



ORIGINAL RESEARCH

Ultrasound assessment of degenerative muscle sarcopenia: the University of Barcelona ultrasound scoring system for sarcopenia

Ingrid Möller Parera,^{1,2} Maribel Miguel,² Joan Blasi,³ Riccardo Piccasso,⁴ Hilde Berner Hammer ,^{5,6} Juan Ortiz-Sagrasta,⁷ Federico Zaottini,⁸ Carlo Martinoli,^{4,8} Lene Terslev ^{9,10}

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ABSTRACT

Aim This study aimed to (1) determine the intraobserver and interobserver reliability of ultrasonographic measurement of muscle thickness (MT) and cross-sectional area (CSA) of the rectus femoris and biceps brachii, correlating these values with manual measurements on dissected cadavers and (2) develop the first semiquantitative musculoskeletal ultrasound (MSUS) scoring system of muscle morphology in sarcopenia and assess its intraobserver and interobserver reliability. In addition, the MSUS morphology score was compared with the corresponding histological images to verify concurrent validity.

Methods Ten cryopreserved limbs of 10 cadavers aged 68–91 years were evaluated. The MSUS scoring system was based on the severity of muscle degeneration on a 3-point qualitative scale: grade 1 (normal), grade 2 (moderate changes) and grade 3 (severe changes). Reliability was assessed with intraclass correlation coefficient (ICC) for the MT and CSA and with Cohen's kappa coefficients (κ) for the MSUS scoring system. Concurrent validity was analysed with ICC.

Results The results showed excellent intraobserver and interobserver reliability for both the MSUS evaluation of MT and CSA (ICC ≥ 0.93). The MSUS scoring system showed excellent intraobserver reliability ($\kappa=1.0$) and very good interobserver reliability ($\kappa=0.85$). There was also a high intra- and inter-observer reliability for the histological scorings ($\kappa \geq 0.85$ and mean $\kappa=0.70$, respectively), as well as high reliability between the histology and MSUS scoring systems (ICC=0.92). All results were statistically significant ($p \leq 0.001$).

Conclusion MSUS measures of MT and CSA and the novel MSUS scoring system for degenerative muscle changes in sarcopenia was found to be reliable and strongly associated with histological findings.

INTRODUCTION

Sarcopenia is a multifaceted degenerative muscle disease with different aetiologies and has been defined by the European Working Group on Sarcopenia in Older

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Sarcopenia is increasing in the population and may be related to age or comorbidities such as inflammatory arthritides. Ultrasound is able to show muscle echotexture and changes therein.

WHAT THIS STUDY ADDS

⇒ The study provides a reliable scoring system for sarcopenia, validated against gross anatomy and histology with high inter-reader and intrareader reliability.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The study provides a tool for assessing presence and severity of sarcopenia that may be relevant in daily clinical practise and clinical trials

People as a deterioration in muscle quantity and quality associated with pathological diminution of muscle strength.¹ Primary sarcopenia is the physiological decrease in skeletal muscle mass associated with ageing. Secondary sarcopenia is seen in relation to other diseases such as diabetes, systemic autoimmune inflammatory diseases (ie, rheumatoid arthritis) and to medical treatment such as steroids or cytostatica.² Sarcopenia constitutes a challenging health problem, representing an independent factor of morbidity and mortality that may entail growing health-care costs in the coming years, since its prevalence is expected to increase following the progressive ageing of the population and the increasing incidence of conditions like degenerative, metabolic and inflammatory diseases.^{3–5} Moreover, the occurrence of sarcopenia has a significant impact on the quality of life of patients with chronic



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For numbered affiliations see end of article.

Correspondence to
Professor Lene Terslev;
terslev@dadlnet.dk

pathologies as it greatly affects their performance in daily activities.^{6,7}

Imaging plays a critical role in the evaluation of sarcopenic patients with the main purpose of objectively assessing the quality and the quantity of skeletal muscles. Dual-energy X-ray absorptiometry (DXA) represents the most commonly employed imaging modality for the evaluation of sarcopenic patients and has been demonstrated to predict and monitor skeletal muscle and fat masses by mean of specific parameters such as the skeletal muscle mass, the appendicular skeletal mass and the appendicular lean mass, adjusted for patients' anthropometrics in different ways, using height squared, weight or body mass index. However, this technique is unable to provide an accurate measure because its results are strongly influenced by several factors, such as technical parameters, operator's experience, hydration status. Furthermore, data provided by DXA only reflect the muscle quantity without considering quality and function. Hence, the results only partially correlate with patient performance and prognosis.⁸⁻¹¹

Thus, an objective, reliable and cost-effective diagnostic tool enabling an accurate evaluation of skeletal muscles is essential not only for diagnostic purposes but also for follow-up of patients in clinical practice and clinical trials. DXA, MRI, CT, bioelectric impedance and ultrasound (US) are used in the evaluation of sarcopenic patients. Nevertheless, there is no consensus on which technique is globally the best though DXA is currently the most frequently used in clinical studies due to its lower cost. However, for all imaging techniques the accuracy, reliability, feasibility and standardisation issues are the same including also limitations in obese patients.^{8,9} Musculoskeletal US (MSUS) is an imaging technique that is increasingly used in routine care and in research contexts. The examination is without ionising radiation at a low cost and can be performed bedside. In addition to allowing quantification of skeletal muscles, MSUS may demonstrate changes in muscle quality by changes in the sonographic appearance determined by the respective number of hypoechoic myofibrils and hyperechoic internal aponeuroses and fat. For this reason, the structural changes observable in muscles affected by sarcopenia, consist of a progressive substitution of myofibrils by adipose tissue, which finally results in an increasing echogenicity of skeletal muscles. These changes are readily detectable by MSUS with high sensitivity.¹²

To the best of our knowledge, there is no validated MSUS scoring system for sarcopenia. The development of a semiquantitative scoring could facilitate the diagnosis and monitoring of sarcopenia and improve therapy-related decisions. The aims of the current study were to determine the validity of US for sarcopenia by assessing 1. intraobserver and interobserver reliability of ultrasonographic assessment of muscle

thickness (MT) and cross-sectional area (CSA) of the rectus femoris and biceps brachii and to correlate these measures with manual measurements on dissected cadavers and 2. to develop the first semiquantitative MSUS scoring system of the sarcopenic muscle and to assess its intraobserver and interobserver reliability. In addition, we wished to evaluate the concurrent validity of the MSUS scoring system by correlating the MSUS scores with the corresponding scores of histological examinations.

METHODS

Participants

The cadavers used in the current study had been cryopreserved. Cryopreservation seeks to slowly reach low temperatures (-18°C) without causing concomitant cell rupture and tissue damage, which may occur if ice crystals are formed in the cells during freezing. Prior to the assessments in the study the cadavers were slowly thawed, the muscles now resembling live muscle in texture.

This cross-sectional study included 10 cryopreserved upper (4 right and 1 left) and lower (3 right and 2 left) limbs of 10 cadavers (5 women and 5 men, aged 68–91 years) without evident pathological findings such as previous injury or surgery. Each specimen was accurately labelled with references to the side, age and sex of the donor. All participants had voluntarily donated their bodies for teaching and research purposes to the donor dissection room of the Faculty of Medicine and Health Sciences.

US assessment

For the MSUS study, a GE Logiq E10 (Wisconsin, USA, 2019) was used. The grey-scale settings of the machine were kept unchanged for the grey-scale map, gain and dynamic range to ensure a homogeneous assessment of muscle echotexture. Fixed frequencies for superficial and depth structures were used (15 MHz upper extremity, 12 MHz lower extremity).

For all MSUS examinations, generous use of contact gel and minimum pressure with the probe were adopted to avoid muscle compression. Particular attention was given to the correct placement of the probe perpendicular to the area to be examined, in order to avoid changes in muscle echogenicity related to anisotropy.

US measurements of MT and CSA

One muscle of the lower extremity (rectus femoris) and one of the upper extremity (biceps brachii) were chosen due to their frequent involvement in sarcopenic studies.^{8,10-13}

Each portion of the muscle that was measured was indicated with a dermatographic pencil and subsequently with a needle to facilitate their identification at the time of US and later dissection.

The MSUS measurements of the MT and the CSA of both the rectus femoris and the biceps brachii were performed independently by two assessors with

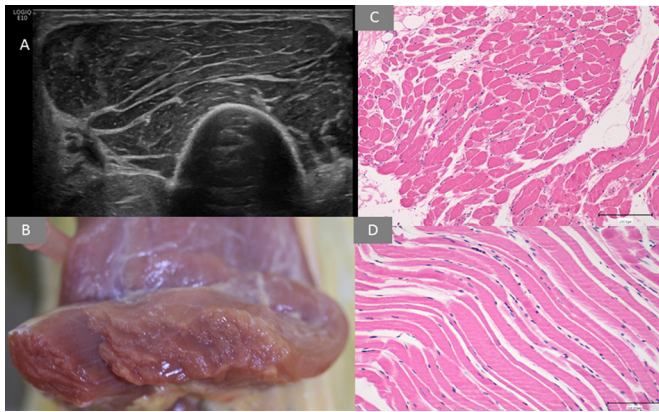


Figure 1 Grade 1 (normal muscle) biceps brachii. (A) MSUS short axis view, (B) anatomical image, (C, D) histology. MSUS, musculoskeletal ultrasound.

experience in MSUS—each blinded to the results of the other assessor.

Placing the probe along the anatomical long axis of the structure in question, MT was defined as the distance between the internal border of the superior and inferior perimysium of the rectus femoris/biceps brachii. Placing the probe in the anatomical short axis of the muscle, an automatic CSA calculation was obtained tracing the internal margin of the muscular perimysium using the calliper.

US scoring of muscle pathology

The MSUS semiquantitative scoring system for sarco- penic muscles was agreed on by the investigators and the external reviewers and was based on the severity of muscle degeneration on a three-point scale: grade 1 defined as normal (preserved muscle architecture and echogenicity); grade 2 defined as moderate changes (moderate/partial loss of muscle architec- ture and increased echogenicity) and grade 3 defined as severe changes (severe or complete loss of muscle architecture and extensively increased echogenicity) (figures 1–3). The muscle changes that were identi- fied by MSUS were the connective web which provides

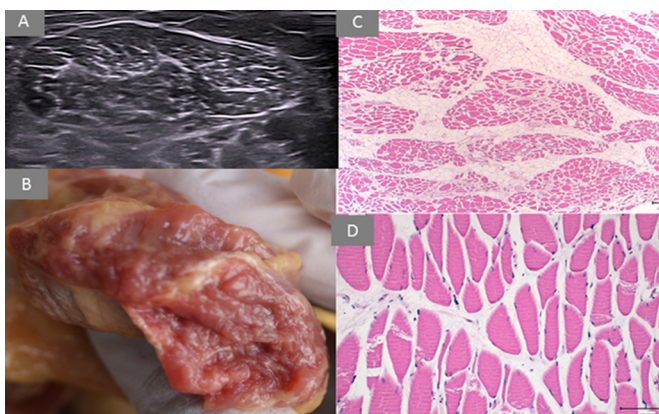


Figure 2 Grade 2 (moderate sarcopenia) biceps brachii. (A) MSUS short axis view, (B) anatomical image, (C, D) histology. MSUS, musculoskeletal ultrasound.

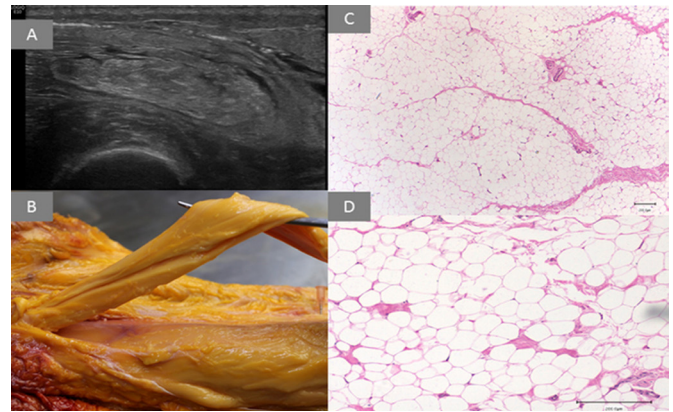


Figure 3 Grade 3 (severe sarcopenia) rectus femoris. (A) MSUS short axis view, (B) anatomical image, (C, D) histology. MSUS, musculoskeletal ultrasound.

an architecture characteristic to the muscle and the degree of fat/fibrous tissue infiltration.

The scoring of the images was performed blinded to the results of the other observer and was repeated after half an hour by each of the investigators to obtain intraob- server and interobserver agreement.

Gross anatomy measurement of the thickness and CSA of the muscles

Following the MSUS assessments, dissection was performed to directly measure the muscles. The spec- imen measurements were performed by a Mytutoyo ABSOLUTE Solar Caliper Series 500 with ABSOLUTE technology (USA) at the level indicated by the needle after the dissection.

The measurements of MT and CSA of the dissected muscles were performed by two assessors and repeated on the same muscle as the first assessment after half an hour to assess intraobserver and interobserver reli- ability. The assessors were blinded to the results of the other observer.

Histology

For validation of the semiquantitative MSUS score, histology was used as a gold standard applying a semi- quantitative score of 1–3 as used for the MSUS assess- ments.

The histological samples were obtained from the previously marked area of the cadaver specimens and were fixated in blocks with a 20% formalin solution. These blocks were then processed for H&E staining to obtain histological slices for general morpholog- ical analysis. All preparations were examined and measured with a Leica DMD 108 microscope.

The histological scoring was carried out inde- pendently by the same two assessors that performed the MSUS examinations together with a third external observer who was an experienced histologist. All were blinded to the others' evaluation and blinded to the results of the MSUS and the gross anatomy measure- ments. The same process was repeated after half an

Table 1 Interobserver and intraobserver reliability of the ultrasound and anatomic measurements of muscle thickness and cross-sectional area

Variable	Intraobserver reliability		Interobserver reliability	
	ICC	P value	ICC	P value
Ultrasound measurements				
Biceps brachii MT (mm)	0.99	<0.0001	0.94	<0.0001
Rectus femoris MT (mm)	0.99	<0.0001	0.97	<0.0001
Biceps brachii CSA (mm ²)	0.99	<0.0001	0.97	<0.0001
Rectus femoris CSA (mm ²)	0.99	<0.0001	0.93	<0.0001
Anatomic measurements				
Biceps brachii MT (mm)	0.99	<0.0001	0.97	<0.0001
Rectus femoris MT (mm)	0.99	<0.0001	0.97	<0.0001
Biceps brachii CSA (mm ²)	0.99	<0.0001	0.98	<0.0001
Rectus femoris CSA (mm ²)	0.99	<0.0001	0.99	<0.0001

CSA, cross-sectional area; ICC, intraclass correlation coefficient; MT, muscle thickness.

hour by each investigator to assess inter- and intra-observer reliability.

The histological characteristics of the muscle, the fat/fibrous infiltration and the organisation of muscular tissue were considered for semiquantitatively scoring of the muscles as follows: (1) normal, (2) moderate sarcopenia (moderate fat infiltration and fibrosis) and (3) severe sarcopenia (severe fat infiltration and fibrosis) (figures 1–3).

Statistical analysis

Intraclass correlation coefficients (ICCs) were calculated to assess intra- and inter-observer reliability of the measurements scored by MSUS and gross anatomy for MT and CSA of the biceps brachii and rectus femoris. The associations between the MSUS score and the anatomical measures of muscles were also explored using ICC. ICC values above 0.75 indicate good reliability.¹⁴

The results of the scoring systems are shown in tables and intra- and inter-observer reliabilities were assessed with Cohen's kappa (κ). The κ -coefficient values were

interpreted using the guidelines suggested by Landis and Koch,¹⁵ according to which κ -coefficient values are between 0.41–0.60, 0.61–0.80 and 0.81–1 indicate moderate, good and excellent agreement, respectively.

A $p < 0.05$ was considered statistically significant. Data were analysed using statistical software SPSS V.20 (IBM).

RESULTS

Reliability of MT and CSA measurements

Table 1 shows the intraobserver and interobserver reliabilities for MT and CSA assessed by MSUS and anatomical measurements. The MSUS and anatomical measurements showed almost perfect agreement for both MT and CSA (ICC=0.99 for the intraobserver agreement and 0.93–0.97 for the interobserver).

Reliability of the MSUS and histological scoring systems for degenerative muscle changes

Table 2 displays the MSUS scores made by the two assessors. Almost identical US scores for sarcopenia were seen

Table 2 Reliability of ultrasound scoring of 10 specimens

Specimen	Ultrasound first observer 1. round	Ultrasound second observer 1. round	Ultrasound first observer 2. round	Ultrasound second observer 2. round
1	2	2	2	2
2	3	3	3	3
3	1	1	1	1
4	2	2	2	2
5	1	1	1	1
6	3	3	3	3
7	2	3	2	3
8	2	2	2	2
9	1	1	1	1
10	3	3	3	3

Table 3 Results from the histology reliability study

Specimen	Histology first observer	Histology second observer	Histology third observer	Histology first observer	Histology second observer	Histology third observer
	1. round	1. round	1. round	2. round	2. round	2. round
1	2	2	2	2	2	2
2	3	3	3	3	3	3
3	1	1	1	1	1	1
4	2	3	2	2	3	2
5	1	1	1	1	1	1
6	3	3	3	3	3	3
7	2	2	3	3	2	3
8	2	2	2	2	2	2
9	1	1	1	1	1	1
10	2	2	3	2	2	3

for all 10 specimens, with intra- and inter-reliability kappa of 1.0 and 0.85, respectively. All the three sarcopenia severity scores were presented in the 10 specimens.

Table 3 displays the histological scores made by the three assessors and shows that for 7 of the 10 specimens, the 3 assessors had identical histology scores for sarcopenia both in the first and second round, resulting in overall excellent intra-rater reliability $\kappa=0.85\text{--}1.00$ and good inter-rater reliability with mean $\kappa=0.70$, $p<0.001$.

Reliability of MSUS and histology scores (concurrent validity)

There was a very strong agreement between the semi-quantitative MSUS and histology scoring systems with ICC of 0.92.

DISCUSSION

In this study, MSUS was highly reliable in determining sarcopenia muscle volume-related parameters such as MT and CSA. Further, the MSUS scoring system for assessing sarcopenic muscle changes was found to have good to excellent intraobserver and interobserver reliability as well as being strongly associated with histological scoring of sarcopenia severity, supporting its concurrent validity. Thus, MSUS may play a future role in the screening, diagnosis and follow-up of sarcopenic patients.

Apart from DXA scanning applied routinely for assessment of sarcopenia, other imaging modalities such as MRI and CT have been consistently reported to present the highest sensibility and specificity in detecting muscle sarcopenia, as they allow for a comprehensive investigation of several aspects like volume, anatomical orientation and internal structure.¹⁶ Nevertheless, the extensive use of these modalities for detecting and monitoring sarcopenia in large cohorts of patients is critically limited by their high cost, no portability, the necessity of high-trained personnel to perform and interpret the examinations, and, in the case of CT, exposure to ionising radiations. Therefore, the use of MSUS for assessing sarcopenia is highly relevant as an alternative imaging tool in

routine care. MSUS has already been shown to be reliable in examining muscles in young and old adults.^{10 17} In a previous study, using CT as the gold standard for evaluating the reliability of MSUS in the assessment of the CSA of the rectus femoris muscle, found no differences between the two techniques.¹⁸ However, MSUS measurements of MT and CSA have previously shown low interobserver reproducibility and moderate/low ICCs.¹⁹ These studies may be limited by differences in patient position and changes in machine settings (mainly gain), which could have reduced the reliability of repeated imaging evaluations.^{20–23}

Other studies have shown that muscle activity does not influence subsequent MSUS muscle measurements and supine rest is not required to obtain a reliable MSUS-based evaluation of skeletal muscle.^{24 25} The influence of probe position and the dimension of the evaluated region has been explored in a reproducibility study where the reliability of MSUS measurements of MT and muscle echogenicity resulted in moderate to high reliability and this was further improved when using the transverse view of the muscle as well as larger regions of interest.²⁶ This plane has indeed shown to affect the reliability of muscle measurement and a panoramic view has been proposed to increase the reproducibility of the evaluation in the scanning of patients.²⁷

The strength of our study is that it was performed on cadavers allowing direct comparison between the MSUS and clinical measurements of muscle quality and between the MSUS score and the histological score, thus enhancing the validity of the findings.

Our study has some limitations. The use of cadavers is a limitation of this study as cadavers may be easier to assess than healthy subjects and patients as their position remains unchanged for the intraobserver and interobserver examinations. Having half hour in between the two assessments could have impacted the intraobserver reliability. However, the interobserver reliability, which is normally the lower of the two, was good to excellent

for all assessments. The present methodological study indicates that our MSUS scoring system for sarcopenia is reliable and should, therefore, be further explored in patients. Including only two aspects of muscle architecture (ie, fat infiltration and fibrosis) in the scoring system may reduce its specificity but also makes the classification easier to apply. Furthermore, the study of two muscles, one in the lower limb and one in the upper limb, may not allow the results to be extrapolated to the entire appendicular musculature.

In conclusion, MSUS was found to be an accurate and reliable tool for the measurement of MT and CSA of appendicular muscles. Our novel semiquantitative MSUS scoring system for sarcopenia was found to be highly reliable and in excellent agreement with histology. These results suggest that the scoring system is relevant for assessments of degenerative muscle changes in patients with or suspected of having sarcopenia. The next step is to test the proposed scoring system in patients and to test the sensitivity to change in longitudinal studies.

Author affiliations

- ¹Reumatologia, Instituto Poal de Reumatologia, Barcelona, Spain
- ²Unidad de Anatomía y Embriología Humana, Departamento de Patología y Terapéutica Experimental, Facultad de Medicina y Ciencias de la Salud (Campus de Bellvitge), University of Barcelona, Barcelona, Spain
- ³Unidad de Histología, Facultad de Medicina y Ciencias de la Salud (Campus de Bellvitge), University of Barcelona, Barcelona, Spain
- ⁴Department of Health Sciences (DISSAL), University of Genoa, Genoa, Italy
- ⁵Center for Treatment of Rheumatic and Musculoskeletal Diseases (REMEDY), Diakonhjemmet Sykehus AS, Oslo, Norway
- ⁶Faculty of Medicine, University of Oslo, Oslo, Norway
- ⁷Department of Anesthesia, Fundacio Puigvert, Barcelona, Spain
- ⁸IRCCS Ospedale Policlinico San Martino, Genoa, Italy
- ⁹Center for Rheumatology and Spine Diseases, Rigshospitalet Glostrup, Glostrup, Denmark
- ¹⁰Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

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Contributors IMP, MM, FZ, RP, HBH, CM and LT have made substantial contributions to study conception and design. IMP, MM and JB have participated in acquisition of data. JO-S and HBH have performed all statistical analyses. IMP, MM, HBH, RP, FZ, CM and LT have interpreted data and drafted the article. All coauthors have revised the article critically for important intellectual content and final approval of the version of the article to be published. CM and LT are joint last authors. IMP is the guarantor.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study was conducted in accordance with the 'Guidelines for Good Scientific Practice of the University of Barcelona' and was approved by the bioethics commission of the University of Barcelona (Institutional Review Board-IRB 00003099).

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ORCID iDs

Hilde Berner Hammer <http://orcid.org/0000-0001-7317-8991>
Lene Terslev <http://orcid.org/0000-0001-8193-9471>

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Correction: *Ultrasound assessment of degenerative muscle Sarcopenia: the university of barcelona ultrasound scoring system for Sarcopenia*

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The author name Riccardo Picasso has been updated.

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