FASCICULATION INTENSITY AND DISEASE PROGRESSION IN AMYOTROPHIC LATERAL SCLEROSIS

Authors: Jun Tsugawa, MD, Thanuja Dharmadasa, FRACP, Yan Ma, MD, William Huynh, FRACP, PhD, Steve Vucic PhD and Matthew C. Kiernan, DSc, FRACP

Brain and Mind Centre, Sydney Medical School, University of Sydney; and Institute of Clinical neurosciences, Royal Prince Alfred Hospital, Sydney, Australia

Title character count: 72

Number of references: 31

Number of tables: 2

Number of figures: 2

Word count abstract: 199

Word count paper: 2912

Supplemental Data: video 1

Corresponding author:

Professor Matthew C. Kiernan

Bushell Chair of Neurology

Brain and Mind Centre, University of Sydney

94 Mallett Street, Camperdown, Sydney, NSW 2040, Australia

E-mail: matthew.kiernan@sydney.edu.au

Statistical Analysis conducted by Dr. Jun Tsugawa, MD.

Author Disclosures:

Drs. Tsugawa, Dharmadasa, Huynh, Ma report no disclosures. Professor Matthew Kiernan serves as Editor-in-Chief of the Journal of Neurology, Neurosurgery and Psychiatry.

Funding:

This work was supported by funding to Forefront, a collaborative research group dedicated to the study of motor neuron disease, from the National Health and Medical Research Council of Australia program grant (#1037746). JT is a recipient of Grant of The Clinical Research Promotion Foundation 2017. TD was supported by the University of Sydney Australian Postgraduate Award, a Rotary Club Cronulla Funding Partner Scholarship, a MNDRIA PhD Top-Up Grant and a Yuglibar Foundation Alzheimer`s Research Program PhD Top-Up Award. WH was supported by the University of Sydney Post-Doctoral Fellowship.

Role of the funding source:

The funding organizations played no role in study design, data collection, review and interpretation of data, or preparation or approval manuscript.

Abstract

Objective: To investigate the association between the frequency and intensity of fasciculations with clinical measures of disease progression in amyotrophic lateral sclerosis (ALS).

Methods: Twenty-four consecutive patients with ALS underwent clinical review and neuromuscular ultrasound assessment to detect intensity of fasciculations. Results were correlated with clinical markers of disease severity, as measured by the ALS Functional Rating Scale-revised (ALSFRS-R) and rate of disease progression (ΔFS), in addition to assessment of cortical motor function.

Results: Disease duration negatively correlated ($R = -0.530$, $p < 0.01$) with fasciculation intensity, while the ΔFS positively correlated with the fasciculation number ($R = 0.626$, $p < 0.01$). In terms of potential central contributions to ectopic impulse generation, patients were classified into cohorts based on their fasciculation intensity and short interval intracortical inhibition (SICI). ΔFS was significantly higher in patients with established hyperexcitability (low SICI) with high fasciculation intensity compared to those patients with minimal SICI change.

Conclusions: Fasciculation intensity appears linked to disease progression and separately to markers of cortical dysfunction, specifically the advent of cortical hyperexcitability.

Significance: Assessment of the intensity of patient fasciculations is a noninvasive approach that may provide further insight disease pathophysiology in ALS.

Keywords: amyotrophic lateral sclerosis, disease progression rate, fasciculation, neuromuscular ultrasound, short interval intracortical inhibition

ABBREVIATIONS

AH, abductor hallucis; ALS, amyotrophic lateral sclerosis; ALSFRS-R, the revised ALS Functional Rating Scale; APB, abductor pollicis brevis; BB, biceps brachii; FCU, flexor carpi ulnaris; GC, gastrocnemius; MEP, motor evoked potential; MUS, muscle ultrasound; PS, 10th cervical paraspinal muscle; RMT, Resting motor threshold; TA, tibialis anterior; TPZ, trapezius; TMS, transcranial magnetic stimulation; TTTMS, Paired-pulse Threshold tracking TMS; SICI, short interval intracortical inhibition; UMNS, upper motor neuron clinical score; VL, vastus lateralis.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that causes progressive degeneration of upper and lower motor neurons, with resultant muscle weakness and paralysis. Fasciculations are considered an early harbinger of ALS and form an important part of various criteria linked to clinical diagnosis **(de Carvalho et al., 2017)**. Despite being a cornerstone of clinical diagnosis, knowledge about implications of the intensity of fasciculations in ALS has not yet been established.

In terms of definition, fasciculations may best be considered as brief, apparently random and spontaneous contractions of muscle fibers, and remain a characteristic finding of patients diagnosed with ALS. Since the establishment of the Awaji criteria, the detection of fasciculations have been established as an important neurophysiological feature for the diagnosis of ALS **(Hardiman et al., 2011; Kiernan et al., 2011; Turner et al., 2013)**. Although the presence of fasciculations in ALS has traditionally been confirmed by electromyography, neuromuscular ultrasonography has emerged over more recent years as a sensitive, non-invasive method to detect fasciculations **(Walker et al., 1990; Arts et al., 2012; Misawa et al., 2011; Noto et al., 2018; Noto et al., 2017b)**.

In terms of their origin, studies to date have suggested that fasciculations may arise from proximal and distal segments of the peripheral nerve, or alternatively from within the motor neuron itself. Fasciculations may also arise or be triggered by central processes, linked to the development of cortical hyperexcitability in ALS **(de Carvalho et al, 2017)**. If accepted that fasciculations may be driven by the advent of hyperexcitability, such ectopic impulse generation may arise peripherally (linked to membrane instability of motor axons) or alternatively from a hyperexcitable corticospinal system, or from both compartments.

Regardless, the presence of fasciculations can typically be described from a diagnostic

perspective as being ‘present’ or ‘absent’ , based for instance on the detection of two or more twitches in a muscle using neuromuscular ultrasound. However, because of the ability of ultrasound to observe a large muscle surface area, different types of fasciculations have been detected, defined as intermittent or continuous twitching. Separately, although ultrasound may seem useful for confirming the presence of fasciculation, it cannot determine the origin or generator of the neural activity linked to the manifestation of fasciculation. As such, the aim of the present study was to utilize ultrasound to confirm the presence and intensity of fasciculation across a range of affected muscles, innervated by different levels of the neural axis. Furthermore, the present study investigated the correlation between fasciculation intensity and disease progression, linked to a multimodal approach incorporating markers of peripheral and cortical function and specifically, the presence of cortical hyperexcitability as identified with transcranial magnetic stimulation (TMS).

2. Material and Methods

2.1 Subjects

In this prospective study, 24 consecutive patients who were referred to the Forefront Multidisciplinary ALS Clinic (NHMRC Sydney Health Partners Academic Healthcare and Translation Centre) were recruited. This study was approved by the Human Research Ethics Committee of the University of Sydney, and all participants gave written informed consent prior to the study. This study has been carried out in accordance with The Code of Ethics of the World Medical Association (declaration of Helsinki)

Patients underwent a comprehensive clinical assessment with subsequent investigation including ultrasound, neurophysiological assessment, including nerve conduction studies, electromyography, and central studies of corticomotoneuronal function using threshold tracking

TMS (TT-TMS). Muscles examined with needle EMG were selected based on the symptom profile for each patient, in order to confirm whether the clinical presentation fulfilled a diagnosis of ALS based on available criteria. Patients fulfilled criteria for a diagnosis of probable or definite ALS according to Awaji criteria **(de Carvalho et al., 2008)**, and investigations excluded mimic disorders such as multifocal motor neuropathy and spinobulbar muscular atrophy.

To better determine disease severity and progression, patients were assessed using the revised ALS Functional Rating Scale (ALSFRS-R) **(Cedarbaum et al., 1999)**. Disease duration (months) was defined as time between first symptom onset to the visit date, with the rate of disease progression (ΔFS) calculated as follows:

ΔFS = 48 – (Total ALSFRS-R at initial visit) / Symptom duration (months) **(Labra et al., 2016)**.

An upper motor neuron clinical score (UMNS) was utilized to classify patients, determined by the presence of pathologically brisk reflexes (biceps, supinator, triceps, finger, knee, ankle, extensor plantar responses assessed bilaterally, and brisk facial and jaw jerks; maximum possible score = 16) **(Turner et al., 2004)**. Patients with an UMN score of >13 were classified into an upper motor neuron predominant group. Separately, patients were classified into phenotypes according to the initial region of clinical involvement (bulbar, upper, or lower limb onset).

2.2 Neuromuscular ultrasound

Ultrasound was performed by a neurologist (JT) with five years of experience in neuromuscular ultrasound, and who was blinded to the clinical history and neurological examination findings. Studies were performed using the MyLabTM Alpha ultrasound machine

(Esoate, Genova, Italy) with a 9-22 MHz broadband linear array transducer (SL2325). Patients were tested in the supine position with their arms and legs extended and with their muscles completely relaxed **(Arts et al., 2010)**. Each muscle was scanned transversely using B-mode with standard transducer locations corresponding to muscle bellies **(Misawa et al.,2011)**. Initial settings were kept constant for all examinations except depth, which was adjusted depending on the individual variations such as thickness of their subcutaneous fat. Ultrasound was undertaken on the following muscles bilaterally: the trapezius (TPZ), biceps brachii (BB), flexor carpi ulnaris (FCU), abductor pollicis brevis (APB), abductor digiti minimi, 10th thoracic paraspinal (PS), vastus lateralis (VL), tibialis anterior (TA), gastrocnemius (GC), and abductor hallucis (AH) muscles, and the tongue (genioglossus muscle). To avoid any impact of exercise of fasciculation intensity, all patients confirmed the absence of vigorous exercise in the days prior to evaluation of fasciculations. Each muscle was recorded as following sequential order; tongue, right upper limb, left upper limb, right lower limb, left lower limb and 10^{th} thoracic paraspinal. The protocol remained uniform for all patients. Recordings for each muscle were maintained for a period of at least 60 seconds to accurately determine the presence of fasciculation **(Noto et al., 2017a)** and were stored as video records. In total, 20 videos per subject were obtained. The presence of fasciculation was defined as two or more involuntary twitches per muscle **(Walker et al., 1990)**. In some cases it may seem difficult to determine whether muscle activity reflects spontaneous fasciculation or alternatively, motor unit activity under voluntary control. In these instances, voluntary activity may become more evident when patients better understood their ability to relax their limbs during evaluation. Fasciculation intensity was calculated as the number of fasciculations over a 60 second period (**video 1**). Based on recent observations of fasciculation intensity **(Noto et al., 2017a)**, ultrasound was recorded for 60 seconds, with fasciculation numbers subsequently counted using a 13.3-inch display screen, visualised in a

179 darkened room. For each patient, a sum fasciculation score was calculated for each muscle as
180 follows: the summed tongue and TPZ muscle fasciculation score (cranial region score), upper
181 limb sum score, lower limb sum score, and total sum score of all muscles altogether (overall
182 muscle fasciculation score). These values were also summed to produce an overall fasciculation
183 sum score. Clinical evaluation, ultrasound and video-recording were undertaken by a single
184 neurologist (JT).

185 2.3 Assessment of the central nervous system

186 Cortical function was assessed using TT-TMS, conducted using a 90-mm circular coil (for
187 upper limbs) and 110-mm double cone coil (for lower limbs) applied to the motor cortex, using
188 two magnetic stimulators connected via a BiStim 200^2 system (Magstim Co., Whitland, South
189 West Wales, UK). The resultant motor evoked potential (MEP) responses were recorded over
190 the APB muscles bilaterally. The APB muscle was used for recording responses to TMS to
191 provide a general measure of cortical function, while in contrast, fasciculation intensity was
192 assessed across a broad range of upper and lower limb muscles. The threshold tracking method
193 was used as previously reported **(Vucic et al., 2006a)**, with resting motor threthold (RMT) was
194 defined as the stimulus intensity required to generate a fixed MEP – response of 0.2 mV, when
195 preceded by a subthreshold conditioning stimulus of 70% RMT were tracked.

196 Short interval intracortical inhibition (SICI) was measured at increasing interstimulus
197 intervals (ISIs; 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, and 7 ms), with stimuli delivered until two consecutive
198 target MEPs were detected. SICI was calculated using the following equation **(Vucic et al.,**
199 **2006b)**:

200 $$\text{SICI} = (\text{Conditioned test stimulus intensity} - \text{RMT}) / \text{RMT} \times 100$$

201 As in previous studies, the averaged SICI response (ISIs 1-7 ms) was used for analysis of
202 cortical function.

203

204 2.4 Statistical analysis

205 SPSS version 22 (SPSS Corporation Chicago, USA) was used for all statistical analyses. For
206 each muscle, correlations were sought between fasciculation intensity and clinical parameters
207 (disease duration, ALSFRS-R score, and ΔFS) using Spearman's rank correlation coefficient.
208 Fasciculation intensity was confirmed between upper motor dominant and lower motor
209 dominant groups using an unpaired t-test and between ALS subgroups (bulbar onset, upper limb
210 onset, and lower limb onset) using a one-way ANOVA. Tukey's range test was used to compare
211 the fasciculation detection rate between these three subgroups. The association between the
212 intensity of fasciculations and average SICI were analyzed using Spearman's rank correlation
213 coefficient. An unpaired t-test was used to confirm difference of combined subgroup which was
214 classified into two group based on fasciculation intensity and averaged SICI value. Statistical
215 significance was considered as $p < 0.05$.

216

217 **3. Results**

218 3.1 Subject characteristics

219 In total, 24 patients were recruited, with the patients forming a representative ALS cohort
220 **(Table 1)**. No patient had a family history of ALS. From this cohort, 58% of patients were
221 diagnosed with definite ALS and 42% with probable ALS according to the Awaji criteria.
222 Approximately 21% of patients were bulbar-onset, 33% upper limb and 46% lower limb onset.
223 In terms of predominant influence evident on clinical assessment, 37.5% were classified as
224 upper motor neuron dominant, while 62.5% were lower motor neuron dominant.

225

226 3.2 Fasciculation detection

227 Ultrasound evaluation of fasciculations was performed across a total of 480 muscles (all
228 patients), with more than 25,000 seconds of video recording utilized for reviewing the
229 fasciculation count for each tested muscle. Overall, fasciculations were observed in more than
230 half of the tested muscles (56.3%; 270/480 muscles; **Figure 1**). Compared to the overall
231 detection rate of fasciculation (56.3%), the biceps brachii had the highest fasciculation detection
232 rate (75%; $p < 0.05$), and the paraspinal muscles had the lowest fasciculation detection rate
233 (29.2%; $p < 0.05$). Across the total sample, the mean fasciculation number was 13.0 ± 12.9
234 (mean ± SD) (**Table 2**). Separately, abductor hallucis had the lowest number of fasciculations
235 (6.8 ± 7.0, $p < 0.01$) of all fasciculation-positive muscles.
236
237 3.3 Correlation of fasciculations with clinical parameters
238 Clinical parameters (disease duration, ALSFRS-R score, and ΔFS) were correlated with the
239 presence of fasciculations for each muscle. Disease duration negatively correlated with
240 fasciculation intensity for both the total sum (R = -0.530, $p < 0.01$) and lower limb sum scores
241 (R = -0.632, $p < 0.01$). The ΔFS positively correlated with fasciculation intensity in total sum (R
242 = 0.626, $p < 0.01$; **Figure 2**.) and in specific tested muscles [Upper limb sum: R=0.504 ($p<0.05$),
243 Lower limb sum: R=0.523 ($p<0.01$), Lt FCU: R=0.482 ($p<0.05$), Lt TA: R=0.551 ($p<0.01$), Lt
244 Gastro: R=0.411 ($p<0.05$)]. No correlation was observed between fasciculation frequency and
245 ALSFRS-R score.
246 In terms of clinical phenotype, the fasciculation detection rate was similar across all
247 subgroups, with a rate of 57.0% in the bulbar-onset group, 65.6% in the upper limb-onset group,
248 and 50.9% in the lower limb-onset group (p=0.385). There were no significant differences
249 between the upper and lower motor neuron dominant groups in regards to the fasciculation
250 detection rate or intensity.

3.4 Fasciculation intensity and cortical function

Assessment of central function established a reduction in averaged SICI (ISI 1-7 ms, %), consistent with cortical hyperexcitability [right-APB 7.1 ± 5.8 (normal value; 10.9±0.8, $p<0.05$), left-APB 3.3 ± 4.7 (normal value; 10.9±0.8, $p<0.0001$)] **(Shibuya et al., 2016a)**. To evaluate the association between fasciculation intensity and cortical function, intensity was correlated with averaged SICI values. There was no correlation between the intensity of fasciculation for each of the total sum scores and SICI [right-APB; R=0.213 (p=0.464), left-APB; R=0.350 (p=0.184)].

In combined subgroup analysis, patients were classified into two groups based on fasciculation intensity and SICI data. Specifically, the difference of ΔFS between low SICI with high fasciculation intensity group was compared to the remaining cohort to determine whether ΔFS could be influenced by changes in cortical hyperexcitability combined with high fasciculation intensity. In the patient subgroup with prominent cortical hyperexcitability (defined as an average SICI of <5.5%) with a high number of fasciculations (defined as more than 150 fasciculations in total), the ΔFS was significantly higher than for the remaining ALS patients (low SICI and high fasciculations: 0.93 ± 0.28; remaining ALS patients 0.45 ± 0.35, $p <$ 0.05).

4. Discussion

In this cross-sectional clinical, ultrasound and neurophysiological study, the association between fasciculation intensity was considered relative to clinical parameters, particularly disease duration and the rate of disease progression in ALS. Analysis of fasciculations as determined by neuromuscular ultrasound determined that ΔFS positively correlated with the

intensity of fasciculation and that the combination of cortical hyperexcitability and high fasciculation intensity combined to promote faster disease progression in ALS.

4.1 Fasciculation rate

Overall, fasciculations were observed in more than half of the muscles tested, with the greatest detection rate identified in the biceps brachii, consistent with previous studies **(Takamatsu et al., 2016; Tsuji et al., 2017)**. In contrast, abductor hallucis showed the lowest number of fasciculations in all fasciculation-positive muscles, as well as a low fasciculation detection rate. The present series suggests that bigger muscles (such as BB and VL) tended to have higher fasciculation intensity than those of smaller muscles (AH) [BB vs AH; 18.6±17.5 vs 6.8±7.0 $p<0.0001$, VL vs AH; 15.6±13.9 vs 6.8±7.0 $p<0.0001$:Unpaired t test]. Of further interest, the present study has revealed that fasciculation intensity was lowest in AH in the cohort of ALS patients. In contrast, fasciculations are most common in AH in healthy subjects (Mitsikistas et al., 1998; Van et al., 1994; Fermont et al., 2010). This observation may have some clinical relevance, in that it makes isolated fasciculations involving AH appear particularly benign.

Separately, disease duration negatively correlated with the fasciculation intensity identified by ultrasound. Such a finding is consistent with the concept that fasciculations are more common during the early disease stages and typically become less prominent as the functional impairment increases, associated with the loss of motor units **(Fermont et al., 2010; de Carvalho and Swash., 2016a)**. The present study also identified a positive correlation between the ΔFS and the intensity of fasciculations as determined by neuromuscular ultrasound, highlighting the utility of ultrasound to serve as a potential marker of disease progression.

Perhaps surprisingly, there was no difference in fasciculation intensity across ALS

phenotypes when assessed by site of onset. In addition, there was no difference between patients with high UMN scores compared to lower UMN scores. Before commencing this study, it may have been considered more likely that lower UMN score patients would manifest more frequent fasciculation, if considered that fasciculations were primarily generated by increased lower motor neuron excitability **(de Carvalho and Swash., 2016b)**. While the origin of fasciculations remains unclear **(de Carvalho et al., 2017)**, in formal diagnostic criteria, fasciculations are identified by EMG features of neurogenic change and have typically been considered as evidence for lower motor neuron involvement **(de Carvalho et al., 2008)**. However, it remains conceivable that fasciculations arise linked to the generation of cortical hyperexcitability in ALS **(de Carvalho et al., 2017)**. Furthermore, fasciculations may develop linked to both upper and motor neuronal impairment, a concept supported by findings from the present study.

4.2 Central and peripheral function

Although the exact mechanisms and timing of underlying motor neuron death in ALS remain unclear, cortical hyperexcitability has been proposed as a contributory mechanism **(Brujin et al., 1997; Trotti et al., 1999)**. Cortical hyperexcitability, specifically a reduction in averaged SICI, seems to be the most robust biomarker for ALS and may provide prognostic insight **(Shibuya et al., 2016b; Menon et al., 2015; Simon et al., 2014; Shibuya et al., 2017)**. In the present series, ALS patients with evidence of cortical hyperexcitability who also demonstrated a high fasciculation intensity experienced the fastest clinical decline. Such findings suggest that this multimodal combination of malignant factors may promote disease progression in ALS.

In terms of potential limitations, it is accepted that the fasciculation intensity may be underestimated in muscles with very frequent fasciculations. To minimize such issues as much

as possible, whole recordings (more than 25,000 seconds of video recordings) were each reviewed twice for each muscle before formalizing fasciculation intensity. However, if anything, underestimating counts may have only served to reduce the strength of the relationships detected. Separately, it is accepted that the study incorporated a cross-sectional design with data obtained from a single tertiary centre.

5. Conclusion

The present study has established that fasciculation intensity as detected by neuromuscular ultrasound was associated with disease progression in ALS. Furthermore, the combination of fasciculation intensity and the advent of cortical hyperexcitability may prove useful for identifying those patients with more malignant disease and fast progression. Such identification may be useful in the future stratification of patients in a clinical trial setting. Although, overall patient numbers were not large in the present series, the studies incorporate ultrasound observations across 480 muscles and more than 25000 seconds of video recording. Future studies may explore differences in fasciculation intensity across a range of upper and lower motor dominant presentations, and amongst ALS subtypes classified by their initial symptomatology, including familial differences.

References

- Arts IM, Overeem S, Pillen S, Kleine BU, Boekestein WA, Zwarts MJ,et al. Muscle ultrasonography: a diagnostic tool for amyotrophic lateral sclerosis. Clin Neurophysiol 2012;123:1662–7.
- Arts IM, Pillen S, Schelhaas HJ, Overeem S, Zwarts MJ. Normal values for quantitative muscle ultrasonography in adults. Muscle Nerve 2010;41:32–41.
- Bruijn LI, Becher MW, Lee MK, Anderson KL, Jenkins NA, Copeland NG, et al. ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. Neuron 1997; 18: 327–38.
- Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B, et al. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J Neurol Sci 1999;169:13–21.
- de Carvalho M, Dengler R, Eisen A, England JD, Kaji R, Kimura J, et al. Electrodiagnostic criteria for diagnosis of ALS. Clin Neurophysiol 2008;119:497–503.
- de Carvalho M, Kiernan MC, Swash M. Fasciculation in amyotrophic lateral sclerosis: origin and pathophysiological relevance. J Neurol Neurosurg Psychiatry 2017;88:773-779.
- de Carvalho M and Swash M. Fasciculation discharge frequency in amyotrophic lateral sclerosis and related disorders. Clin Neurophysiol 2016;127:2257-62.
- de Carvalho M and Swash M. Lower motor neuron dysfunction in ALS. Clin Neurophysiol 2016 ;127:2670-81
- Fermont J, Arts IM, Overeem S, Kleine BU, Schelhaas HJ, Zwarts MJ. Prevalence and distribution of fasciculations in healthy adults: Effect of age, caffeine consumption and

366 exercise. Amyotroph Lateral Scler 2010;11:181-6.
367 ● Hardiman O, van den Berg LH, Kiernan MC. Clinical diagnosis and management of
368 amyotrophic lateral sclerosis. Nat Rev Neurol 2011;7:639–49.
369 ● Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O, et al.
370 Amyotrophic lateral sclerosis. Lancet. 2011;377:942-55.
371 ● Labra J, Menon P, Byth K, Morrison S, Vucic S. Rate of disease progression: a
372 prognostic biomarker in ALS. J Neurol Neurosurg Psychiatry 2016;87:628-32
373 ● Menon P, Geevasinga N, Yiannikas C, Howells J, Kiernan MC, Vucic S. Sensitivity
374 and specificity of threshold tracking transcranial magnetic stimulation for diagnosis of
375 amyotrophic lateral sclerosis: a prospective study. Lancet Neurol 2015 ;14:478-84.
376 ● Misawa S, Noto Y, Shibuya K, Isose S, Sekiguchi Y, Nasu S, et al. Ultrasonographic
377 detection of fasciculations markedly increases diagnostic sensitivity of ALS. Neurology.
378 2011;77:1532-7.
379 ● Mitsikostas DD, Karandreas N, Coutsopetras P, Piperos P, Lygidakis C, Papageorgiou
380 C. Fasciculation potentials in healthy people. Muscle Nerve. 1998 ;21:533-5.
381 ● Noto YI, Shibuya K, Shahrizaila N, Huynh W, Matamala JM, Dharmadasa T, et al.
382 Detection of fasciculations in amyotrophic lateral sclerosis: The optimal ultrasound
383 scan time. Muscle Nerve 2017 ;56:1068-71.
384 ● Noto YI, Simon NG, Selby A, Garg N, Shibuya K, Shahrizaila N, et al. Ectopic impulse
385 generation in peripheral nerve hyperexcitability syndromes and amyotrophic lateral
386 sclerosis. Clin Neurophysiol. 2018 ;129:974-980.
387 ● Noto YI, Simon N, Shibuya K, Matamala JM, Dharmadasa T, Kiernan MC. Dynamic
388 muscle ultrasound identifies upper motor neuron involvement in amyotrophic lateral
389 sclerosis. Amyotroph Lateral Scler Frontotemporal Degener. 2017 ;18:404-410.

- Shibuya K, Park SB, Geevasinga N, Huynh W, Simon NG, Menon P, et al. Threshold tracking transcranial magnetic stimulation: Effects of age and gender on motor cortical function.Clin Neurophysiol 2016;127 :2355-61.
- Shibuya K, Simon NG, Geevasinga N, Menon P, Howells J, Park SB, et al. The evolution of motor cortical dysfunction in amyotrophic lateral sclerosis. Clin Neurophysiol. 2017 ;128:1075-1082.
- Shibuya K, Park SB, Geevasinga N, Menon P, Howells J, Simon NG, et al. Motor cortical function determines prognosis in sporadic ALS. Neurology. 2016 ;87:513-20.
- Simon NG, Turner MR, Vucic S, Al-Chalabi A, Shefner J, Lomen-Hoerth C, et al. Quantifying disease progression in amyotrophic lateral sclerosis. Annals of Neurology 2014;76: 643-57.
- Takamatsu N, Nodera H, Mori A, Maruyama-Saladini K, Osaki Y, Shimatani Y, et al. Which muscle shows fasciculations by ultrasound in patients with ALS? J Med Invest 2016;63:49-53.
- Trotti D, Rolfs A, Danbolt NC, Brown RH Jr, Hediger MA. SOD1 mutants linked to amyotrophic lateral sclerosis selectively inactivate a glial glutamate transporter. Nat Neurosci 1999; 2: 427–33.
- Tsuji Y, Noto YI, Shiga K, Teramukai S, Nakagawa M, Mizuno T. A muscle ultrasound score in the diagnosis of amyotrophic lateral sclerosis. Clin Neurophysiol. 2017 ;128:1069-1074.
- Turner MR, Cagnin A, Turkheimer FE, Miller CC, Shaw CE, Brooks DJ, et al. Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: an [(11)C] (R)-PK11195 positron emission tomography study. Neurobiol Dis 2004;15:601–9.
- Turner MR, Hardiman O, Benatar M, Brooks BR, Chio A, de Carvalho M, et al.

Controversies and priorities in amyotrophic lateral sclerosis. Lancet Neurol. 2013 ;12:310-22.

- Van der Heijden A, Spaans F, Reulen J. Fasciculation potentials in foot and leg muscles of healthy young adults. Electroencephalogr Clin Neurophysiol. 1994 ;93:163-8.
- Vucic S and Kiernan MC. Novel threshold tracking techniques suggest that cortical hyperexcitability is an early feature of motor neuron disease. Brain 2006;129:2436–46.
- Vucic S, Howells J, Trevillion L, Kiernan MC. Assessment of cortical excitability using threshold tracking techniques. Mescle Nerve 2006; 33: 477-86.
- Walker FO, Donofrio PD, Harpold GJ, Ferrell WG. Sonographic imaging of muscle contraction and fasciculations: a correlation with electromyography. Muscle Nerve 1990;13:33–9.

		n	Male:female	Age (y)	Disease duration (m)	ALSFRS-R	ΔFS
Site of onset	Bulbar	5	2:3	66.4±5.4	46.2±65.9	43.0±3.8	0.49±0.44
	Upper limb	8	6:2	64.7±12.0	24.5±22.5	38.4±4.3	0.59±0.30
	Lower Limb	11	7:4	55.9±13.4	47.5±88.9	39.5±4.8	0.60±0.50
ALS (overall)		24	15:9	61.0±12.3	39.6±66.8	39.8±4.6	0.56±0.41

Table 1: Demographic characteristics of study subjects. The site of onset was classified as either bulbar, upper limb and lower limb onset. The data for age, disease duration, ALSFRS-R and ΔFS are provided as mean ± standard deviation (SD). ALSFRS-R; revised ALS Functional Rating, ΔFS; the rate of disease progression, m; month, y; year,

Regions	Muscle	The mean number of fasciculation in fasciculation detected muscle.
Brainstem	Tongue	13.6 ± 16.4
	TPZ	13.2 ± 11.6
Cervical	BB	18.6 ± 17.5
	FCU	12.37 ± 9.3
	APB	11.5 ± 15.3
	ADM	9.8 ± 12.1
Thoracic	PS	7.3 ± 6.6
Lumbosacral	VL	15.6 ± 13.9
	TA	12.6 ± 12.0
	GC	13.3 ± 11.8
	AH	6.8 ± 7.0 **
	Overall	13.0±12.9

Table 2: Mean fasciculation number of each muscle (mean ±SD). Of the 276 muscles the mean fasciculation number was 13.0 ± 12.9. AH had the lowest fasciculation number (6.8 ± 7.0, $p < 0.01$) of all fasciculation-positive muscles.

Supplemental data (video 1): ultrasound at vastus lateralis. There can be seen muscle fasciculations at center slightly left part. (4 fasciculations are counted within recording) Some pulse artifacts are detected over on the right in this video.

Highlights

- Fasciculation intensity in patients with amyotrophic lateral sclerosis (ALS) was measured using neuromuscular ultrasound.
- Fasciculation frequency appears linked to disease progression.
- Assessment of fasciculations is a noninvasive approach that may provide insight into ALS disease pathophysiology.

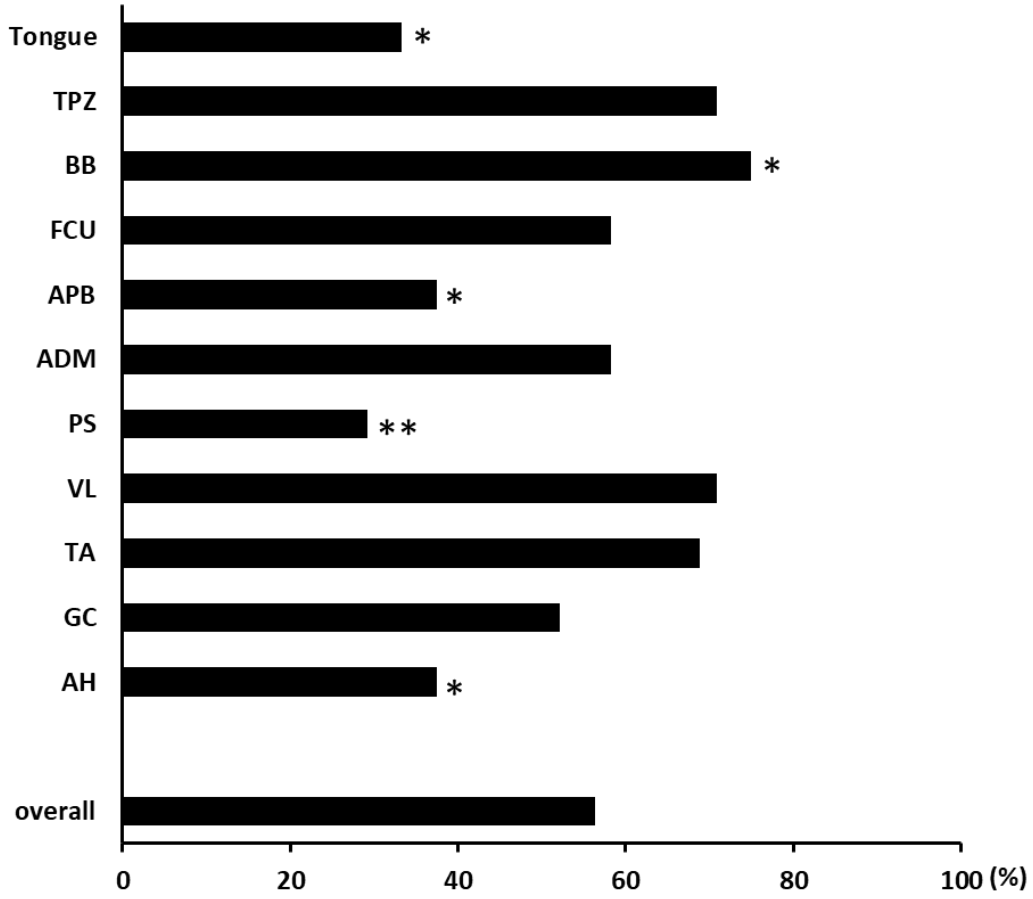

Figure 1: The fasciculation detection rate (%) using ultrasound in each muscle. Overall, muscle fasciculations were observed in more than half of the tested muscles (56.3%; 270/480 muscles). Compared to the overall detection rate of fasciculation the BB had the highest fasciculation detection rate (75%; $p < 0.05$), and the PS muscle had the lowest fasciculation detection rate (29.2%; $p < 0.05$). * $p<0.05$, ** $p<0.01$,

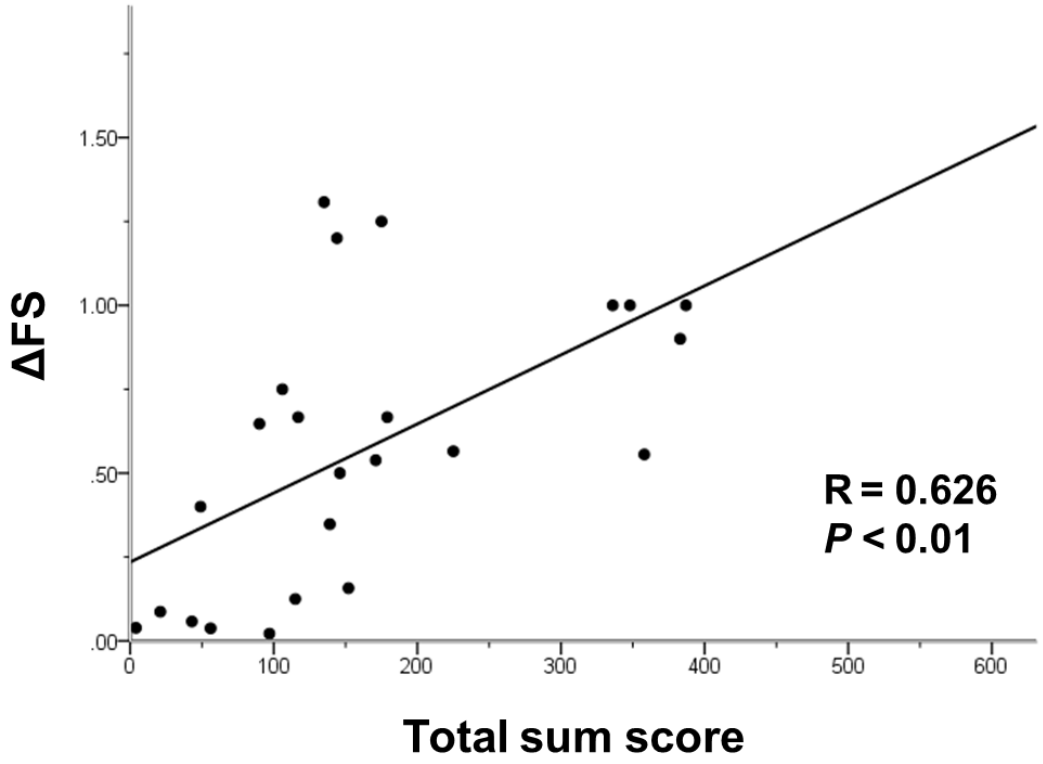

Figure 2: Correlation between fasciculation intensity of total sum score and Δ FS. The ΔFS was positively correlated with the fasciculation number in total sum (R = 0.626, p < 0.01).