Fasciculations were more frequent in muscles that showed acute or chronic denervation per EMG than in non-denervated muscles (83.9% vs 55.6%; exp(Estimate) = 2.33 [1.43, 3.77], p = 0.001).

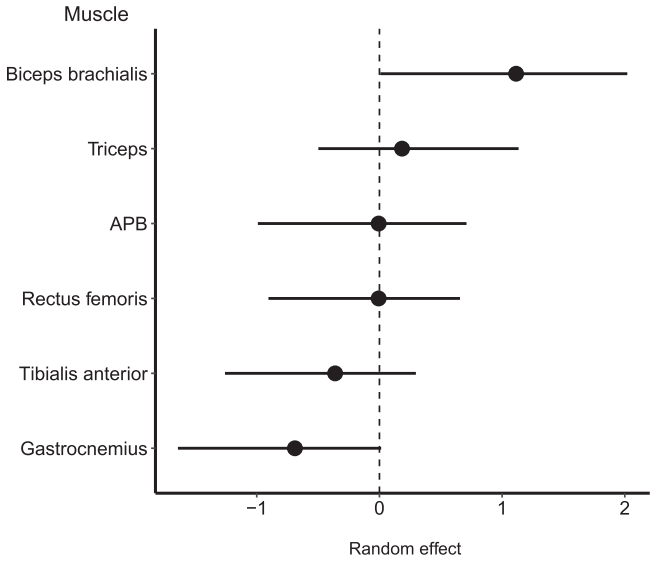

Fig. 3. Number of fasciculations in the different muscle groups predicted by the model of Table 3. Muscles in the graphic are shown in a descendant order based on the number of fasciculations according to the model and after adjusting by other co-variables. In other words, this graphic represents the independent effect of each muscle on the fasciculations number. The graphic was developed considering the variable "Muscle" as a random effect with random intercept to correct for the non-independence of the data. The variance of the fasciculations' number between the selected muscles was 0.867 CI95% [0.44, 1.77]. APB: abductor pollicis brevis.

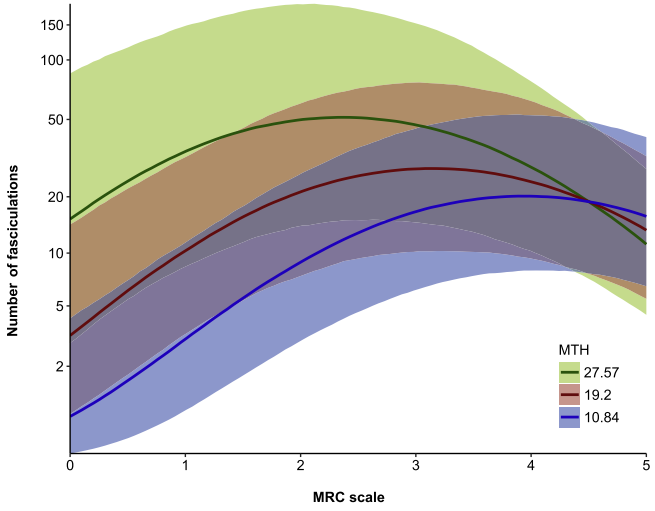

Fig. 4. Graphic representation of the association between the fasciculations number and the interaction of muscle strength (MRC) and thickness (MTh) predicted by the model of Table 3. On the one hand, greater MTh associates with greater fasciculations number (colours). On the other hand, the model shows an inverted U-shape association of the fasciculations' number with muscle strength. Namely, the number of fasciculations initially increases with the loss of muscle strength until a particular point (determined by the MTh), where it progressively decreases. The interaction between muscle strength and thickness means that thicker muscles (such as biceps or triceps brachialis) need to be severely weak to show a decrease in fasciculations, whereas thinner muscles (such as abductor policis brevis) show an early decrease in fasciculations with the onset of weakness. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. Association of fasciculations and BMI

The first model showed that the association of fasciculations and BMI loss was independent of other muscular variables (muscle strength or thickness). Therefore, we hypothesized that this BMI loss would be independent of the muscle mass loss. To test this hypothesis we studied the association between fasciculations and BMI using creatinine, a proxy of the muscle mass (Chiò et al., 2014), as a covariable. The second model confirmed previous associations and showed that BMI was negatively associated with fasciculations (0.86 [0.81, 0.92]) independently of creatinine (Table 5). Moreover, in this model, bulbar onset was also associated with fewer fasciculations than UL onset (0.52 [0.29, 0.87]).

Table 5
Model analysing the association between fasciculations and demographic and clinical variables.

	exp(Estimate)	Credibility interval 95%	
Age	1.015	0.985	1.047
Male gender	0.777	0.475	1.276
BMI	0.860	0.809	0.919
Creatinine	0.323	0.099	1.071
ALSFRS-R	1.076	0.993	1.166
UMN score	1.067	1.013	1.127
Bulbar onset	0.516	0.296	0.871
LL onset	0.335	0.210	0.543

In this model, ALSFRS-R and UMN score associate directly with fasciculations, whereas BMI, and bulbar and LL onset associate with less fasciculations. Those variables highlighted in bold show an independent effect on the fasciculations' number. BMI: body mass index; LL: lower limbs; MRC: muscle strength based on the medical research council scale; MTh: muscle thickness; UMN: upper motor neuron.

4. Discussion

Fasciculations are a hallmark of ALS and have been linked, in previous electrophysiological studies, to excitability changes in the LMN and UMN (Iwai et al., 2016; de Carvalho et al., 2017). Our study confirms the US potential to deepen on the clinical and pathophysiological meaning of fasciculations (Noto et al., 2018).

4.1. Where are fasciculations more frequent?

Our study shows that fasciculations are frequent, multifocal and widespread (beyond the region and side of onset) at diagnosis in most ALS patients, as previously shown with the LMN and UMN impairment (Ravits et al., 2007; Krarup, 2011; Vázquez-Costa et al., 2018). The distribution and frequency of fasciculations, predominating in the UL muscles and proximal vs distal muscles, confirms previous findings (Krarup, 2011; Misawa et al., 2011; Noto et al., 2017, 2018; Tsuji et al., 2017) and has been shown specific for the ALS diagnosis (Tsuji et al., 2017). Here we show that this muscle preference is independent of other factors such as the UMN score and muscle strength or thickness, i.e. of the degree of UMN and LMN impairment. Interestingly, UL onset patients were also those with the greatest number of fasciculations, followed by bulbar and lower limb onset patients. A previous study showed similar, although not statistically significant, results (Tsuji et al., 2017).

Fasciculations were detected in the tongue in 63.6% of patients, including 83.3% of the bulbar onset patients. This confirms the utility of US for fasciculations' detection in the tongue (Misawa et al., 2011). Conversely, fasciculations in cricothyroid were scarce. The detection of fasciculations among cranial nerve innervated muscles has been found to be highly variable (O'gorman et al., 2017). However, the lack of fasciculations in cricothyroid is intriguing because it is clinically impaired in ALS patients. Moreover, the nucleus ambiguous, as happens with the hypoglossal nucleus, shows a severe pathologic involvement in ALS patients (Brettschneider et al., 2013) and receives bilateral direct CM projections from motor cortex (Eisen et al., 2017).

Trapezius has been proposed as an alternative muscle to the tongue for the detection of denervation and fasciculations in the

bulbar region (Sonoo et al., 2009). However, in most individuals, trapezius has both bulbar and cervical innervation (Pu et al., 2008). In our study, fasciculations in trapezius were found in 62.5% of patients without fasciculations in the tongue. Considering the high sensitivity of US (83%) to detect fasciculations in the bulbar region in bulbar onset patients, our study suggests that the presence of fasciculations in trapezius frequently represents cervical rather than bulbar impairment. In fact, in 40% of patients showing fasciculations in tongue, they were lacking in trapezius. Moreover, while fasciculations in bulbar onset patients were more frequent in the tongue than in trapezius, in UL onset patients this predominance pattern was inverted (Fig. 1).

4.2. Which demographic factors are associated with fasciculations?

Fasciculations are not associated with demographic factors such as age, sex or premorbid BMI. Conversely, we demonstrate for the first time that fasciculations associate with BMI loss. Two hypotheses, which are not mutually exclusive, could explain this association. First, that fasciculations result in increased energy expenditure. Second, that fasciculations act just as a marker of the extent of the LMN impairment and associate with weight loss due to muscle atrophy. In the latter hypothesis, BMI loss would largely depend on muscle mass loss. However, in our second model, the association of fasciculations with BMI was found to be independent of creatinine, a proxy of the muscle mass (Chiò et al., 2014).

This reinforces the first hypothesis, suggesting that fasciculations, a feature of hyperexcitability, are actually a source and not a bystander of the weight loss. Also in ALS animal models, fasciculations have been proposed to cause an increase of energy expenditure, which could ultimately lead to motor neuron degeneration due to metabolic stress (Dupuis et al., 2011; Vandoorne et al., 2018). This link between hyperexcitability, fasciculations and energy expenditure leading to weight loss and ultimately to motor neuron degeneration could explain that all these factors have been linked to poor prognosis in ALS (Krarup, 2011; Marin et al., 2011; Paganoni et al., 2011; Shimizu et al., 2014; Shibuya et al., 2016; Noto et al., 2018; Steyn et al., 2018).

4.3. Which clinical factors are associated with fasciculations?

Two types of fasciculations have been previously described in different disease stages, according to the EMG characteristics (de Carvalho et al., 2017), and both have been linked with hyperexcitability either in the cortex or in the LMN (de Carvalho et al., 2017; Eisen et al., 2017; Noto et al., 2018). Our study confirms that both the UMN and LMN impairment contribute independently to the occurrence of fasciculations. Moreover, it shows limb and muscle-specific variations in the number of fasciculations that are independent of the other clinical or ultrasonographical studied factors and probably reflect divergences in the excitability of the different innervating motor neurons.

4.3.1. UMN impairment

Our finding of a direct and independent contribution of UMN impairment to the rise of fasciculations in ALS is in line with previous studies that have found a supraspinal origin of some fasciculations (de Carvalho et al., 2017), which have been related to central hyperexcitability (Noto et al., 2018). Conversely, our study and others (Higashihara et al., 2012; Tsuji et al., 2017) show that fasciculations are equally frequent despite the lack of overt UMN signs, but are somewhat less frequent in the absence of LMN impairment (e.g. in the primary lateral sclerosis) (de Carvalho and Swash, 2016). This suggests that, although the UMN impairment can cause fasciculations, it is not its only source in ALS patients at diagnosis.

4.3.2. LMN impairment

Several consecutive muscle changes are thought to arise throughout the course of ALS. Fasciculations appear early on the disease, before the emergence of LMN loss (Iwai et al., 2016; de Carvalho et al., 2017). Later, the LMN loss begins, but it is initially compensated by collateral sprouting and reinnervation changes (chronic denervation), which are visible electromyographically at that moment, together with fasciculations arising distally in the LMN (de Carvalho et al., 2017). With the disease progression, acute denervation signs may appear due to the inability of collateral sprouting to compensate for the loss of motor neurons (Krarup, 2011). When more than a third of LMN have degenerated, mild muscle weakness becomes evident (Wohlfart, 1958), which is usually followed by a decrease in muscle thickness (Arts et al., 2011). Previous EMG studies suggest that, while both the axonal hyperexcitability (Iwai et al., 2016) and the fasciculations' rate and frequency increase with the initial LMN impairment (Mills, 2010; Krarup, 2011), they decrease in severely denervated and weak muscles (Krarup, 2011; de Carvalho and Swash, 2016). Our results (Fig. 4) fit this pathophysiologic model, showing an initial increase of fasciculations with denervation and mild weakness (attributable to the progressive recruitment of hyperexcitable motor units), which is followed by a late decrease with progressive atrophy and moderate to severe weakness, attributable to a progressive reduction of both motor units and axonal excitability (Krarup, 2011; de Carvalho and Swash, 2016).

Interestingly, according to our model, the number of fasciculations in thicker muscles peaks later than in thinner ones (Fig. 4). Since muscle cross-sectional area has not been found to influence the number of fasciculations (Noto et al., 2017), other factors should explain these differences. The thinnest studied muscles in our study were APB and gastrocnemius, which frequently show acute and chronic denervation in ALS, in comparison to thicker and more proximal muscles such as biceps or quadriceps, which show more frequently fasciculations (Krarup, 2011; Higashihara et al., 2012; Babu et al., 2017; Noto et al., 2018). If the cortical input (Eisen et al., 2017) explains this characteristic denervation/fasciculation muscle pattern found in ALS, deserves further investigation. However, what seems clear from our and previous works (Krarup, 2011; Higashihara et al., 2012; Babu et al., 2017) is that muscles becoming preferentially denervated suffer an earlier decrease of fasciculations, probably due to an earlier loss of hyperexcitable motor units.

4.3.3. Disability

Finally, the inverse association of fasciculations with disability is compatible with the common observation that fasciculations are an early sign in ALS patients that disappear with disease progression, probably also as the result of the loss of hyperexcitable motor units.

4.4. Strengths and limitations

The main strength of our study is its design with a systematic collection of demographic, clinical and analytical variables in a cohort of patients at an early disease stage and of electrophysiological and ultrasonographical variables in a great pre-specified number of muscles in each patient. The main limitation of the study is that the origin of fasciculations (UMN or LMN) cannot be distinguished by US and consequently, the fasciculations' number is probably the sum of both kinds of fasciculations. However, the statistical model, based on a predefined hypothesis, allows capturing the complexity of the fasciculations' pathophysiology in ALS and the independent contribution of each studied variable. Another limitation is that the premorbid weight was not available in the medical record in every patient. However, only mild discrepancies between the

recalled and recorded weight were found in those patients with available records. Consequently, this should not significantly impact our results and conclusions. Furthermore, our study was not specifically designed to compare the sensitivity of US vs. EMG for fasciculations detection, since the study protocol was only applied to the US measurements, whereas EMG was performed as a routine for diagnostic purposes. However, our results are similar to previous studies showing that US is more sensitive than EMG for fasciculations' detection (Walker et al., 1990; Misawa et al., 2011; Higashihara et al., 2012). Finally, the UMN burden was measured only clinically by means of the UMN score, and no biomarker of UMN impairment was used. In some ALS patients with severe LMN impairment, the UMN signs can be masked by the LMN impairment. However, our patients were all at an early stage of the disease and the degree of LMN impairment was overall mild to moderate, making less probable this masking phenomenon. Moreover, the same experienced neurologist performed the examinations, minimizing the variability of the assessment.

5. Conclusion

Our study suggests a link between fasciculations and BMI loss, which could be mediated by hyperexcitability. Although fasciculations are driven by both UMN and LMN impairment, they decrease with increasing disability and weakness, probably as a consequence of the loss of hyperexcitable motor units. Nonetheless, there are limb and muscle-specific variations in the frequency of fasciculations that are independent of these factors. We suggest that, differences in fasciculations rates within and between muscles could be explained by the different degrees of LMN impairment. If this is the result of a variable degree of corticomotoneuronal input, will deserve further investigation.

Conflict of interest

None of the authors have potential conflicts of interest to be disclosed.

Acknowledgments

We would like to thank patients for their participation in this study. Elvira Millet y Victoria Cortes contributed to data acquisition.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2018.09.014.

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