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Proposed protocol for fasciculations detection by muscle ultrasound in amyotrophic lateral sclerosis

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Abstract

Objective: To investigate potential diagnostic value of fasciculations among amyotrophic lateral sclerosis (ALS) patients and determine the most optimal protocol based on selection of a combination of muscles with highest yield in clinical practice. *Methods:* The cross-sectional study comprised 149 ALS and 54 non-ALS patients, all underwent muscular ultrasound (MUS) on muscle groups in four body regions to find potential indicators for ALS diagnosis. Fasciculations intensity was divided into five grades based on firing frequency and number in the involved muscle groups. The fasciculations diagnostic score was defined according to the indicators with high specificity for ALS. *Results:* Detection rate of fasciculations was highest in the lumbosacral (970/1622, 59.8%) and cervical (841/1516, 55.5%) muscle groups, followed by the thoracic muscles (148/548, 34.3%), and bulbar muscle groups (102/652, 15.6%) among ALS patients ($p<0.05$). The detection of fasciculations in bulbar and thoracic muscle groups and detection of high-grade fasciculations in cervical and lumbosacral muscle groups were highly specific among ALS patients. Through detailed screening, a total of 13 muscle groups were involved in the fasciculations diagnostic score. Receiver operating characteristic (ROC) analysis showed that the area under curve (AUC) was 0.961 (95%CI 0.927-0.996). The optimal cut-off value was 1 point with 95.8% of sensitivity and 88.9% of specificity.
Conclusions: A practical protocol was feasible with optimal diagnostic yield in suspected ALS to help detect fasciculations. This could complement routine clinical evaluation and electrodiagnostic work-up and be performed as a practical bedside test with little patient burden and low cost.

Keywords: Amyotrophic lateral sclerosis, fasciculations, ultrasonography

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease involving upper and lower motor neurons. ALS patients usually manifest progressive muscle weakness and atrophy, bulbar paralysis.[1,2] The heterogeneity in clinical presentation and course of ALS causes considerable difficulty in ALS diagnosis. Currently the diagnosis of ALS is made based on clinical manifestations (Gold Coast Criteria)[3], which requires exclusion of other diagnosis and this may result in delays in reaching a timely diagnosis. Reducing diagnostic delay is of vital importance to help initiate the appropriate care and identify patients that may benefit from potentially novel treatment strategies (e.g. subset is now eligible for genetic treatment modalities, and several other new compounds are currently being tested).[4,5]

Fasciculation in electromyography (EMG) is described as transient, involuntary muscle activity, representing the spontaneous discharge of one or more motor units.[6] Fasciculation potentials (FPs) have been considered a very early marker of ALS, which even anticipate motor unit potentials (MUPs) instability or reinnervation and are consistent with a very early phase of increased axonal excitability.[7] However, invasiveness and time-consuming limit the application of EMG in detecting fasciculation in more muscles. With the advancement of high-frequency ultrasound technology, the observation of fasciculation under muscle ultrasound (MUS), an important non-invasive auxiliary method, has been gradually adopted in clinical practice. Fasciculation under

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MUS refers to an involuntary twitch of a small part of one muscle with a 0.2-0.5 second duration.[8] Benefiting from the capability of examining a wide muscle area and observing the contraction of multiple muscles simultaneously, recent studies have shown that the sensitivity of MUS to detect fasciculation is significantly higher than EMG and physical examination.[9-12]

There have been several studies of MUS on the differences in fasciculation between ALS and ALS-mimics.[13-16] Based on the firing frequency and site number in the specific muscle group involved in each examination, we defined a criterion including four grades of fasciculation, which could effectively differentiate ALS from non-ALS patients.[17] However, corresponding to previous studies[15,16], in order to improve sensitivity and specificity, we performed MUS on as many muscles as possible. This is not feasible in clinical practice, and might cause significant wastage of limited medical resources. In this study, we aimed to provide a novel protocol for detecting fasciculation by MUS in clinical practice, with less muscle examined while the same sensitivity for detecting fasciculation. Also, a diagnostic process based on fasciculation was proposed to provide fresh insights into the ALS diagnosis.

METHODS

Subjects

This study was a cross-sectional study conducted at the Department of Neurology, Peking Union Medical College Hospital (PUMCH). Patients were recruited consecutively between March 2017 and September 2022 at inpatient wards and outpatient clinics. We set no restriction on onset age, disease course or medication of included patients. Needle EMG and relevant ancillary examinations were conducted on each patient to reach the final diagnosis.

ALS patients according to the Awaji criteria[18] were included in our analysis. All patients were followed up for at least six months for final diagnosis (definite or probable ALS). Non-ALS patients included those with lower motor neuron syndrome caused by other etiology or showing definite responsiveness to immunotherapy or experience spontaneous remission, mainly including multifocal motor neuropathy (MMN), immunotherapy-responding chronic motor axonal polyneuropathy with undefined causes. Besides, motor-dominant cervical spondylosis and lumbar spondylosis were included as disease controls. Benign fasciculations from anxiety syndrome was also included for comparisons. The main exclusion criterion was definite clinical or electrophysiological evidence of sensory involvement. Of note, several other causes of lower motor neuron syndromes with a confirmed systemic etiology, common metabolic disorders and intoxications were excluded as potential mimics.

The following information was collected for each patient: (1) general information [name, gender, body mass index (BMI), disease duration, and region of onset]; (2) detailed neurological examinations; (3) results of EMG. The Medical Research Council (MRC) score, including bilateral assessment of shoulder abduction, elbow flexion, elbow extension, wrist flexion, wrist extension, finger flexion, finger extension, thumb abduction, little finger abduction, hip flexion, knee flexion, knee extension, ankle dorsal flexion, ankle plantar flexion, toe dorsal flexion, and toe plantar flexion was calculated. The total MRC score was 160. The revised ALS functional rating scale (ALSFRS-r) was also assessed.[16]

This study was approved by the Ethics Committee of the PUMCH (JS1218). All enrolled patients provided written, informed consent to be included in the study.

Ultrasound examination

All patients would undergo ultrasound examinations at the first visit to the clinic. MUS was performed by a single examiner with 3-year experience in neuromuscular ultrasound, blinded to the clinical history, findings from neurological examination and ancillary test results including EMG. Included patients were examined in the same examination room with a constant temperature of 26-28°C. All included patients were told to avoid intensive physical activity and intake of potential foods/drinks (e.g. caffeine contents) for at least a week before the examination.

MUS examination was performed equipped with an 8-12 MHz linear array transducer (LOGIQ e; General Electric company, Wuxi, China). The initial settings were kept constant during all examinations. The probe was gently placed on the targeted muscle group to avoid external pressure. The gain was set to automatic mode, the depth and focus were adjusted depending on the muscle and individual variations. Zoom function was omitted to avoid the changes in visual field of each muscle group. All patients were placed in a supine position. The patients were asked to relax

for at least 30 minutes before MUS examination was initiated. The view under the probe always included more than one muscle, especially for the forearm. Therefore, in this study, the target of observation was fasciculations of muscle groups rather than specific muscles. We recorded any fasciculations detected in the area of the muscle group being assessed.

Initially, we selected as many as possible muscle groups for MUS examination. As presented in Supplementary Table 1 and Supplementary Figure 1, nine muscle groups in bulbar region, twenty muscle groups in cervical, fourteen muscle groups in thoracic region and twenty-eight muscle groups in lumbosacral regions for each participant. In the process of enrolling patients, we gradually excluded muscle groups with low detection rates. Each muscle was imaged transversely using the B-mode. The transducer was adjusted to be perpendicular to the belly of the muscle groups, which also was the standard insertion site for the needle used for EMG assessment. This specific orientation allowed the maximal cross-sectional image of the muscles. The transducer was held in the one position for 60 seconds. The presence of fasciculations was recorded for each muscle group. The whole process was recorded in videos for all muscles tested. The patient kept the muscles relaxed and silent during the MUS examination.

The intensity of fasciculations was divided into 5 grades (0 to 4) based on our defined criteria that included firing frequency and site number in the specific muscle group involved in each examination (Supplementary Table 2). Besides, grade 1 and 2 fasciculations were defined as the low-grade fasciculations while grade 3 and 4 fasciculations were defined as the high-grade fasciculations. The videos of MUS fasciculations grading are provided in supplementary materials (Supplementary videos). One experienced examiner (blinded to other ancillary test results, especially EMG) would carefully review the videos and identify the highest fasciculations grade in any 10 seconds within 60 seconds as the final result. The fasciculations grade for each muscle group was recorded after each assessment.

In the process of MUS examination, we tried to find diagnostic indicators with high specificity for ALS, and defined the fasciculations diagnostic score comprising these indicators.

Statistics

Continuous variables were expressed as mean±standard deviation (SD), while fasciculations grade was expressed as median (inter-quartile range, IQR). χ^2 Test was used for the comparisons between rates. Bonferroni correction was used after comparison between multiple groups. Differences in demographic factors and median fasciculations grade between ALS and non-ALS patients were examined using unpaired t-tests. Two-sided P values were calculated for all analyses. In all comparisons, a p-value of less than 0.05 was considered significant. All p-values in the manuscript are values obtained after statistical correction. Receiver operating characteristic (ROC) curve was used for selecting cut off values of ultrasonic indicators for diagnosis and the sensitivity and specificity would be calculated. All statistical analyses were performed using SPSS 23.0 software (Armonk, NY, USA).

RESULTS

Clinical characteristics in ALS and non-ALS patients

A total of 149 ALS patients were finally included, all followed up for at least 6 months, and diagnosed with probable/definite ALS according to Awaji criteria.[18] Non-ALS patients included 8 MMN, 14 pure motor PN with unknown causes, 28 cervical spondylosis or lumbar spondylosis (8 with C5-6 radiculopathy, 4 with C6-7 radiculopathy, 7 with L4-5 radiculopathy and 9 with both C5-6 and L4-5 radiculopathy), and 4 benign fasciculations syndrome patients. The clinical features were presented in Table 1. There were significant differences in age of onset, gender ratio, disease duration and BMI while no clinically meaningful difference was revealed in baseline total MRC score between ALS and non-ALS patients.

Fasciculations characteristics in ALS and non-ALS patients

To provide feasible protocol for clinical MUS examination, we conducted MUS detection for fasciculations on a total of seventy-one muscle groups in four body regions for each participant initially (Supplementary Table 1). During the detection process of the first 50 patients, we found that the entire process might take up to 90 minutes and the positive rate of detection in some muscle groups was relatively low, as shown in Table 2. Therefore, we selected the following muscle groups for the further MUS detection: bilateral sternocleidomastoideus and suprahyoid muscle for bulbar region; bilateral proximal/distal flexor/extensor in upper limbs for cervical region;

Table 1. Clinical characteristics of included patients

	ALS patients	Non-ALS patients	Comparisons
N	149	54	
Onset Age (years old)	54.32±11.74	47.52±18.25	**0.002**
Gender (M/F)	79/70	16/38	**0.027**
BMI (kg/m^2)	23.75±3.96	29.18±30.00	**0.031**
Disease duration (months)	16.01±14.62	36.80±48.34	**<0.001**
Total MRC score	134.34±19.76	129.00±22.48	0.120
ALSFRS-r score	40.98±5.23		
Region of onset (n, %)			
Bulbar	25 (16.8%)		
Cervical	77 (51.7%)		
Lumbosacral	47 (31.5%)		
Awaji criteria (n, %)			
Probable ALS	72 (48.3%)		
Definite ALS	77 (51.7%)		

Note: Continuous variables were expressed as mean±standard deviation (SD) and compared with t-test. χ^2 Test was used for the comparisons between rates. A p-value of less than 0.05 was considered significant, which was bold.
Abbreviations: ALS amyotrophic lateral sclerosis; ALSFRS-r revised ALS functional rating scale; BMI body mass index; F female; M male; MRC Medical Research Council.

bilateral T10 paraspinal muscle in thoracic region; bilateral proximal/distal flexor/extensor in lower limbs and L1 paraspinal muscle for lumbosacral region (Figure 1).

Fasciculations were detected in 42.7% (2326/5444) of included muscle groups, including 49.6% (2141/4328) among ALS patients and 16.6% (185/1116) among non-ALS patients. For ALS patients, the detection rate of fasciculations was highest in the lumbosacral (59.8%) and cervical (55.5%) muscle groups, followed by the thoracic muscles (34.3%), and the bulbar muscle groups (15.6%) ($p<0.05$). The median intensity of thoracic or bulbar muscle groups was also significantly lower than that of cervical or lumbosacral muscle groups ($p<0.001$). Besides, in cervical region, the detection rate of fasciculations of the flexors was significantly higher than that of the extensors ($p<0.05$). In lumbosacral region, there was clinically meaningful difference in the detection rate of fasciculations between proximal (76.5%) and distal (64.4%) muscle groups ($p<0.05$), while not in the comparison between flexors and extensors.

As shown in Table 2, the detection rates of included muscle groups among ALS patients were all significantly higher than that of non-ALS patients. Similar predominant differences also existed in median fasciculations grade between ALS and non-ALS patients ($p<0.001$). Besides, high grade fasciculations were common in ALS than non-ALS patients ($p<0.001$).

The main targets of fasciculations detection by MUS examination

To better differentiate ALS from non-ALS patients, high sensitivity and specificity MUS indicators were needed. Given that the detection rates of fasciculations of muscle groups were higher than 10% in cervical and lumbosacral regions, it might be more appropriate for high-grade of fasciculations to be a potential diagnostic indicator for ALS. For muscle groups in bulbar region and thoracic region, the detection of fasciculations was of high specificity to ALS patients and could act as diagnostic indicators for ALS.

To conclude, the following four potential indicators deserved main attention during MUS examination: (1) Detection of fasciculations in bulbar muscle groups; (2) Detection of high-grade fasciculations in cervical muscle groups; (3) Detection of fasciculations in thoracic muscles; (4) Detection of high-grade fasciculations in lumbosacral muscle groups.

Proposed muscle groups for detection

We sorted the included muscle groups according to the detection rate of fasciculations or high-grade fasciculations, and further observed the total detection rate of diagnostic indicators discussed above by increasing one tested muscle group in sequence each time both in ALS and non-ALS patients.

As shown in Figure 2, we recommended the

Table 2. Detection of fasciculations among ALS and non-ALS patients

Region	Muscles (groups)	Detection rate of any fasciculation (n/N, %)			Median (IQR) of fasciculation			Detection rate of high-grade fasciculation (n/N, %)		
		ALS	Non-ALS	p	ALS	Non-ALS	p	ALS	Non-ALS	p
Bulbar region	Frontalis	0/112 (0.00%)			0 (0-0)					
	Facialis	2/70 (2.86%)			0 (0-0)					
	Masticatory muscle	3/112 (2.67%)			0 (0-0)					
	Sternocleidomastoideus	48/212 (22.64%)	0/90 (0.00%)		0 (0-0)	0 (0-0)	**0.002**	14/212 (6.60%)	0/90 (0.00%)	
	Suprahyoid muscle	49/146 (33.56%)	0/54 (0.00%)		0 (0-1)	0 (0-0)	**0.002**	9/146 (6.16%)	0/54 (0.00%)	
Cervical region	Proximal flexors in upper limb	237/298 (79.53%)	13/108 (12.04%)	**<0.001**	2 (1-4)	0 (0-0)	**<0.001**	136/298 (45.64%)	1/108 (0.93%)	**<0.001**
	Proximal extensors in upper limb	212/298 (71.14%)	12/108 (11.11%)	**<0.001**	2 (0-4)	0 (0-0)	**<0.001**	111/298 (37.25%)	1/108 (0.93%)	**<0.001**
	Distal flexors in upper limb	180/228 (78.95%)	34/108 (31.48%)	**<0.001**	2 (1-4)	0 (0-1)	**<0.001**	106/228 (46.49%)	2/108 (1.85%)	**<0.001**
	Distal extensors in upper limb	160/228 (70.18%)	27/108 (25.00%)	**<0.001**	2 (0-3)	0 (0-0.75)	**<0.001**	67/228 (29.39%)	4/108 (3.70%)	**<0.001**
	Abductor pollicis brevis	30/52 (57.69%)			1 (0-2)					
	Abductor digiti minimi	22/50 (44.00%)			1 (0-2)					
	The first dorsal interosseous muscle	23/42 (54.76%)			1 (0-2)					
	C5 paraspinal muscle	13/120 (10.83%)			0 (0-0)					
	C6 paraspinal muscle	6/50 (12.00%)			0 (0-0)					
	C7 paraspinal muscle	3/50 (6.00%)			0 (0-0)					
Thoracic region	T2 paraspinal muscle	5/50 (10.00%)			0 (0-0)					
	T6 paraspinal muscle	6/50 (12.00%)			0 (0-0)					
	T8 paraspinal muscle	11/70 (15.71%)			0 (0-0.5)					
	T10 paraspinal muscle	131/228 (57.46%)	2/108 (1.85%)	**<0.001**	0 (0-1)	0 (0-0)	**<0.001**	27/228 (11.84%)	0/108 (0.00%)	
	T6 rectus abdominis	11/40 (27.50%)			0 (0-1)			0/40 (0.00%)		
	T8 rectus abdominis	16/70 (22.86%)			0 (0-0)			4/70 (5.71%)		
	T10 rectus abdominis	8/40 (20.00%)			0 (0-0)			2/40 (5.00%)		

Lumbosacral region	Proximal flexors in lower limb	172/228 (75.44%)	18/108 (16.67%)	**<0.001**	2 (1-4)	0 (0-0)	**<0.001**	102/228 (44.74%)	4/108 (3.70%)	**<0.001**
	Proximal extensors in lower limb	177/228 (77.63%)	18/108 (16.67%)	**<0.001**	2 (1-3)	0 (0-0)	**<0.001**	74/228 (32.46%)	3/108 (2.78%)	**<0.001**
	Distal flexors in lower limb	189/298 (63.42%)	29/108 (26.85%)	**<0.001**	1 (0-2)	0 (0-1)	**<0.001**	54/298 (18.12%)	2/108 (1.85%)	**<0.001**
	Distal extensors in lower limb	195/298 (65.44%)	32/108 (29.63%)	**<0.001**	1 (0-2)	0 (0-1)	**<0.001**	53/298 (17.79%)	3/108 (2.78%)	**<0.001**
	L1 paraspinal muscle	30/50 (60.00%)			1 (0-2)					
	L2 paraspinal muscle	28/50 (56.00%)			1 (0-2)					
	L3 paraspinal muscle	22/50 (44.00%)			1 (0-2)					
	L4 paraspinal muscle	41/120 (34.17%)			0 (0-1)					
	L5 paraspinal muscle	27/50 (54.00%)			1 (0-3)					
	S1 paraspinal muscle	20/50 (40.00%)			0 (0-3)					
	S2 paraspinal muscle	26/50 (52.00%)			1 (0-3)					
	S3 paraspinal muscle	20/50 (40.00%)			0 (0-1)					
	S4 paraspinal muscle	15/50 (30.00%)			0 (0-1)					
	S5 paraspinal muscle	8/50 (16.00%)			0 (0-0)					

Note: Fasciculations grade was expressed as median (inter-quartile range, IQR) and compared with unpaired t-test. χ^2 Test was used for the comparisons between rates. A p-value of less than 0.05 was considered significant, which was bold.

Abbreviations: ALS amyotrophic lateral sclerosis.

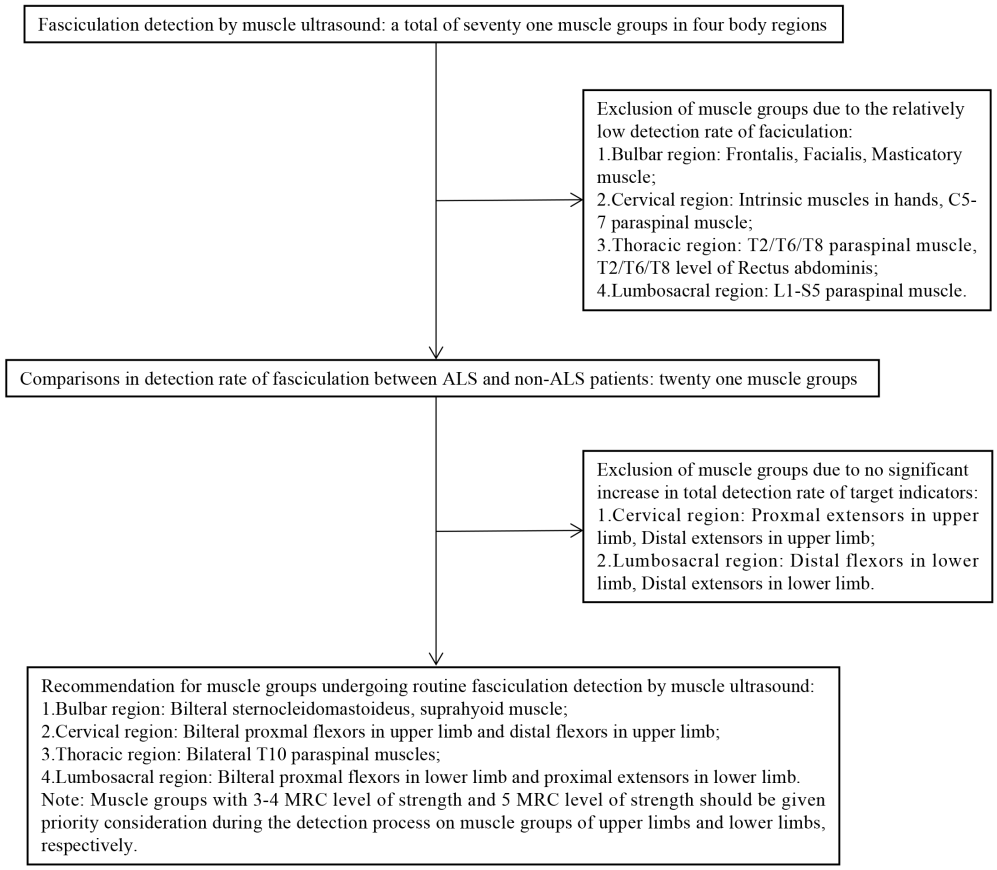


Figure 1. The selection of muscle groups for fasciculations detection by muscle ultrasound.
Abbreviations: ALS amvotrophic lateral sclerosis: MRC the Medical Research Coucil

following muscle groups as routine choices for fasciculations detection by MUS examination: (1) Bulbar region: Suprahyoid muscles, Sternocleidomastoideus; (2) Cervical region: Proximal flexors in upper limbs, Distal flexors in upper limbs; (3) T10 paraspinal muscles; (4) Lumbosacral region: Proximal flexors in lower limbs, Proximal extensors in lower limbs. Detection of proximal extensors in upper limbs, distal flexors in lower limbs and distal extensors in both upper and lower limbs added no significantly diagnostic value for ALS and might not be conducted routinely.

Comparisons in detection rate of fasciculations (for bulbar and paravertebral muscle groups) or high-grade fasciculations (for limb muscle groups) between unilateral and bilateral muscle groups among ALS patients were presented in Supplementary Table 3. The clinically meaningful differences between unilateral and bilateral detection of targeted fasciculations suggested that MUS examination of bilateral muscle groups might be more reasonable.

Besides, we divided included patients with ALS according to region of onset and MRC muscle strength of tested muscle groups. Relevant results were presented in Supplementary Table 4. There was no prominent divergence in distribution characteristics of fasciculations in bulbar muscle groups and thoracic muscles among patients with different regions of onset. Compared to muscle groups in lower limbs, detection rate of high-grade fasciculations in upper limbs was higher. As for muscle strength, detection rate of high-grade fasciculations of cervical muscle groups was highest among those with 3-4 MRC level of strength ($p<0.001$), while for lumbosacral muscle groups, detection rate of high-grade fasciculations was highest among those with 5 MRC level of strength ($p<0.001$).

Judging from the discussion above, we recommended thirteen muscle groups for fasciculations detection by MUS examination for each participant, as presented in Figure 1. This could reduce scanning time of each patient from 90 minutes to less than 10 minutes, and significantly increase the tolerance of patients.

Diagnostic value of fasciculations

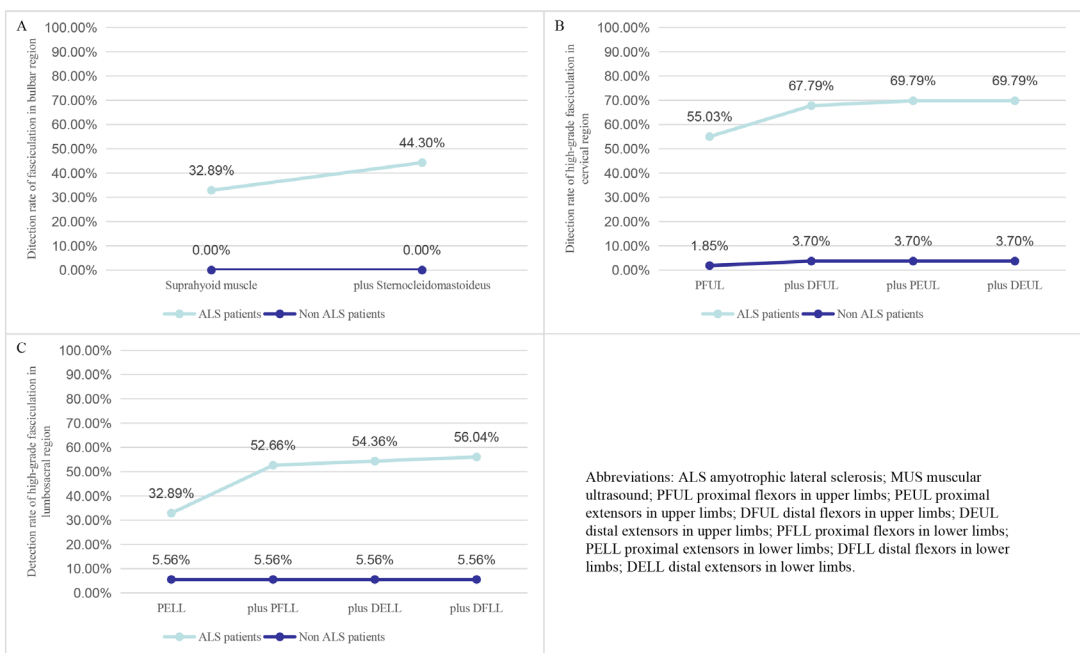
Based on the above results, we further explore the diagnostic value of fasciculations in differentiating ALS from ALS-mimics. ALS patients (N=71) and non-ALS patients (N=45) with the results of fasciculations of all recommended thirteen muscles were included in the diagnostic analysis. Fulfillment of each diagnostic indicator scored one point and the total score was defined as the fasciculations diagnostic score (0-4).

The mean (SD) fasciculations diagnostic score for included ALS patients was 2.5 (1.2), while for non-ALS patients, it was 0.2 (0.6). ROC analysis showed that the area under curve (AUC) was 0.961 (95%CI 0.927-0.996) (Figure 3). The optimal cut-off value was 1 point with 95.8% of sensitivity and 88.9% of specificity. The misdiagnosed patients included 4 MMN patients and 1 lumbar spondylosis patient (false positive rate: 11.1%).

DISCUSSION

The first objective of this study was to analyze the distribution of fasciculations in ALS patients using MUS technology. We found that the highest detection rate of fasciculations was in the lumbosacral region, followed by the cervical region and thoracic region, and the lowest in the bulbar region. The median fasciculations diagnostic grade in cervical muscle groups was the highest, while the lowest was in bulbar muscle groups. Consistent with our results, Vazquez-Costa *et al*. found that in ALS patients, fasciculations occurred most frequently in the limb muscles, especially the cervical muscles, and biceps brachii was the muscle with the highest fasciculations frequency.[11] Noto *et al*. found that the proportion of bulbar muscles (tongue and trapezius) with fasciculations was lower than cervical and lumbosacral muscles.[13] EMG studies of fasciculations also reported similar results.[17] We used the entire muscle group as a unit when detecting fasciculations, which might partly explain the higher detection rate of cervical and lumbosacral fasciculations than prior studies.

The second objective of this study was to provide a feasible protocol for MUS examination that maximized specificity for ALS. There were many prior studies that have explored the diagnostic value of fasciculations by MUS in the ALS, some of which proposed their scanning protocols. Hannaford *et al*. established a simplified 5 muscle screening protocol exhibited an AUC of 0.94 (95 %CI 0.89-0.99) in discriminating ALS from mimics.[19] Ma *et al*. used the fasciculations score comprising unilateral 10 muscles in each patient for the diagnosis of ALS, which showed high sensitivity and specificity.[20] Through machine learning, Fukushima *et al*. developed and validated MUS-fasciculations-based diagnostic models with high positive predictive value



Abbreviations: ALS amyotrophic lateral sclerosis; MUS muscular ultrasound; PFUL proximal flexors in upper limbs; PEUL proximal extensors in upper limbs; DFUL distal flexors in upper limbs; DEUL distal extensors in upper limbs; PFLL proximal flexors in lower limbs; PELL proximal extensors in lower limbs; DFLL distal flexors in lower limbs; DELL distal extensors in lower limbs.

Figure 2. The detection rate of potentially diagnostic indicators by MUS among ALS and non-ALS patients

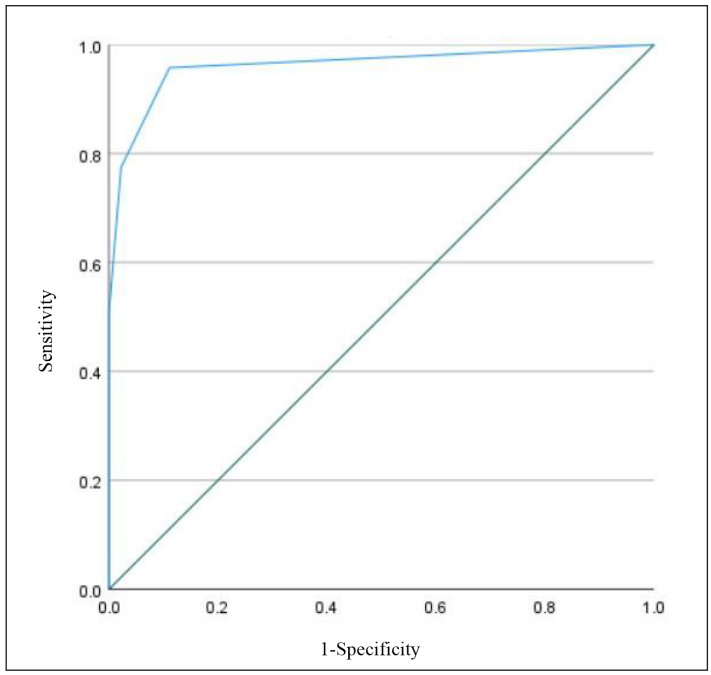


Figure 3. ROC curve of the fasciculations diagnostic score in differentiating ALS from non-ALS patients
Abbreviations: ALS amyotrophic lateral sclerosis; ROC receiver operating characteristic.

(precision) for both early- and later-stage ALS patients.[21] In this study, we used semi-quantitative analysis of the fasciculations and found that the detection of fasciculations in bulbar and thoracic muscle groups and detection of high-grade fasciculations in cervical and lumbosacral muscle groups have high specificity for the diagnosis of ALS. This could not only aid in the exclusion of some benign fasciculations that were commonly among PN or anxious patients, but also simplify the whole detection process. Besides, recommendation of tested muscles based on body regions might be more in line with the clinical and electrophysiological characteristics of ALS patients, that was, multiple regions of neuronal damage in both upper and lower motor neurons.[22] We initially chose 71 muscle groups for fasciculations detection per patient, which resulted in over 90-minute detection time for each participant. Through analysis, we recommended routine detection of 13 muscle groups for each patient, which significantly decreased the detection time to less than 15 minutes. Besides, during the analysis process, we found that the three-level classification system (no fasciculations, low level of fasciculations and high level of fasciculations) could meet the diagnostic needs regarding ALS, which might be more easily mastered by clinicians. The duration for fasciculations detection in one muscle group could be further limited to around 10-20 seconds. Through these measures, the estimated detection time for each patient would not be longer than 10 minutes.

We found high grade fasciculations were more frequently detected in limb muscles, especially upper limb, with preserved strength. Consistent with our study, Bokuda *et al*.[23] reported the FPs were more common in biceps brachii with 4 level of MRC strength and tibialis anterior with 5 level of MRC strength. We suspected that fasciculations were more prevalent in denervated muscle with active re-innervation and therefore more prevalent in muscles with preserved strength.[24] This indicated that a window of optimal detection (beyond this too few surviving motor neurons might be present that could generate fasciculations) and muscle groups with relatively preserved muscle strength should be prioritized in the MUS examination.

For other muscle groups, such as cervical and lumbosacral paraspinal muscles or rectus abdominis, could be chosen for fasciculations detection to assist in clinical localization and differentiation peripheral neuropathy from radiculopathy. It was noteworthy that fasciculations by MUS examination was a flexible auxiliary examination, and the final tested muscle groups should be determined based on clinical scenarios.

In addition, we hoped to facilitate the utilization

of fasciculations by MUS in the diagnosis and mimic-diagnosis of ALS. We defined the fasciculations diagnostic score involving 13 muscle groups for each patient, which were able to differentiate ALS from non-ALS patients. The proposed diagnostic process was shown in Figure 4. The main advantage of MUS was its timesaving, simplicity and non-invasiveness. We recorded fasciculations from muscle groups instead of individual muscles, which saved time, and was easily performed. It was worth noting that the diagnosis of ALS was mainly based on clinical information. The role of fasciculations detection by MUS might be more in the early screening of patients with limb weakness, and the differential diagnosis of ALS patients with neurogenic damage in one body region in the early stages, similar to that of EMG. However, with the increasing studies on fasciculations, we hoped that MUS could partly replace the role of needle EMG in the diagnosis of ALS in the future.

The misdiagnosis (false positives) mainly came from MMN patients, which deserved more attention in clinical practice and needed further EMG examination or follow-up. Considering that ALS is an excluded diagnosis, more strength should be placed on reducing the misdiagnosis rate and missed diagnosis rate. We found when a cut-off value was set at 2 points, 77.5% of ALS could be diagnosed while only one MMN patient was misdiagnosed. This indicated that when high-grade fasciculations were detected in limbs, enough attention was deserved for the diagnosis of MMN in addition to ALS. And when a cut-off value was set at 3 points, none of non-ALS patient would be misdiagnosed as ALS. The optimal cut-off will depend on the clinical context and pre-test probability for ALS. Besides, we found fasciculations were detected in all ALS patients, meaning that no detection of fasciculations might serve as an excluded standard for ALS.

This study had several limitations. First, the significantly longer disease duration in non-ALS patients limited the diagnostic significance of our results in early stages. Second, we did not combine fasciculations results of MUS with other parameters, such as EMG, to provide an overall comprehensive analysis. Other sonographic features such as presence of atrophy, changes in echogenicity of muscles and presence of fibrillations were not part of present study. Third, the cross-sectional design disabled us to analyze the dynamic changes of fasciculations in ALS. Lastly, we only included part of motor-dominant radiculopathy or peripheral neuropathy in control group, the comparisons between ALS and more ALS-mimics needed to conduct. Besides, change of study protocol, heterogeneity patient characteristics and potential selection bias in controls might also cause influence of our results, which needed further validation.

In conclusion, our study showed that a practical MUS protocol was feasible with optimal diagnostic yield in suspected ALS to help detect fasciculations. This could complement routine clinical evaluation and electrodiagnostic work-up and could be performed as a practical bedside test with little patient burden and low cost. Multiple-

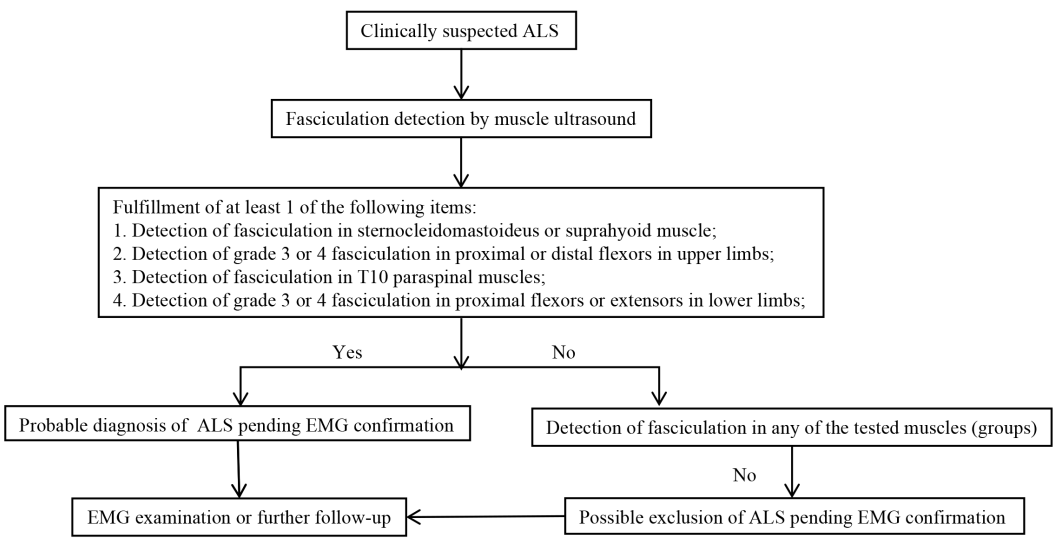


Figure 4. The detection rate of potentially diagnostic indicators by MUS among ALS and non-ALS patients
Abbreviations: ALS amyotrophic lateral sclerosis; EMG electromyography.

center, large-sample and longitudinal studies were needed to further explore the diagnostic value of fasciculations by MUS, especially those combined EMG and other ultrasonic indicators.

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DISCLOSURE

Ethics: This study was approved by the Ethics Committee of the PUMCH (JS1218). All enrolled patients provided written, informed consent to be included in the study.

Data availability: The data in the study are available by contacting the corresponding author.

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Conflict of interest: None

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Supplementary Table 1. Regions and muscle groups assessed with MUS

Regions	Muscle groups	Muscles
Bulbar	Frontalis	
	Facialis	
	Masticatory muscle	Temporal muscle, masseter muscle, medial pterygoid muscle, lateral pterygoid muscle
	Sternocleidomastoideus	
	Suprahyoid muscle	Musculus digastricus, mylohyoid, geniohyoid, tongue muscle
Cervical	Proximal flexors in upper limb	Biceps brachii, brachialis
	Proximal extensors in upper limb	Triceps brachii
	Distal flexors in upper limb	Flexor carpi radialis, palmaris longus, Flexor carpi ulnaris, flexor digitorum superficialis, flexor digitorum profundus
	Distal extensors in upper limb	Extensor digitorum, extensor carpi radialis brevis, extensor carpi ulnaris, extensor pollicis longus, abductor pollicis longus
	Intrinsic muscles in hands	Abductor pollicis brevis, abductor digiti minimi, the first dorsal interosseous muscle
	Paravertebral muscles	C5 paraspinal muscle, C6 paraspinal muscle, C7 paraspinal muscle
Thoracic	Paravertebral muscles	T2 paraspinal muscle, T6 paraspinal muscle, T8 paraspinal muscle, T10 paraspinal muscle
	Rectus abdominis	T6 level, T8 level, T10 level
Lumbosacral	Proximal flexors in lower limb	Vastus lateralis, rectus femoris, vastus intermedius, tensor fasciae latae
	Proximal extensors in lower limb	Semitendinosus, semimembranosus, biceps femoris
	Distal flexors in lower limb	Gastrocnemius, soleus, flexor hallucis longus, flexor digitorum longus, tibialis posterior
	Distal extensors in lower limb	Tibialis anterior, extensor hallucis longus, extensor digitorum longus
	Paravertebral muscles	L1 paraspinal muscle, L2 paraspinal muscle, L3 paraspinal muscle, L4 paraspinal muscle, L5 paraspinal muscle, S1 paraspinal muscle, S2 paraspinal muscle, S3 paraspinal muscle, S4 paraspinal muscle, S5 paraspinal muscle

Supplementary Table 2. The criteria for fasciculations grade

Fasciculations intensity	Definition
Grade 0	No fasciculations in the area tested
Grade 1	Fasciculations presented at ≤ 2 sites in the area tested, and ≤ 3 times in 10 seconds at any site.
Grade 2	Fasciculations presented at ≤ 2 sites in the area tested, and > 3 times in 10 seconds at least at 1 site.
Grade 3	Fasciculations presented at ≥ 3 sites in the area tested, and ≤3 times in 10 seconds at any site.
Grade 4	Fasciculations presented at ≥ 3 sites in the area tested, and >3 times in 10 seconds at least at 1 site.

Supplementary Table 3. Comparisons in detection rate of fasciculations (for bulbar and thoracic muscle groups) or high-grade fasciculations (for limb muscle groups) between unilateral and bilateral muscle groups among ALS patients

Part I. Detection rate of fasciculations

Region of the muscles (groups)	Region of onset (n/N, %)			
	Bulbar	Cervical	Lumbosacral	p
Bulbar region	13/69 (18.84%)	59/180 (32.78%)	25/109 (22.94%)	**0.032**
Thoracic region	10/28 (35.71%)	81/128 (63.28%)	42/74 (56.76%)	**<0.001**

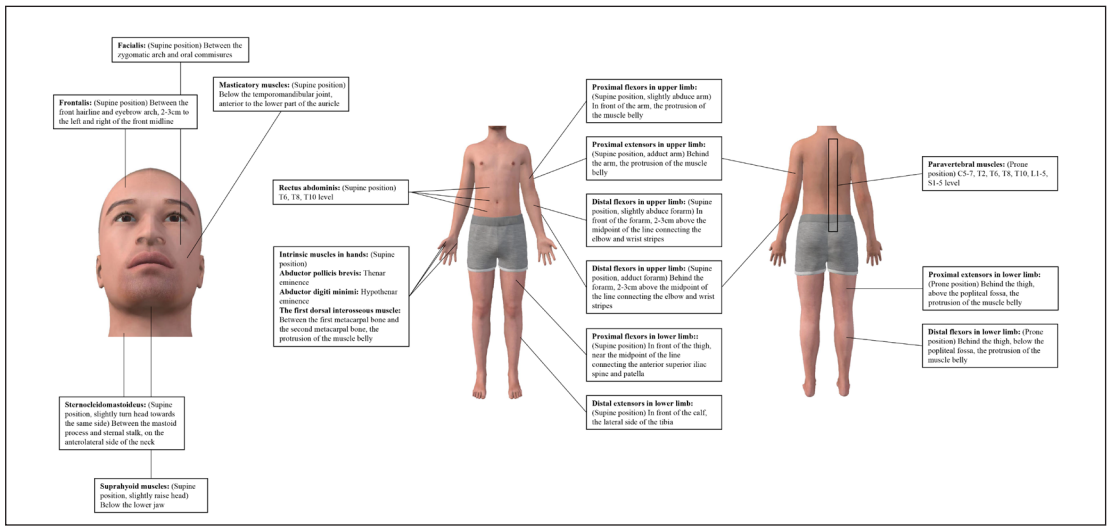
Part II. Detection rate of high-grade fasciculations

Region of the muscles (groups)	Region of onset (n/N, %)				Muscle strength (MRC) (n/N, %)			
	Bulbar	Cervical	Lumbosacral	p	0-2	3-4	5	p
Cervical region	52/156 (33.33%)	264/560 (47.14%)	94/336 (27.98%)	**<0.001**	38/104 (36.54%)	202/421 (47.98%)	171/579 (29.53%)	**<0.001**
Lumbosacral region	13/156 (8.33%)	171/560 (30.54%)	99/336 (29.46%)	**<0.001**	19/82 (23.17%)	82/275 (29.82%)	275/695 (39.57%)	**<0.001**

Note: χ^2 Test was used for the comparisons between rates. Bonferroni correction was used after comparison between multiple groups. A p-value of less than 0.05 was considered significant, which was bold.
Abbreviations: ALS amyotrophic lateral sclerosis; MRC Medical Research Council.

Supplementary Table 4. Results of subgroup analysis

	Left (n, %)	Right (n, %)	Bilateral (n, %)
Sternocleidomastoideus	25 (16.78%)	23 (15.44%)	33 (22.15%)
Proximal flexors in upper limb	72 (48.32%)	64 (42.95%)	82 (55.03%)
Distal flexors in upper limb	55 (36.91%)	51 (34.23%)	65 (43.62%)
T10 paraspinal muscle	63 (42.28%)	68 (45.64%)	81 (54.36%)
Proximal flexors in lower limb	45 (30.20%)	57 (38.26%)	66 (40.27%)
Proximal extensors in lower limb	37 (24.83%)	37 (24.83%)	49 (32.89%)



Supplementary Figure 1. Sites of muscle ultrasound detection for fasciculation.

Supplementary videos:

video 1: Fasciculation grade 0 http://neurology-asia.org/content/30/3/neuroasia-2025-30(3)-825-v1.mp4
video 2: Fasciculation grade 1 http://neurology-asia.org/content/30/3/neuroasia-2025-30(3)-825-v2.mp4
video 3: Fasciculation grade 2 http://neurology-asia.org/content/30/3/neuroasia-2025-30(3)-825-v3.mp4
video 4: Fasciculation grade 3 http://neurology-asia.org/content/30/3/neuroasia-2025-30(3)-825-v4.mp4
video 5: Fasciculation grade 4 http://neurology-asia.org/content/30/3/neuroasia-2025-30(3)-825-v5.mp4