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ORIGINAL ARTICLE

ULTRASOUND ASSESSMENT OF LOWER-LIMB MUSCLE ARCHITECTURE IN SEVERE ACQUIRED BRAIN INJURY. PROSPECTIVE STUDY

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LEVEL OF EVIDENCE: 3

ABSTRACT

Objectives: Ultrasound (US) is a validated tool for the *in vivo* assessment of muscle architecture. Individuals with severe acquired brain injury (sABI) often develop profound alterations in muscle structure and function due to neurological impairment, prolonged immobilization, and systemic factors. This prospective study aims to (i) describe a standardized ultrasound protocol for lower-limb muscle architecture assessment in patients with sABI, (ii) illustrate typical ultrasound features with different clinical presentations compared with a healthy subject, and (iii) discuss the potential clinical implications for neurorehabilitation practice.

Methods: Cross-sectional area (CSA), muscle thickness, and pennation angle were assessed bilaterally at the rectus femoris (RF) and medial gastrocnemius (MG) muscles using B-mode ultrasound, following a standardized protocol and anatomical landmarks.

Results: The patient with a more severe clinical condition and spasticity showed marked alterations in muscle echostructure, characterized by a pronounced reduction in CSA, muscle thickness, and pennation angle, together with increased connective tissue and loss of normal fiber organization. In contrast, the patient with earlier functional recovery and absence of spasticity demonstrated only partial muscle architecture alterations, with overall preservation of muscle structure compared with the healthy subject.

Conclusions: The proposed ultrasound protocol appears feasible, reliable, and suitable for patients with sABI across different rehabilitation settings. When integrated with clinical assessment, muscle ultrasound may support the evaluation of muscle status, guide rehabilitation strategies, and provide complementary prognostic information.

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Further studies on larger samples are warranted to confirm these preliminary observations.

KEYWORDS
Skeletal muscle ultrasound, disorder of consciousness, muscle cross-sectional area, muscle thickness, pennation angle

INTRODUCTION

In recent years, ultrasound imaging (USI) has gained increasing relevance in neurorehabilitation as a noninvasive, accessible, and reliable tool for assessing skeletal muscle architecture. USI allows the evaluation of architectural parameters including cross-sectional area (CSA), muscle thickness, pennation angle, fascicle length, echo intensity, and tissue stiffness, providing valuable information on muscle structure and function *in vivo*[1–5]. Ultrasound can be applied at rest, during isometric contraction, or dynamically during functional tasks, and can be implemented in different clinical settings, including bedside evaluation, inpatient rehabilitation, and outpatient care[2,6]. These characteristics make USI particularly suitable for patients with neurological conditions, where conventional strength testing may be limited by poor cooperation, impaired consciousness, or severe motor deficits.

Neurological disorders associated with altered motor control induce significant modifications in muscle architecture and function, reflecting deficits in neuromuscular coordination, motor output and muscle efficiency[7–9]. These changes are further influenced by age, nutritional status, pharmacological treatment, and physical activity levels[10–12]. Prolonged immobilization, a common condition in patients with severe acquired brain injury (sABI), results in reductions in muscle CSA, thickness, and pennation angle, along with increased intramuscular connective tissue and echo intensity[4,13]. Despite its potential clinical value, muscle ultrasonography remains underutilized in patients with sABI. In this population, the assessment of muscle architecture may offer insights into the extent of musculoskeletal involvement, enable longitudinal monitoring of structural changes, and complement clinical evaluation during rehabilitation.

Alterations in muscle architecture affect key physiological properties, including force–length and force–velocity relationships, maximal force generation, muscle stiffness, and susceptibility to injury, all of which may influence functional recovery and rehabilitation outcomes[2,11,13]. This technical note describes a standardized and clinically feasible ultrasound protocol for assessing lower-limb muscle architecture in patients with sABI. Through two illustrative cases with different clinical conditions and comparison with a healthy subject, we provide technical considerations, describe typical ultrasound findings, and discuss potential clinical implications of integrating muscle ultrasound into neurorehabilitation practice.

MATERIALS AND METHODS

Study design

This manuscript is structured as a technical note with illustrative cases and is reported in accordance with the CARE guidelines. The aim was to describe a standardized ultrasound (US) protocol for assessing lower-limb muscle architecture in patients with sABI and to illustrate typical findings through representative clinical cases.

Aims

This prospective study aims to describe a standardized ultrasound protocol for the assessment of lower-limb muscle architecture in patients with sABI, illustrate ultrasound findings in patients with different clinical presentations compared with a healthy subject for descriptive purposes, and outline potential clinical implications for neurorehabilitation practice.

Participants

Three subjects were evaluated:

Subject 1: 55-year-old man, assessed 78 days after the neurological event, presenting with a severe clinical condition, marked functional impairment, and severe lower-limb spasticity.

Subject 2: 32-year-old woman, assessed 33 days after the event, showing a more favorable clinical condition with progressive motor recovery and absence of spasticity.

Subject 3: 34-year-old healthy man with no relevant medical history who practiced regular physical activity, included for illustrative purposes only.

Functional assessment

For each patient with sABI, functional outcome measures reflecting impairments and global disability within an ICF-based assessment framework were collected[14]. These included the Level of Cognitive Functioning (LCF)[15], Coma Recovery Scale–Revised (CRS-R)[16], Disability Rating Scale (DRS)[17,18], and Motricity Index for the Lower Limb (MI-LL)[19]. Anthropometric data were recorded.

Ultrasound assessment

Ultrasound examinations were performed using a HI VISION Avius ultrasound diagnostic scanner (Hitachi, Japan), equipped with a linear probe (bandwidth frequency 4–16 MHz; ultrasound surface area 49 × 10 mm; maximum depth 100 mm). Scanner settings were standardized (frequency 8–9.6 MHz; single focal zone; depth 4 cm). All ultrasound settings, including frequency, depth, focal zone,

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and gain, were kept constant across subjects to ensure image standardization and comparability of measurements.
Ultrasound assessment was performed bilaterally on the rectus femoris (RF) and medial gastrocnemius (MG) muscles, with subjects in a supine position and muscles in a passive state. The supine position was selected because it was better suited to the patients' clinical conditions, as they were often unable to cooperate or tolerate alternative positions due to neurological impairment. Hip extension was maintained at 180°, knee extension at 0°, and ankle position at 90°. The probe was positioned perpendicular to the skin surface with controlled pressure to avoid tissue compression.
Standardized anatomical landmarks were used. For RF, the probe was positioned at the midpoint between the anterior superior iliac spine and the proximal border of the patella. For MG, the probe was positioned at the proximal one-third of the distance between the medial knee crease and the medial malleolus *(Fig. 1)*.

Ultrasound parameters

The following parameters were assessed:
Cross-sectional area (CSA): anatomical CSA measured on transverse images (cm^2). CSA is directly related to muscle force capacity. Because CSA measurements do not require voluntary muscle activation, they are useful for quantifying muscle status in non-cooperative patients *(Fig. 2)*.
Muscle thickness: distance between the superficial and deep aponeuroses on longitudinal images (mm). Thickness measurements show high reliability and reproducibility and correlate with muscle mass and strength *(Fig. 3)*.
Pennation angle: angle between muscle fascicles and the deep aponeurosis on longitudinal images (degrees). Pennation angle is associated with the amount of contractile tissue packed within a given volume and is related to force-generating capacity *(Fig. 3)*.

RESULTS

Results are reported descriptively, without inferential statistical analysis.

Subject 1

A 55-year-old man with acquired brain injury following a left cerebellar hemorrhage complicated by hydrocephalus. Medical history included hypertension and a minor stroke 4 years prior without sequelae. The patient was admitted to the rehabilitation unit 72 days after the event in a minimally conscious state (CRS-R 7), with spastic tetraparesis, tracheostomy, and enteral nutrition. Rehabilitation program included passive mobilization, postural positioning, static standing, robotics, and semi-immersive virtual reality stimulation. Ultrasound evaluation was performed 78 days after the event and revealed overall muscle atrophy, reduced subcutaneous adipose tissue, and marked increase in connective tissue with disorganized muscle architecture (see *Tables 1*, *2* and *Fig. 4*, *5*).

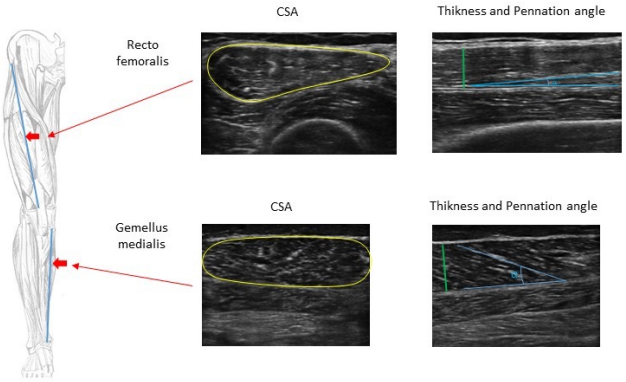


Figure 1. *Ultrasound landmarks.*
Rectus femoris: midpoint between the anterior superior iliac spine and proximal patella border. Medial gastrocnemius: proximal one-third between the medial knee crease and medial malleolus.

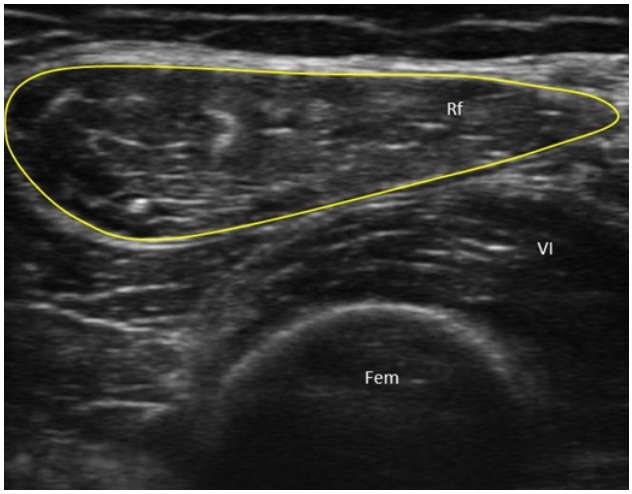


Figure 2. *Cross-sectional area (CSA) measurement of the rectus femoris (transverse view).*

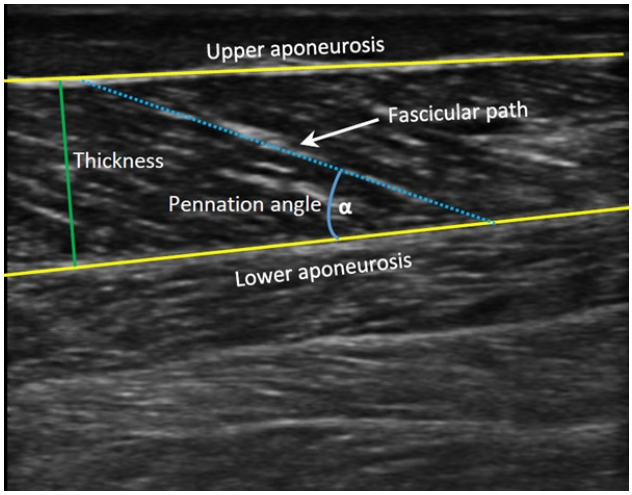


Figure 3. *Muscle thickness and pennation angle measurements of the medial gastrocnemius (longitudinal view).*

Subject 2

A 32-year-old woman with acquired brain injury due to meningoencephalitis followed by status epilepticus; she remained in coma for 7 days. She was admitted to the neurorehabilitation unit 28 days after the event in stable clinical condition, with tracheostomy, nasogastric tube, and

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Subject	Sex	Age (y)	Weight (kg)	Height (cm)	BMI (kg/m²)	Event-T0 (days)	DRS	LCF	CRS-R	MI-LL (R/L)
S1	M	55	86	180	26.6	78	24	3	7	0 / 0
S2	F	32	50	175	18.3	33	12	5	23	63 / 57
S3	M	34	72	174	23.8	NA	NA	NA	NA	NA

Table 1. *Anthropometric and functional data.*
Abbreviations: DRS, Disability Rating Scale; LCF, Level of Cognitive Functioning; CRS-R, Coma Recovery Scale–Revised; MI-LL, Motricity Index for Lower Limb; NA, not applicable. Subject IDs: S1, subject 1; S2, subject 2; S3, subject 3.

bladder catheter. Rehabilitation program included assisted active mobilization, postural transitions, strengthening, gait training with assistance and a forearm walker, robotics, and speech therapy for dysphagia. At the time of assessment, tracheostomy had been removed and oral feeding resumed with a dysphagic diet. Ultrasound evaluation at 33 days post-event showed partially hypotrophic muscle with overall preserved architecture, reduced subcutaneous adipose tissue, and increased connective tissue (see *Tables 1, 2* and *Fig. 4, 5*).

Subject 3

A 34-year-old healthy man with no significant medical history, not taking medications, and practicing regular physical activity (1.5 hours/day, three times/week, with a focus on strengthening). Ultrasound examination revealed normal muscle trophism and echostructure (see *Tables 1, 2* and *Fig. 4, 5*).

DISCUSSION

This technical note illustrates the feasibility and clinical relevance of ultrasound assessment of lower-limb muscle architecture in patients with sABI. By applying a standardized protocol and presenting illustrative cases with different clinical conditions, we highlight how muscle ultrasound can provide meaningful information on muscle structure in a population characterized by severe neurological impairment, reduced cooperation, and

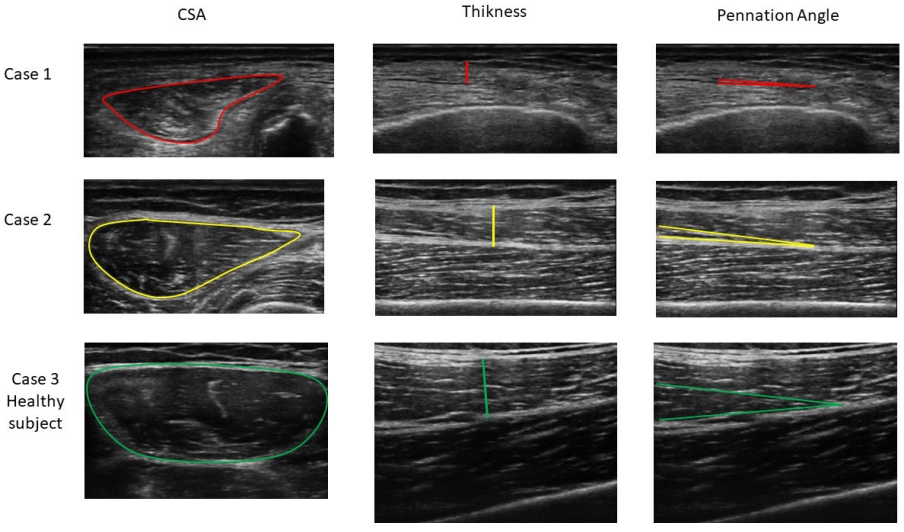


Figure 4. *Ultrasound evaluation of the rectus femoris (CSA, thickness, and pennation angle).*

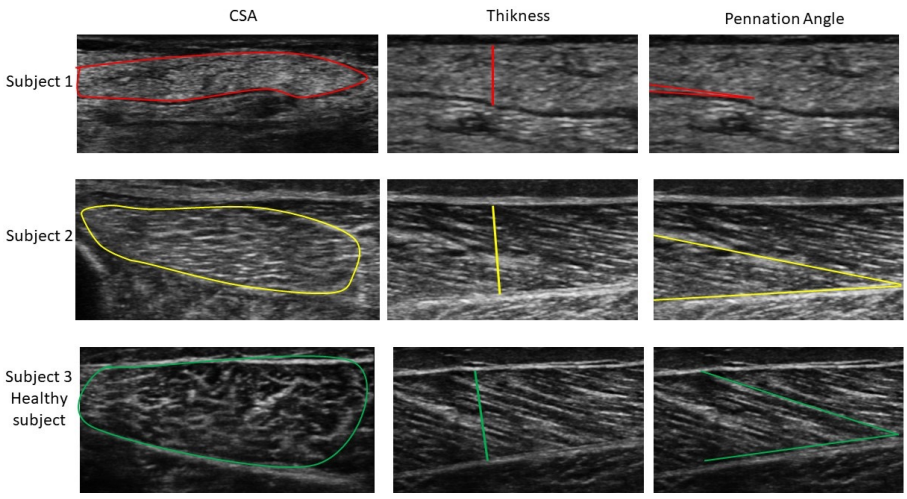


Figure 5. *Ultrasound evaluation of the medial gastrocnemius (CSA, thickness, and pennation angle).*

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	RF Right			RF Left			MG Right			MG Left		
Subject	CSA (cm^2)	Th (mm)	Angle (°)	CSA (cm^2)	Th (mm)	Angle (°)	CSA (cm^2)	Th (mm)	Angle (°)	CSA (cm^2)	Th (mm)	Angle (°)
S1	2.7	4	1.6	3.2	5	2.0	2.0	5	1.5	2.9	9	2.4
S2	4.2	8	4.8	4.5	9	5.0	3.8	13	18.0	3.5	10.5	18.5
S3	7.6	15	8.0	7.3	14	7.2	5.8	16	24.7	6.0	17	21.0

Table 2. *Ultrasound data (bilateral).*

Abbreviations: RF, rectus femoris; MG, medial gastrocnemius; CSA, cross-sectional area; Th, thickness.

prolonged immobilization.

Patients with sABI often present with a complex and heterogeneous clinical picture influenced by multiple factors, including etiology, lesion characteristics, comorbidities, age, and premorbid conditions conditioning the outcome[20]. These factors, combined with reduced mobility, contribute to profound alterations in muscle architecture and function. In this context, ultrasound is a valuable tool for noninvasively assessing muscle structure and monitoring musculoskeletal changes during rehabilitation. The parameters selected in this protocol (CSA, muscle thickness, and pennation angle) are among the most reliable indicators of muscle architecture and are closely related to muscle mass and force-generating capacity[2,21].

The proposed protocol is feasible across different settings, including bedside, outpatient, and rehabilitation room, and can be applied even when cooperation is limited or devices are present. Appropriate identification of anatomical landmarks and standardized probe placement are crucial for reproducible measurements. Lower-limb muscle groups are particularly susceptible to architectural deterioration[4,12]; therefore, RF and MG were selected because of their size and accessibility. MG typically exhibits a larger pennation angle, facilitating measurement even when architectural involution is marked.

In the presented cases, the patient with more severe impairment and spasticity exhibited more pronounced alterations, whereas the patient with a more favorable trajectory showed partial preservation of muscle architecture. Although causal inferences cannot be drawn, ultrasound-derived parameters may reflect overall clinical severity, functional status, duration of immobilization, age, and nutritional condition, consistent with prior literature[4,12,23].

Musculoskeletal ultrasound can be used not only as a diagnostic tool but also as a quantitative outcome measure capable of capturing structural muscle changes over time[24]. Altered muscle architecture affects physiological parameters including force–length and force–velocity relationships, maximal force output, active and passive stiffness, and susceptibility to injury[2,11,13], all of which may influence neurorehabilitation outcomes. Integrating clinical assessment with ultrasound evaluation may support rehabilitation planning and provide complementary prognostic information[23].

LIMITATIONS AND FUTURE PERSPECTIVES

The small sample size and illustrative nature of this technical note limit generalizability. Findings should be regarded as exploratory and hypothesis-generating. Ultrasound was performed at a single time point, and intra- and inter-rater reliability were not formally assessed. Future studies should include larger cohorts, longitudinal follow-up, and reliability analyses.

CONCLUSIONS

This technical note describes a standardized, feasible, and replicable ultrasound protocol for assessing lower-limb muscle architecture in patients with sABI. When integrated with clinical evaluation, muscle ultrasound may support rehabilitation planning, monitoring, and prognostic considerations. Further studies with larger sample sizes are necessary to better define these preliminary observations.

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REFERENCES

1. Boissonnault È, Jeon A, Munin MC, Filippetti M, Picelli A, Haldane C, Reebye R. Assessing muscle architecture with ultrasound: implications for spasticity. Eur J Transl Myol. 2024;34(2):12397.
2. Timmins RG, Shield AJ, Williams MD, Lorenzen C, Opar DA. Architectural adaptations of muscle to training and injury: a narrative review outlining the contributions by fascicle length, pennation angle and muscle thickness. Br J Sports Med. 2016;50(23):1467-1472.
3. Kwah LK, Pinto RZ, Diong J, Herbert RD. Reliability and validity of ultrasound measurements of muscle fascicle length and pennation in humans: a systematic review. J Appl Physiol (1985). 2013;114(6):761-769.
4. Formenti P, Umbrello M, Coppola S, Froio S, Chiumello D. Clinical review: peripheral muscular ultrasound in the ICU. Ann Intensive Care. 2019;9(1):57.
5. Tran A, Gao J. Quantitative ultrasound to assess skeletal muscles in post-stroke spasticity. J Cent Nerv Syst Dis. 2021;13:1179573521996141.
6. Hodges PW, Pengel LH, Herbert RD, Gandevia SC. Measurement of muscle contraction with ultrasound imaging. Muscle Nerve. 2003;27(6):682-692.
7. Iosa M, Morelli D, Marro T, Paolucci S, Fusco A. Ability and stability of running and walking in children with cerebral palsy. Neuropediatrics. 2013;44(3):147-154.
8. Iosa M, Morone G, Fusco A, Marchetti F, Caltagirone C, Paolucci S, Peppe A. Loss of fractal gait harmony in Parkinson's disease. Clin Neurophysiol. 2016;127(2):1540-1546.
9. Kim JM, Tay MRJ, Rajeswaran DK, Tham SL, Lui WL, Kong KH. Changes in muscle architecture on ultrasound in patients early after stroke. NeuroRehabilitation. 2021;49(4):565-572.
10. Lieber RL, Fridén J. Functional and clinical significance of skeletal muscle architecture. Muscle Nerve. 2000;23(11):1647-1666.
11. Wisdom KM, Delp SL, Kuhl E. Use it or lose it: multiscale skeletal muscle adaptation to mechanical stimuli. Biomech Model Mechanobiol. 2015;14(2):195-215.
12. Perkisas S, Bastijns S, Baudry S, Bauer J, Beaudart C, Beckwée D, et al. Application of ultrasound for muscle assessment in sarcopenia: 2020 SARCUS update. Eur Geriatr Med. 2021;12(1):45-59.
13. Wu R, De Vito G, Delahunt E, Ditroilo M. Age-related changes in motor function (I). Mechanical and neuromuscular factors. Int J Sports Med. 2020;41(11):709-719.
14. Prodinger B, Tennant A, Stucki G. Standardized reporting of functioning information on ICF-based common metrics. Eur J Phys Rehabil Med. 2018;54(1):110-117.
15. Malkmus D, Booth B, Kodimer C. Rehabilitation of the head-injured adult: comprehensive cognitive management. Downey, CA: Rancho Los Amigos Hospital; 1980.
16. Giacino JT, Kalmar K, Whyte J. The JFK Coma Recovery Scale-Revised: measurement characteristics and diagnostic utility. Arch Phys Med Rehabil. 2004;85(12):2020-2029.
17. Rappaport M, Hall KM, Hopkins K, Belleza T, Cope DN. Disability rating scale for severe head trauma: coma to community. Arch Phys Med Rehabil. 1982;63(3):118-123.
18. Gouvier WD, Blanton PD, LaPorte KK, Nepomuceno C. Reliability and validity of the Disability Rating Scale and the Levels of Cognitive Functioning Scale in monitoring recovery from severe head injury. Arch Phys Med Rehabil. 1987;68(2):94-97.
19. Demeurisse G, Demol O, Robaye E. Motor evaluation in vascular hemiplegia. Eur Neurol. 1980;19(6):382-389.
20. Fusco A, Galluccio C, Castelli L, Pazzaglia C, Pastorino R, Pires Marafon D, Bernabei R, Giovannini S, Padua L. Severe acquired brain injury: prognostic factors of discharge outcome in older adults. Brain Sci. 2022;12(9):1232.
21. Nijholt W, Scafoglieri A, Jager-Wittenaar H, Hobbelen JSM, van der Schans CP. The reliability and validity of ultrasound to quantify muscles in older adults: a systematic review. J Cachexia Sarcopenia Muscle. 2017;8(5):702-712.
22. Sarwal A, Parry SM, Berry MJ, Hsu FC, Lewis MT, Justus NW, et al. Interobserver reliability of quantitative muscle sonographic analysis in the critically ill population. J Ultrasound Med. 2015;34(7):1191-1200.
23. Özçakar L, Ata AM, Kaymak B, Kara M, Kumbhare D. Ultrasound imaging for sarcopenia, spasticity and painful muscle syndromes. Curr Opin Support Palliat Care. 2018;12(3):373-381.
24. Pillen S, Van Alfen N. Muscle ultrasound from diagnostic tool to outcome measure—quantification is the challenge. Muscle Nerve. 2011;43(5):712-724.

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