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A Procedure for Measuring the Anterior Scalene Morphology and Quality with Ultrasound Imaging: An Intra- and Inter-Rater Reliability Study

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Abstract:	Objective: Ultrasound imaging (US) an essential tool for clinicians due to its cost-effectiveness and accessibility for assessing multiple muscle metrics including muscle quality, size and shape. Although previous studies highlighted the importance of the anterior scalene muscle (AS) in patients with neck pain, studies analyzing the reliability of US measurements for this muscle are lacking. This study aimed to develop a protocol for assessing the AS muscle shape and quality measured with US and investigate its intra- and inter-examiner reliability. Methods: Using a linear transducer, B-mode images of the antero-lateral neck region at C7 level were acquired in 28 healthy volunteers by two examiners (one experienced and one novel). Cross-sectional area, perimeter, shape descriptors and mean echo-intensity were measured twice by each examiner in randomized order. Intra-class correlation coefficients (ICC), standard error of measurement (SEM) and minimal detectable changes (MDC) were calculated. Results: Results showed no muscle side-to-side asymmetries (p>0.05). Gender differences were found for muscle size (p<0.01), but muscle shape and brightness were comparable (p>0.05). Intra-examiner reliability was good-to-excellent for all the metrics for the experienced and the novel examiners (ICC>0.846 and ICC>780 respectively). Although the inter-examiner reliability was good for most of the metrics (ICC>0.709), the estimates for assessing solidity and circularity were unacceptable (ICC<0.70). Conclusion:This study found that the described ultrasound procedure for locating and measuring the anterior scalene muscle morphology and quality is highly reliable in asymptomatic subjects.

Editor-in-Chief, ULTRASOUND IN MEDICINE AND BIOLOGY

A Procedure for Measuring the Anterior Scalene Morphology and Quality with Ultrasound Imaging.

Here we submit a paper that we believe can be of interest for the readers of the journal. We submit the paper as *Original Research* as we believe that the present finding will be of interest to the readers of **ULTRASOUND IN MEDICINE AND BIOLOGY** and we will look forward to receiving your comments.

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All authors have participated in the study design, data collection, data analysis, data interpretation, writing the manuscript and approved the submitted version of the paper and its submission to **ULTRASOUND IN MEDICINE AND BIOLOGY.**

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Response Letter manuscript UMB-D-23-00037

A Procedure for Measuring the Anterior Scalene Morphology and Quality with Ultrasound Imaging: An Intra- and Inter-Rater Reliability Study

We would like to thank the reviewers for their comments, which we believe have clarified many aspects of the manuscript. We have edited the text according to the suggestions from the reviewers. We have highlighted all changes in yellow throughout the manuscript. A point-by-point response is presented below.

Reviewer 1

This is a well-organized study, and results are straightforward and will have reference value for the field. Following comments for reference for polishing the manuscript:

1. How different parameters were obtained should be elaborated using drawings on some typical ultrasound images, for example using the Fig 1 left example image, the manually drawn border should be labelled. Actually, it is rather challenging to identify the border, how it was identified by the two examiners. This is a very important reference information for the readers.

Response: Thank you for your feedback. We added a new figure (Figure 2) following your recommendations, contouring the anterior scalene muscle and showing the software measurements.

2. It was mentioned "solidity and circularity metrics 281 demonstrated unacceptable reliability (ICC<0.70)." in the abstract and discussion section, but not elaborated in the

result section (only appears in Table 3, with not elaboration), not sure why.

Response: We addressed this comment in Results as follows:

"…However, circularity and solidity metrics did not reach the minimally acceptable ICC (ICC<0.7)."

3. The reproducibility of solidity and circularity was unacceptable, why the conclusion is still that positive, because for morphological information only, and solidity and circularity not belongs to that? In addition, the authors did not go into explain why the solidity and circularity are not reliable, and I think it is very important to understand and discuss why, otherwise the paper is in very low level. Can the authors conclude that the ultrasound imaging technique should not be used for assessment of solidity and circularity for this muscle? If this is the case, what is the clinical implications, as i guess these two parameters have been commonly used in the field.

Response: Conclusions are positive since the most important metrics showed good statistical estimates. It's true that circularity and solidity belong to shape descriptors, but all shape descriptors are complementary to each other. This means that, even if circularity and solidity are not reliable, still aspect ratio and roundness can be used to indicate the muscle shape.

The most probably hypothesis explaining why these two descriptors are less reliable than the other metrics has been included in discussion: "One potential reason explaining the limited reliability for these two metrics could be attributed to a higher contour sensitivity. For example, slight imperfections during the contour process have lower impact in the aspect ratio (as only assess the longest vertical and horizontal distances to describe if the area selected is as width as height) in contrast with circularity (where instead of two distances, the full contour is considered to obtain the metric)."

A low reliability for assessing these two metrics is not determinant for the clinical practice since, as commented before, other reliable metrics less sensitive to contour errors can be used for describing muscle shape.

4. As discussed by the authors, the conclusion made in this study can also be used for asymptomatic cases, as the study only include normal subjects. But actually some symptomatic cases have been tested, but excluded from the analysis, while the authors pointed out it is a limitation for not including symptomatic cases, but on the hand, excluding them for analysis. Why not simply include them to see the results, which will be more real and more useful. I suggest the authors should include symptomatic cases for comparison and for facilitating the discussion.

Response: We acknowledge the recommendations as we believe is necessary analyzing clinical populations, not only for reliability studies but also for case control studies. However, the number of participants with neck pain excluded was not high enough to build another group. In addition, the eligibility criteria for including clinical populations should be selected carefully, as different histological characteristics could be presented depending on the pain aetiology. For instance, increased fatty infiltration and increased cross-sectional area located in deep neck extensors can be find in patients with whiplash associated disorders while patients with idiopathic neck pain show decreased muscle size with no alterations in fatty infiltration.

We are currently working on case control studies considering different causes of neck pain. We hope the journal contact you again for revising that manuscript in the future.

Reviewer 2

The paper "A Procedure for Measuring the Anterior Scalene Morphology and Quality with Ultrasound Imaging: An Intra- and Inter-Rater Reliability Study" has a relevant topic of investigation. It is methodological study important to futures clinical trials involving ultrasound.

Some comments/suggestions are necessary be considered to improve the clarity of study:

1) In statistical analysis and results, you mention the side by side and gender comparisons but they were not considered in objectives and discussion. I suggest making it clearer. Maybe it is necessary to include secondary objectives.

Response: Thank you for the kind feedback. We included the comparison between genders and side to provide descriptive information of the sample analyzed for this study. Although we met the sample size required to obtain enough statistical power regarding the reliability analyses, we believe that our sample of 28 subjects (13 males and 15 females) is quite small to discuss normative values in healthy subjects. As we would prefer to limit our recommendations and comments to those supported with enough statistical power.

2) In inclusions criteria, the age ranged from 18-65 years old. In results, the standard deviation of age was 5 years. Why did you choose too wide age range? I believe that muscles of elderly could influence the reliability because they are constituted of more fat and connective tissue.

Response: Actually, we included that range for better results generalizability. A previous study investigated these concerns. This paper found that age, even if it was associated with lean mass and water volume, was not correlated with cervical multifidus ultrasound cross-sectional area, perimeter, circularity, aspect ratio, roundness or solidity. However, we belive this is an interesting topic to be studied in the future. We included the findings of this study in Discussion, and included the necessity of further research targeting the anterior scalene muscle.

3) I suggest to include an image showing the measurements of shape of the anterior scalene for improve the reproducibility of protocol.

Response: We included a new figure including one raw US image and one contouring the anterior scalene muscle using the software for these calculations.

4) Pg. 8, L241: rewrite "muscle"

Response: We apologize for the misspelling. Now is corrected.

5) Pg. 11, L283: "morphology" is repeated in phrase.

Response: We corrected.

6) I suggest to include more citations involving reliability of other muscles assessed by ultrasound to compare with yours results.

Response: We included that information as suggested. "The reliability estimates obtained in this study were similar to other muscles located in the neck region in asymptomatic populations such as the cervical multifidus, showing excellent reliability for assessing muscle size, shape and brightness **(Valera Calero et al., 2020d; 2021a)** and better than other muscles such as the longus colli, the rectus capitis posterior major and the semispinalis capitis **(McGaugh & Ellison, 2011; Øverås et al., 2017)**."

7) Review the formatting of references (some of them were in italic, another no).

Response: We corrected

We hope that the current version of the paper can be finally accepted for publication in the Journal of Ultrasound in Medicine & Biology

Kind Regards,

The authors

Title Page

Title

A Procedure for Measuring the Anterior Scalene Morphology and Quality with Ultrasound Imaging: An Intra- and Inter-Rater Reliability Study

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A Procedure for Measuring the Anterior Scalene Morphology and Quality with Ultrasound Imaging: An Intra- and Inter-Rater Reliability Study

Abstract

Objective: Ultrasound imaging (US) an essential tool for clinicians due to its cost-effectiveness and accessibility for assessing multiple muscle metrics including muscle quality, size and shape. Although previous studies highlighted the importance of the anterior scalene muscle (AS) in patients with neck pain, studies analyzing the reliability of US measurements for this muscle are lacking. This study aimed to develop a protocol for assessing the AS muscle shape and quality measured with US and investigate its intra- and inter-examiner reliability. **Methods:** Using a linear transducer, B-mode images of the antero-lateral neck region at C7 level were acquired in 28 healthy volunteers by two examiners (one experienced and one novel). Cross-sectional area, perimeter, shape descriptors and mean echo-intensity were measured twice by each examiner in randomized order. Intra-class correlation coefficients (ICC), standard error of measurement (SEM) and minimal detectable changes (MDC) were calculated. **Results:** Results showed no muscle side-to-side asymmetries ($p>0.05$). Gender differences were found for muscle size ($p<0.01$), but muscle shape and brightness were comparable ($p>0.05$). Intra-examiner reliability was good-to-excellent for all the metrics for the experienced and the novel examiners (ICC>0.846 and ICC>780 respectively). Although the inter-examiner reliability was good for most of the metrics (ICC>0.709), the estimates for assessing solidity and circularity were unacceptable (ICC<0.70). **Conclusion:** This study found that the described ultrasound procedure for locating and measuring the anterior scalene muscle morphology and quality is highly reliable in asymptomatic subjects.

Keywords: Anterior Scalene; Ultrasound imaging; Diagnostic accuracy studies, Reliability.

A Procedure for Measuring the Anterior Scalene Morphology and Quality with Ultrasound Imaging: An Intra- and Inter-Rater Reliability Study

Introduction

Scalene muscles are a group of up to 4 muscles (anterior, medium, posterior and minimus) allocated in the antero-lateral aspect of the neck, from the transverse processes of the cervical vertebrae to the first and second ribs[1]. Their functions comprise lateral flexion of the cervical spine and controversial cervical spine rotation[2,3] if activated unilaterally, and cervical flexion if activated bilaterally[4]. Additionally, this muscle group is considered an accessory inspiratory muscle group[5,6].

Although these muscles' attachments, surrounding structures, nerve supply and actions are widely described in the literature[7], multiple anatomical variations have been found[8-13]. One of the most relevant clinical interests for this region is the inter-scalene triangle (the space formed by the anterior and middle scalene muscles in the lateral limits and the first rib in the lower limit), since through this space run the roots and trunks of the brachial plexus and the subclavian artery[14].

In addition to the thoracic outlet syndrome, the anterior scalene muscle was individually assessed in previous studies and showed to be a relevant structure associated with neck pain. Patients with chronic neck pain demonstrated greater slow-twitch type-1 fibers conversion to fast-twitch type-2B fibers in comparison with asymptomatic subjects[15], greater electromyographic activity during low-load tasks[16,17], which may explain the greater muscle fatigue specific to the pain side[18]. Although the anterior scale muscle is a clinically relevant structure to be considered in clinical populations and several methods assessed the morphology and function of this muscle, studies using US for investigating the anterior scalene muscle are lacking in contrast with many other muscles in the neck area (e.g., short rotators, cervical multifidus, semispinalis, upper trapezius, levator scapulae or longus colli)[19-21].

Ultrasound imaging is a diagnostic imaging tool widely used in the clinical and research settings since is fast, easy to use, safe and cost-effective compared with other imaging modalities, providing real-time information[22]. Since there are multiple US imaging modes and technologies including B-mode (e.g., for assessing tissues' morphology and quality)[23,24], Doppler US (e.g., for assessing vascular flows)[25], M-mode (e.g., for measuring muscle thickness changes during motor control exercises)[26], shear-wave and strain elastography (e.g., for assessing muscle stiffness properties)[27] or panoramic US (e.g., for assessing muscle size, shape and quality in large structures)[28], the evaluation of this elevate number of objective metrics also contributes to the increasing popularity of US. In addition, offline software also allows the modification of DICOM images (e.g., gain, gray scales, pixel selections…) and their measurement without the need of using the US device for this purpose, providing information about the tissues' histological and morphological characteristics while the device is being used in other exams.

Since clinicians prioritize the use of objective tools with acceptable indices of utility (i.e., validity, reliability, specificity and sensitivity)[29], there is a need of assessing the diagnostic accuracy of US exploratory protocols prior to its use in the clinical and research settings. Therefore, the aim of this study is to design an easy and reproducible protocol for locating and measuring the anterior scalene muscle morphology and brightness using US and assess its intra- and inter-rater reliability in healthy subjects.

Methods

Study Design

A cross-sectional observational study with a diagnostic accuracy design was conducted between September 2022 and December 2022 in a private University located in Ávila (Spain). In order to enhance the presentation quality of this report, the directives for Reporting Reliability and Agreement Studies (GRRAS)[30] and the Enhancing the QUAlity and Transparency Of health Research (EQUATOR) guidelines were followed[31]. Additionally, the Ethics Committee of Universidad Rey Juan Carlos (URJC 3001201801618) supervised and approved the protocol developed for this study prior to the data collection.

Participants

A convenient sample of healthy volunteers were recruiting after posting local announcements in the campus. To be eligible for participation, volunteers had to be aged between 18 and 65 years old and report no history of neck pain symptoms in the previous year. Participants were excluded if they reported history of whiplash, medication intake affecting muscle tone (e.g., muscle relaxants), underwent any surgical procedure, reported any neuropathic condition (e.g., radiculopathy, thoracic outlet syndrome or myelopathy) or showed severe degenerative radiologic finding. Once eligibility criteria were verified, participants had to read and sign an informed written consent to be included in the data collection.

Sample Size Calculation

The sample size was estimated using the directives provided by Walter et al. for estimating the minimum sample size based on intraclass correlation coefficients[32]. Using as a reference the results obtained in previous studies which calculated the reliability of US procedures targeting neck muscles in healthy subjects[21,23], ICC values >0.75 (since this is the accepted cut-off for good-to-excellent reliability[33] were considered as the value minimally acceptable.

Since 1) an expected ICC value =0.9 was hypothesized; 2) an 80% of power and a 5% significance level were set; and 3) 10% losses were assumed considering the longitudinal nature of this study (participants had to be explored twice by two different examiners), the minimum sample size required for this study was set at 37 images.

Examiners

Two examiners participated in this study, one experienced (with +10 years of experience in the use of musculoskeletal US and +10 years of clinical experience with patients reporting neck pain) and one novel (with +10 years of clinical experience with patients reporting neck pain, but no previous experience in the use of musculoskeletal US). Before starting the study, the experienced examiner trained the novel for 10 hours distributed in two sessions (one theoretical with 3 hours of duration and one practical with 7 hours of duration). During these sessions, basic concepts of US, use of the US device and the protocol developed for this study were revised. After finishing the training, the novel examiner had to demonstrate the knowledge and skills acquired by performing a successful trial.

During the study, both examiners were isolated to ensure the blinding by doing the imaging acquisition in two turns (9:00 h to 13:00 h and 15:00 h to 19:00 h), changing the turn in alternate days. Participants were cited twice with 24 hours of difference.

Ultrasound Imaging Acquisition Protocol

All ultrasound images were acquired with a Logiq E9 device and a linear 6-15 MHz transducer ML-6-15-D (General Electric Healthcare, Milwaukee, WI, USA). The console settings were also standard for all the acquisitions (Frequency=12 MHz, Gain=65 dB and Depth=4.5 cm).

All participants were placed in the supine position minimizing their lumbar lordosis by using a pillow under their knees and asked to relax their neck musculature during the procedure for minimizing muscle changes due to muscle contraction[34].

After administering acoustic coupling gel on the supraclavicular region, the transducer was placed transversally and glided laterally to the cricoid cartilage until locating the carotid artery and visualizing it in the lateral border of the image. Then, the transducer was glided cranially and caudally until locating the C6 transverse process in a short-axis view. This osseus reference is easy to recognize since is characterized by a prominent the anterior tubercle and a smaller posterior tubercle[35]. At this point, the probe was caudally glided until locating the transverse process of C7, which is characterized by a prominent posterior tubercle and no anterior tubercle (but sometimes a rudimentary anterior tubercle might be visualized)[36] and the image was frozen and saved for posterior analyses. An example of the images acquired with US and the main structures identified is illustrated in **Figure 1**.

Measurement of Muscle Morphology and Quality

An independent researcher codified, saved and, after exporting the images acquired to DICOM format, sent the files to the examiners. Each examiner measured the images acquired by themselves in a randomized order. For ensuring the blinding, no information was shared between the examiners during this process.

All images were analyzed using the ImageJ offline DICOM software (National Institute of Health, Bethesda, MD, USA, v.1.53a). After transforming the image to a 32-bit images (which is a 256 gray scale image), the anterior scalene was contoured avoiding the inclusion of bone, nerve roots or surrounding fascia as shown in **Figure 2A**. Finally, muscle morphology (cross-sectional area in mm^2 and perimeter in mm), shape (circularity was calculated as 4π*area/perimeter^2 – values range from 0 to 1, where a value of 1 indicates a perfect circle-, aspect ratio was calculated as the division between the major axis and the minor axis and roundness was calculated as 4*Area/(π*major axis^2) and solidity was calculated as the proportion of pixels in the convex hull that are also in the muscle) and quality (mean echo-intensity calculated as the mean average brightness in this 256 gray scale within the region of interest contoured) metrics were automatically calculated by the software as shown in **Figure 2B**.

Statistical Analysis

All analyses were conducted in the Statistical Package for the Social Sciences (SPSS v.27, Armonk, NY, USA) for Mac OS, setting the significance level at $p<0.05$ for all the analyses. Firstly, data distribution was verified using histograms and Shapiro-Wilk tests for continuous variables. P values <0.05 were considered as non-normally distributed and $p>0.05$ as normally distributed[37].

Secondly, descriptive statistics for were used for reporting the total sample's characteristics. Categorical data were reported as frequency and percentage for each category (e.g., number and percentage of women and men). Continuous variables were reported using central tendency metrics (i.e., mean for normal variables and median for non-normal variables) and dispersion metrics (i.e., standard deviation for normal variables and interquartile range for non-normal variables). Additionally, sociodemographic characteristics were independently reported for men and women while muscle morphology and quality characteristics were reported by gender and side. Between-group differences were analyzed using the Student's T-tests for independent samples, reporting the mean difference with a 95% confidence interval and considering a p value <0.05 as statistically significant.

Intra-examiner and inter-examiner reliability analyses consisted of reporting 1) mean average and standard deviation of each metric score, 3) absolute error between attempts for intra-examiner reliability and examiners for inter-examiner reliability (absolute error was calculated since signs could underestimate the disagreement magnitude), 4) intraclass correlation coefficients ($ICC_{3,1}$ for intra-examiner reliability and $ICC_{3,2}$ for inter-examiner reliability, calculated with a 2-way mixed model, consistency type), 5) standard error of measurement (SEM= Standard Deviation of the mean average * $\sqrt{1-ICC}$) and 6) minimal detectable changes (MDC= 1.96* $\sqrt{2}$*SEM)[33].

Results

From a total of 37 subjects interested on participating in this study, 9 were excluded due to history of clinically relevant neck pain episodes within the previous year (n=9). Since 28 asymptomatic volunteers were finally included in the data collection and both the left and right sides were analyzed, 56 anterior scalene muscles were studied.

Table 1 summarizes the sociodemographic characteristics of the sample (and compared by gender) and the US characteristics of the anterior scalene muscle (reported by gender and side). Males and females had comparable age and BMI (both, p>0.05), but males were significantly taller and heavier (both, p<0.001). Regarding the anterior scalene muscle, results showed no side-to-side asymmetries for size, shape or brightness (all metrics, p>0.05). Only muscle size (cross-sectional area and perimeter, p<0.01) showed statistically significant differences between males and females. Shape descriptors and mean echo-intensity were comparable between genders (p>0.05).

Table 2 shows intra-examiner reliability data for both examiners independently assessed. Regarding the novel examiner, ICC were excellent for measuring muscle size (cross-sectional area ICC=0.954 and muscle perimeter ICC=0.940) and muscle quality (mean echo-intensity ICC=0.969) and good for measuring muscle shape (circularity ICC=0.816, AR ICC=0.780, roundness ICC=0.823 and solidity ICC=0.766). On the other hand, ICC values for the experienced examiner were excellent for measuring muscle size cross-sectional area ICC=0.973, muscle perimeter ICC=0.951) and muscle brightness (ICC=0.942) while reliability was good-to-excellent for assessing muscle shape (circularity ICC=0.846, AR ICC=0.924, roundness ICC=0.915 and solidity ICC=0.860). Indicative MDC values are also detailed for each experience level in order to orientate whether changes in longitudinal studies (where a single examiner is involved) assessing the effect of specific interventions on these metrics are attributable to real changes (if changes are greater than MDCs) or measurement errors (if changes are smaller than MDC). Absolute errors were comparable between the novel and the experienced examiners (all metrics, p>0.05).

Finally, inter-examiner reliability estimates are summarized in **Table 3**. These results showed good reliability for assessing cross-sectional area (ICC=0.841), muscle perimeter (ICC=0.705), aspect ratio (ICC=0.745), roundness (ICC=0.709) and excellent reliability for assessing muscle brightness (ICC=0.907). However, circularity and solidity metrics did not reach the minimally acceptable ICC (ICC<0.7). Although absolute errors showed no statistically significant differences between single and average of 2 measurements (all, p>0.05), ICC generally improved if a mean average of 2 measurements was conducted as shown in **Table 3**.

An illustrative comparison between intra-examiner (for both the experienced and novel examiners) and inter-examiner reliability (comparing 1 trial and mean average of 2 measurements) is shown in **Figure 3**, summarizing the obtained ICC scores for each US metric.

Discussion

Up to the authors' knowledge, this is the first study calculating the intra- and inter-examiner reliability of a US procedure for assessing the anterior scalene morphology and brightness. In general, we found good to excellent reliability for assessing anterior scalene muscle size, shape and brightness, independently the examiners' experience. Regarding the inter-examiner agreement, statistical reliability estimates were comparable conducting a single measurement or calculating a mean average of two measurements. The reliability estimates obtained in this study were similar to other muscles located in the neck region in asymptomatic populations such as the cervical multifidus, showing excellent reliability for assessing muscle size, shape and brightness[23,38] and better than other muscles such as the longus colli, the rectus capitis posterior major and the semispinalis capitis[39,40]. Although results showed good reliability for measuring aspect ratio, roundness, muscle brightness, cross-sectional area and perimeter, solidity and circularity metrics demonstrated unacceptable reliability (ICC<0.70). One potential reason explaining the limited reliability for these two metrics could be attributed to a higher contour sensitivity. For example, slight imperfections during the contour process have lower impact in the aspect ratio (as only assess the longest vertical and horizontal distances to describe if the area selected is as width as height) in contrast with circularity (where instead of two distances, the full contour is considered to obtain the metric).

Recent research analyzed the association between sociodemographic and body composition features with US measurement errors[41,42]. Their results showed that age, even if it was associated with lean mass and water volume, was not associated with errors for measuring cervical multifidus cross-sectional area, perimeter, circularity, aspect ratio, roundness or solidity[41]. In contrast, age was significantly correlated with US measurement errors for assessing the lumbar multifidus cross-sectional area, circularity, aspect ratio and roundness[42]. Both studies showed that age was associated with mean echo-intensity errors. Therefore, further research may replicate these studies targeting the anterior scalene muscle.

As introduced previously, most of the available evidence analyzed the morphology of the anterior scalene muscle using magnetic resonance imaging and computed tomography methods[43-50]. Among these studies, Hardy et al.,[45] tested the diagnostic accuracy of MRI for identifying anatomical structures associated with thoracic outlet syndrome. Their results showed that this Gold Standard has enough specificity to provide guidance for planning surgical procedures, and 81% sensitivity for detecting anterior scalene hypertrophy. Since Radosher et al.,[50] found the cross-sectional area (assessed with CT) of superficial neck muscles to be associated with upper limb disability and pain, there is a justified need for developing cost-effective imaging alternatives (such as US).

The anterior scalene muscle is the leading muscle within the anterolateral aspect of the neck (in terms of number and size) type I muscle fibers[47]. Considering the muscle fibers type conversion demonstrated in patients with chronic neck pain[15] and thoracic outlet syndrome[43], this may explain the increased electromyographic activity and fatigue in low-loads tasks shown in these clinical populations[16,17,44]. Since US demonstrated to be a valid tool for assessing muscle composition by specific morphological and brightness analyses[48,49], further studies may consider assessing US differences between cases and controls or analyze the correlation between US and clinical severity indicators for demonstrating the utility of US and, in this case, use US metrics for identifying histological changes in the anterior scalene muscle after specific interventions.

In addition, anterior scalene blocks have been used as a diagnostic test for identifying thoracic outlet syndrome and as a predictor of surgical success[46,51]. A previous study described how perform CT-guided injections, reporting 100% of success in intramuscular needle placement. Although there were no major complications following that procedure, 11% of the patients had minor complications (e.g., Horner sign, dysphagia, muscle weakness, temporary brachial plexus blocks and needle induce pain)[51]. Similarly, the same procedure was tested using US guiding[52]. Although the authors also reached 100% of success with no major complications, some minor complications were also reported (31% temporary partial brachial plexus block and 3% complete brachial plexus block). Although these differences could be attributable to the number of participants for each study (146 and 26 respectively) and the intervention time was better for CT guide compared with US (10 minutes and 30 minutes respectively), other needle interventions such as percutaneous electrical nerve stimulation[53] or dry needling[54] may benefit from US guide since CT is not readily accessible for most of physical therapists. In fact, previous research used US for developing prediction models aiming to assist clinicians in the needle length selection for avoiding adverse effects during invasive procedures where imaging guide is not possible[55-57]. Future studies could investigate prediction models for assisting with needle length selection and puncturing angulation in order to reduce accidental puncture of non-desirable structures (e.g., brachial plexus, phrenic nerve, carotid artery, jugular vein, vague nerve…).

Finally, the reliability estimates obtained in this study were similar to other muscles located in the neck region in asymptomatic populations including the cervical multifidus, showing excellent reliability for assessing muscle size, shape and brightness[23,38] and better than other muscles such as the longus colli, the rectus capitis posterior major and the semispinalis capitis[39,40].

Limitations

This study had some important limitations that should be recognized. First, we limited our sample to asymptomatic subjects. We do not know if these reliability estimates could be extrapolated to patients with neck pain symptoms since some clinical populations showed histological changes which may difficult the visualization of muscles' limits. In addition, we only examined a single level and included a single US device and two examiners. Further research assessing other cervical levels, US brands and including more examiners is needed for confirming these results. Also, we limited the number of measurements per examiner to two trials. Future research is needed for analyzing if increasing the number of trials could improve the inter-examiner reliability of solidity and circularity calculations. Finally, the metrics obtained with US should be compared with a Gold Standard method (i.e., magnetic resonance imaging) for ensuring the US validity.

Conclusion

This study found that the described ultrasound procedure for locating and measuring the anterior scalene muscle morphology and quality is highly reliable in asymptomatic subjects based on the reliability estimates obtained in this study. Intra-examiner reliability was good-to-excellent for assessing all the metrics included in the analyses independently of the examiners' experience and inter-examiner reliability was good for assessing cross-sectional area and perimeter, solidity and circularity and aspect ratio, independently if one trial or a mean average of two trials is calculated. However, the inter-examiner agreement for assessing the anterior scalene muscle circularity and solidity was low. In addition, this paper proposes technical considerations for future studies using this protocol for assessing its discriminative capacity, association with clinical severity or for developing prediction models aiming to assist clinicians on needle length selection and puncture angulation.

Declarations

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Clinical Ethics Committee of Universidad Rey Juan Carlos (ID: URJC 3001201801618).

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Legends of Figures

Figure 1. Sonoanatomy of the structures of the lateral region of the neck at C7 level (A) with outlined structures (B).

Figure 2. Raw Ultrasound imaging acquired at C7 level for assessing the anterior scalene muscle (A) and muscle contouring using ImageJ software for calculating the size, shape and brightness metrics (B).

Figure 3. Radar chart comparing Intraclass Correlation Coefficients between the experienced (blue) and novel (green) examiners for intra-examiner reliability and inter-examiner reliability for a single measurement (yellow) and mean average of two measurements (red).

Title Page

Title

A Procedure for Measuring the Anterior Scalene Morphology and Quality with Ultrasound Imaging: An Intra- and Inter-Rater Reliability Study

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A Procedure for Measuring the Anterior Scalene Morphology and Quality with Ultrasound Imaging: An Intra- and Inter-Rater Reliability Study

Abstract

Objective: Ultrasound imaging (US) an essential tool for clinicians due to its cost-effectiveness and accessibility for assessing multiple muscle metrics including muscle quality, size and shape. Although previous studies highlighted the importance of the anterior scalene muscle (AS) in patients with neck pain, studies analyzing the reliability of US measurements for this muscle are lacking. This study aimed to develop a protocol for assessing the AS muscle shape and quality measured with US and investigate its intra- and inter-examiner reliability. **Methods:** Using a linear transducer, B-mode images of the antero-lateral neck region at C7 level were acquired in 28 healthy volunteers by two examiners (one experienced and one novel). Cross-sectional area, perimeter, shape descriptors and mean echo-intensity were measured twice by each examiner in randomized order. Intra-class correlation coefficients (ICC), standard error of measurement (SEM) and minimal detectable changes (MDC) were calculated. **Results:** Results showed no muscle side-to-side asymmetries ($p>0.05$). Gender differences were found for muscle size ($p<0.01$), but muscle shape and brightness were comparable ($p>0.05$). Intra-examiner reliability was good-to-excellent for all the metrics for the experienced and the novel examiners (ICC>0.846 and ICC>780 respectively). Although the inter-examiner reliability was good for most of the metrics (ICC>0.709), the estimates for assessing solidity and circularity were unacceptable (ICC<0.70). **Conclusion:** This study found that the described ultrasound procedure for locating and measuring the anterior scalene muscle morphology and quality is highly reliable in asymptomatic subjects.

Keywords: Anterior Scalene; Ultrasound imaging; Diagnostic accuracy studies, Reliability.

A Procedure for Measuring the Anterior Scalene Morphology and Quality with Ultrasound Imaging: An Intra- and Inter-Rater Reliability Study

Introduction

Scalene muscles are a group of up to 4 muscles (anterior, medium, posterior and minimus) allocated in the antero-lateral aspect of the neck, from the transverse processes of the cervical vertebrae to the first and second ribs[1]. Their functions comprise lateral flexion of the cervical spine and controversial cervical spine rotation[2,3] if activated unilaterally, and cervical flexion if activated bilaterally[4]. Additionally, this muscle group is considered an accessory inspiratory muscle group[5,6].

Although these muscles' attachments, surrounding structures, nerve supply and actions are widely described in the literature[7], multiple anatomical variations have been found[8-13]. One of the most relevant clinical interests for this region is the inter-scalene triangle (the space formed by the anterior and middle scalene muscles in the lateral limits and the first rib in the lower limit), since through this space run the roots and trunks of the brachial plexus and the subclavian artery[14].

In addition to the thoracic outlet syndrome, the anterior scalene muscle was individually assessed in previous studies and showed to be a relevant structure associated with neck pain. Patients with chronic neck pain demonstrated greater slow-twitch type-1 fibers conversion to fast-twitch type-2B fibers in comparison with asymptomatic subjects[15], greater electromyographic activity during low-load tasks[16,17], which may explain the greater muscle fatigue specific to the pain side[18]. Although the anterior scale muscle is a clinically relevant structure to be considered in clinical populations and several methods assessed the morphology and function of this muscle, studies using US for investigating the anterior scalene muscle are lacking in contrast with many other muscles in the neck area (e.g., short rotators, cervical multifidus, semispinalis, upper trapezius, levator scapulae or longus colli)[19-21].

Ultrasound imaging is a diagnostic imaging tool widely used in the clinical and research settings since is fast, easy to use, safe and cost-effective compared with other imaging modalities, providing real-time information[22]. Since there are multiple US imaging modes and technologies including B-mode (e.g., for assessing tissues' morphology and quality)[23,24], Doppler US (e.g., for assessing vascular flows)[25], M-mode (e.g., for measuring muscle thickness changes during motor control exercises)[26], shear-wave and strain elastography (e.g., for assessing muscle stiffness properties)[27] or panoramic US (e.g., for assessing muscle size, shape and quality in large structures)[28], the evaluation of this elevate number of objective metrics also contributes to the increasing popularity of US. In addition, offline software also allows the modification of DICOM images (e.g., gain, gray scales, pixel selections…) and their measurement without the need of using the US device for this purpose, providing information about the tissues' histological and morphological characteristics while the device is being used in other exams.

Since clinicians prioritize the use of objective tools with acceptable indices of utility (i.e., validity, reliability, specificity and sensitivity)[29], there is a need of assessing the diagnostic accuracy of US exploratory protocols prior to its use in the clinical and research settings. Therefore, the aim of this study is to design an easy and reproducible protocol for locating and measuring the anterior scalene muscle morphology and brightness using US and assess its intra- and inter-rater reliability in healthy subjects.

Methods

Study Design

A cross-sectional observational study with a diagnostic accuracy design was conducted between September 2022 and December 2022 in a private University located in Ávila (Spain). In order to enhance the presentation quality of this report, the directives for Reporting Reliability and Agreement Studies (GRRAS)[30] and the Enhancing the QUAlity and Transparency Of health Research (EQUATOR) guidelines were followed[31]. Additionally, the Ethics Committee of Universidad Rey Juan Carlos (URJC 3001201801618) supervised and approved the protocol developed for this study prior to the data collection.

Participants

A convenient sample of healthy volunteers were recruiting after posting local announcements in the campus. To be eligible for participation, volunteers had to be aged between 18 and 65 years old and report no history of neck pain symptoms in the previous year. Participants were excluded if they reported history of whiplash, medication intake affecting muscle tone (e.g., muscle relaxants), underwent any surgical procedure, reported any neuropathic condition (e.g., radiculopathy, thoracic outlet syndrome or myelopathy) or showed severe degenerative radiologic finding. Once eligibility criteria were verified, participants had to read and sign an informed written consent to be included in the data collection.

Sample Size Calculation

The sample size was estimated using the directives provided by Walter et al. for estimating the minimum sample size based on intraclass correlation coefficients[32]. Using as a reference the results obtained in previous studies which calculated the reliability of US procedures targeting neck muscles in healthy subjects[21,23], ICC values >0.75 (since this is the accepted cut-off for good-to-excellent reliability[33] were considered as the value minimally acceptable.

Since 1) an expected ICC value =0.9 was hypothesized; 2) an 80% of power and a 5% significance level were set; and 3) 10% losses were assumed considering the longitudinal nature of this study (participants had to be explored twice by two different examiners), the minimum sample size required for this study was set at 37 images.

Examiners

Two examiners participated in this study, one experienced (with +10 years of experience in the use of musculoskeletal US and +10 years of clinical experience with patients reporting neck pain) and one novel (with +10 years of clinical experience with patients reporting neck pain, but no previous experience in the use of musculoskeletal US). Before starting the study, the experienced examiner trained the novel for 10 hours distributed in two sessions (one theoretical with 3 hours of duration and one practical with 7 hours of duration). During these sessions, basic concepts of US, use of the US device and the protocol developed for this study were revised. After finishing the training, the novel examiner had to demonstrate the knowledge and skills acquired by performing a successful trial.

During the study, both examiners were isolated to ensure the blinding by doing the imaging acquisition in two turns (9:00 h to 13:00 h and 15:00 h to 19:00 h), changing the turn in alternate days. Participants were cited twice with 24 hours of difference.

Ultrasound Imaging Acquisition Protocol

All ultrasound images were acquired with a Logiq E9 device and a linear 6-15 MHz transducer ML-6-15-D (General Electric Healthcare, Milwaukee, WI, USA). The console settings were also standard for all the acquisitions (Frequency=12 MHz, Gain=65 dB and Depth=4.5 cm).

All participants were placed in the supine position minimizing their lumbar lordosis by using a pillow under their knees and asked to relax their neck musculature during the procedure for minimizing muscle changes due to muscle contraction[34].

After administering acoustic coupling gel on the supraclavicular region, the transducer was placed transversally and glided laterally to the cricoid cartilage until locating the carotid artery and visualizing it in the lateral border of the image. Then, the transducer was glided cranially and caudally until locating the C6 transverse process in a short-axis view. This osseus reference is easy to recognize since is characterized by a prominent the anterior tubercle and a smaller posterior tubercle[35]. At this point, the probe was caudally glided until locating the transverse process of C7, which is characterized by a prominent posterior tubercle and no anterior tubercle (but sometimes a rudimentary anterior tubercle might be visualized)[36] and the image was frozen and saved for posterior analyses. An example of the images acquired with US and the main structures identified is illustrated in **Figure 1**.

Measurement of Muscle Morphology and Quality

An independent researcher codified, saved and, after exporting the images acquired to DICOM format, sent the files to the examiners. Each examiner measured the images acquired by themselves in a randomized order. For ensuring the blinding, no information was shared between the examiners during this process.

All images were analyzed using the ImageJ offline DICOM software (National Institute of Health, Bethesda, MD, USA, v.1.53a). After transforming the image to a 32-bit images (which is a 256 gray scale image), the anterior scalene was contoured avoiding the inclusion of bone, nerve roots or surrounding fascia as shown in **Figure 2A**. Finally, muscle morphology (cross-sectional area in mm^2 and perimeter in mm), shape (circularity was calculated as 4π*area/perimeter^2 – values range from 0 to 1, where a value of 1 indicates a perfect circle-, aspect ratio was calculated as the division between the major axis and the minor axis and roundness was calculated as 4*Area/(π*major axis^2) and solidity was calculated as the proportion of pixels in the convex hull that are also in the muscle) and quality (mean echo-intensity calculated as the mean average brightness in this 256 gray scale within the region of interest contoured) metrics were automatically calculated by the software as shown in **Figure 2B**.

Statistical Analysis

All analyses were conducted in the Statistical Package for the Social Sciences (SPSS v.27, Armonk, NY, USA) for Mac OS, setting the significance level at $p<0.05$ for all the analyses. Firstly, data distribution was verified using histograms and Shapiro-Wilk tests for continuous variables. P values <0.05 were considered as non-normally distributed and $p>0.05$ as normally distributed[37].

Secondly, descriptive statistics for were used for reporting the total sample's characteristics. Categorical data were reported as frequency and percentage for each category (e.g., number and percentage of women and men). Continuous variables were reported using central tendency metrics (i.e., mean for normal variables and median for non-normal variables) and dispersion metrics (i.e., standard deviation for normal variables and interquartile range for non-normal variables). Additionally, sociodemographic characteristics were independently reported for men and women while muscle morphology and quality characteristics were reported by gender and side. Between-group differences were analyzed using the Student's T-tests for independent samples, reporting the mean difference with a 95% confidence interval and considering a p value <0.05 as statistically significant.

Intra-examiner and inter-examiner reliability analyses consisted of reporting 1) mean average and standard deviation of each metric score, 3) absolute error between attempts for intra-examiner reliability and examiners for inter-examiner reliability (absolute error was calculated since signs could underestimate the disagreement magnitude), 4) intraclass correlation coefficients ($ICC_{3,1}$ for intra-examiner reliability and $ICC_{3,2}$ for inter-examiner reliability, calculated with a 2-way mixed model, consistency type), 5) standard error of measurement (SEM= Standard Deviation of the mean average * $\sqrt{1-ICC}$) and 6) minimal detectable changes (MDC= 1.96* $\sqrt{2}$*SEM)[33].

Results

From a total of 37 subjects interested on participating in this study, 9 were excluded due to history of clinically relevant neck pain episodes within the previous year (n=9). Since 28 asymptomatic volunteers were finally included in the data collection and both the left and right sides were analyzed, 56 anterior scalene muscles were studied.

Table 1 summarizes the sociodemographic characteristics of the sample (and compared by gender) and the US characteristics of the anterior scalene muscle (reported by gender and side). Males and females had comparable age and BMI (both, p>0.05), but males were significantly taller and heavier (both, p<0.001). Regarding the anterior scalene muscle, results showed no side-to-side asymmetries for size, shape or brightness (all metrics, p>0.05). Only muscle size (cross-sectional area and perimeter, p<0.01) showed statistically significant differences between males and females. Shape descriptors and mean echo-intensity were comparable between genders (p>0.05).

Table 2 shows intra-examiner reliability data for both examiners independently assessed. Regarding the novel examiner, ICC were excellent for measuring muscle size (cross-sectional area ICC=0.954 and muscle perimeter ICC=0.940) and muscle quality (mean echo-intensity ICC=0.969) and good for measuring muscle shape (circularity ICC=0.816, AR ICC=0.780, roundness ICC=0.823 and solidity ICC=0.766). On the other hand, ICC values for the experienced examiner were excellent for measuring muscle size cross-sectional area ICC=0.973, muscle perimeter ICC=0.951) and muscle brightness (ICC=0.942) while reliability was good-to-excellent for assessing muscle shape (circularity ICC=0.846, AR ICC=0.924, roundness ICC=0.915 and solidity ICC=0.860). Indicative MDC values are also detailed for each experience level in order to orientate whether changes in longitudinal studies (where a single examiner is involved) assessing the effect of specific interventions on these metrics are attributable to real changes (if changes are greater than MDCs) or measurement errors (if changes are smaller than MDC). Absolute errors were comparable between the novel and the experienced examiners (all metrics, $p>0.05$).

Finally, inter-examiner reliability estimates are summarized in **Table 3**. These results showed good reliability for assessing cross-sectional area (ICC=0.841), muscle perimeter (ICC=0.705), aspect ratio (ICC=0.745), roundness (ICC=0.709) and excellent reliability for assessing muscle brightness (ICC=0.907). However, circularity and solidity metrics did not reach the minimally acceptable ICC (ICC<0.7). Although absolute errors showed no statistically significant differences between single and average of 2 measurements (all, $p>0.05$), ICC generally improved if a mean average of 2 measurements was conducted as shown in **Table 3**.

An illustrative comparison between intra-examiner (for both the experienced and novel examiners) and inter-examiner reliability (comparing 1 trial and mean average of 2 measurements) is shown in **Figure 3**, summarizing the obtained ICC scores for each US metric.

Discussion

Up to the authors' knowledge, this is the first study calculating the intra- and inter-examiner reliability of a US procedure for assessing the anterior scalene morphology and brightness. In general, we found good to excellent reliability for assessing anterior scalene muscle size, shape and brightness, independently the examiners' experience. Regarding the inter-examiner agreement, statistical reliability estimates were comparable conducting a single measurement or calculating a mean average of two measurements. The reliability estimates obtained in this study were similar to other muscles located in the neck region in asymptomatic populations such as the cervical multifidus, showing excellent reliability for assessing muscle size, shape and brightness[23,38] and better than other muscles such as the longus colli, the rectus capitis posterior major and the semispinalis capitis[39,40]. Although results showed good reliability for measuring aspect ratio, roundness, muscle brightness, cross-sectional area and perimeter, solidity and circularity metrics demonstrated unacceptable reliability (ICC<0.70). One potential reason explaining the limited reliability for these two metrics could be attributed to a higher contour sensitivity. For example, slight imperfections during the contour process have lower impact in the aspect ratio (as only assess the longest vertical and horizontal distances to describe if the area selected is as width as height) in contrast with circularity (where instead of two distances, the full contour is considered to obtain the metric).

Recent research analyzed the association between sociodemographic and body composition features with US measurement errors[41,42]. Their results showed that age, even if it was associated with lean mass and water volume, was not associated with errors for measuring cervical multifidus cross-sectional area, perimeter, circularity, aspect ratio, roundness or solidity[41]. In contrast, age was significantly correlated with US measurement errors for assessing the lumbar multifidus cross-sectional area, circularity, aspect ratio and roundness[42]. Both studies showed that age was associated with mean echo-intensity errors. Therefore, further research may replicate these studies targeting the anterior scalene muscle.

As introduced previously, most of the available evidence analyzed the morphology of the anterior scalene muscle using magnetic resonance imaging and computed tomography methods[43-50]. Among these studies, Hardy et al.,[45] tested the diagnostic accuracy of MRI for identifying anatomical structures associated with thoracic outlet syndrome. Their results showed that this Gold Standard has enough specificity to provide guidance for planning surgical procedures, and 81% sensitivity for detecting anterior scalene hypertrophy. Since Radosher et al.,[50] found the cross-sectional area (assessed with CT) of superficial neck muscles to be associated with upper limb disability and pain, there is a justified need for developing cost-effective imaging alternatives (such as US).

The anterior scalene muscle is the leading muscle within the anterolateral aspect of the neck (in terms of number and size) type I muscle fibers[47]. Considering the muscle fibers type conversion demonstrated in patients with chronic neck pain[15] and thoracic outlet syndrome[43], this may explain the increased electromyographic activity and fatigue in low-loads tasks shown in these clinical populations[16,17,44]. Since US demonstrated to be a valid tool for assessing muscle composition by specific morphological and brightness analyses[48,49], further studies may consider assessing US differences between cases and controls or analyze the correlation between US and clinical severity indicators for demonstrating the utility of US and, in this case, use US metrics for identifying histological changes in the anterior scalene muscle after specific interventions.

In addition, anterior scalene blocks have been used as a diagnostic test for identifying thoracic outlet syndrome and as a predictor of surgical success[46,51]. A previous study described how perform CT-guided injections, reporting 100% of success in intramuscular needle placement. Although there were no major complications following that procedure, 11% of the patients had minor complications (e.g., Horner sign, dysphagia, muscle weakness, temporary brachial plexus blocks and needle induce pain)[51]. Similarly, the same procedure was tested using US guiding[52]. Although the authors also reached 100% of success with no major complications, some minor complications were also reported (31% temporary partial brachial plexus block and 3% complete brachial plexus block). Although these differences could be attributable to the number of participants for each study (146 and 26 respectively) and the intervention time was better for CT guide compared with US (10 minutes and 30 minutes respectively), other needle interventions such as percutaneous electrical nerve stimulation[53] or dry needling[54] may benefit from US guide since CT is not readily accessible for most of physical therapists. In fact, previous research used US for developing prediction models aiming to assist clinicians in the needle length selection for avoiding adverse effects during invasive procedures where imaging guide is not possible[55-57]. Future studies could investigate prediction models for assisting with needle length selection and puncturing angulation in order to reduce accidental puncture of non-desirable structures (e.g., brachial plexus, phrenic nerve, carotid artery, jugular vein, vague nerve…).

Finally, the reliability estimates obtained in this study were similar to other muscles located in the neck region in asymptomatic populations including the cervical multifidus, showing excellent reliability for assessing muscle size, shape and brightness[23,38] and better than other muscles such as the longus colli, the rectus capitis posterior major and the semispinalis capitis[39,40].

Limitations

This study had some important limitations that should be recognized. First, we limited our sample to asymptomatic subjects. We do not know if these reliability estimates could be extrapolated to patients with neck pain symptoms since some clinical populations showed histological changes which may difficult the visualization of muscles' limits. In addition, we only examined a single level and included a single US device and two examiners. Further research assessing other cervical levels, US brands and including more examiners is needed for confirming these results. Also, we limited the number of measurements per examiner to two trials. Future research is needed for analyzing if increasing the number of trials could improve the inter-examiner reliability of solidity and circularity calculations. Finally, the metrics obtained with US should be compared with a Gold Standard method (i.e., magnetic resonance imaging) for ensuring the US validity.

Conclusion

This study found that the described ultrasound procedure for locating and measuring the anterior scalene muscle morphology and quality is highly reliable in asymptomatic subjects based on the reliability estimates obtained in this study. Intra-examiner reliability was good-to-excellent for assessing all the metrics included in the analyses independently of the examiners' experience and inter-examiner reliability was good for assessing cross-sectional area and perimeter, solidity and circularity and aspect ratio, independently if one trial or a mean average of two trials is calculated. However, the inter-examiner agreement for assessing the anterior scalene muscle circularity and solidity was low. In addition, this paper proposes technical considerations for future studies using this protocol for assessing its discriminative capacity, association with clinical severity or for developing prediction models aiming to assist clinicians on needle length selection and puncture angulation.

Declarations

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Clinical Ethics Committee of Universidad Rey Juan Carlos (ID: URJC 3001201801618).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data derived from this study are presented in the text.

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Legends of Figures

Figure 1. Sonoanatomy of the structures of the lateral region of the neck at C7 level (A) with outlined structures (B).

Figure 2. Raw Ultrasound imaging acquired at C7 level for assessing the anterior scalene muscle (A) and muscle contouring using ImageJ software for calculating the size, shape and brightness metrics (B).

Figure 3. Radar chart comparing Intraclass Correlation Coefficients between the experienced (blue) and novel (green) examiners for intra-examiner reliability and inter-examiner reliability for a single measurement (yellow) and mean average of two measurements (red).

Table 1. Participants' sociodemographic and US characteristics.

Variables	Total sample (n=28)	Gender		Side	
		Male (n=13)	Female (n=15)	Left (n=28)	Right (n=28)
Sociodemographic Characteristics					
Age (y)	19.9 ± 4.8	20.8 ± 6.6	19.0 ± 1.8	-	-
Height (m)*	1.70 ± 0.08	1.76 ± 0.04	1.65 ± 0.06	-	-
Weight (kg)*	69.7 ± 15.6	77.8 ± 12.9	62.4 ± 14.3	-	-
Body Mass Index (kg/m^2)	23.8 ± 4.9	25.0 ± 4.7	22.7 ± 5.0	-	-
Anterior Scalene Ultrasound Characteristics [a]					
Cross-sectional area (mm^2)**	165.0 ± 41.7	176.0 ± 35.8	155.7 ± 44.4	165.3 ± 37.5	164.7 ± 45.7
Muscle Perimeter (mm)**	49.8 ± 6.7	51.6 ± 5.3	48.3 ± 7.4	49.6 ± 7.0	50.1 ± 6.4
Circularity (0-1)	0.83 ± 0.07	0.82 ± 0.06	0.83 ± 0.08	0.83 ± 0.08	0.82 ± 0.07
Aspect Ratio	1.49 ± 0.28	1.51 ± 0.27	1.46 ± 0.28	1.50 ± 0.27	1.47 ± 0.29
Roundness (0-1)	0.69 ± 0.11	0.67 ± 0.10	0.70 ± 0.12	0.70 ± 0.12	0.69 ± 0.11
Solidity (0-1)	0.97 ± 0.03	0.97 ± 0.02	0.97 ± 0.03	0.97 ± 0.03	0.97 ± 0.03
Mean echo-intensity (0-255)	49.3 ± 8.7	48.9 ± 9.5	49.7 ± 8.2	50.4 ± 9.1	48.2 ± 8.2

[a] Reported values are the mean average scores of both trials performed by both examiners
* Statistically significant differences between genders ($p<0.001$)
** Statistically significant differences between genders ($p<0.01$)

Table 2. Intra-rater reliability for the anterior scalene US metrics

Variables	Mean ± SD	Absolute Error	$ICC_{3,1}$ (95% CI)	SEM	MDC_{95}
		Novel Examiner			
Cross-sectional area (mm^2)	170.8 ± 40.1	12.7 ± 11.5	0.954 (0.920;0.973)	8.6	23.8
Muscle Perimeter (mm)	49.8 ± 6.2	2.0 ± 2.2	0.940 (0.898;0.965)	1.5	4.2
Circularity (0-1)	0.85 ± 0.05	0.03 ± 0.03	0.816 (0.685;0.893)	0.02	0.05
Aspect Ratio	1.44 ± 0.22	0.15 ± 0.15	0.780 (0.622;0.872)	0.10	0.28
Roundness	0.71 ± 0.10	0.06 ± 0.05	0.823 (0.696;0.897)	0.04	0.11
Solidity	0.98 ± 0.02	0.01 ± 0.01	0.766 (0.598;0.863)	0.01	0.02
Mean echo-intensity (0-255)	51.2 ± 8.3	2.2 ± 2.2	0.969 (0.946;0.982)	1.46	4.1
		Experienced Examiner			
Cross-sectional area (mm^2)	159.0 ± 43.0	10.7 ± 8.9	0.973 (0.954;0.985)	7.1	19.6
Muscle Perimeter (mm)	49.9 ± 7.3	2.4 ± 2.2	0.951 (0.915;0.972)	1.6	4.5
Circularity (0-1)	0.80 ± 0.08	0.05 ± 0.03	0.846 (0.732;0.912)	0.03	0.09
Aspect Ratio	1.54 ± 0.32	0.13 ± 0.13	0.924 (0.867;0.956)	0.08	0.24
Roundness	0.67 ± 0.13	0.05 ± 0.05	0.915 (0.853;0.951)	0.03	0.10
Solidity	0.96 ± 0.03	0.02 ± 0.01	0.860 (0.757;0.920)	0.01	0.03
Mean echo-intensity (0-255)	47.3 ± 8.8	3.3 ± 2.9	0.942 (0.898;0.967)	2.11	5.9

SEM and MDC_{95} are expressed in the units described for each parameter

Table 3. Inter-rater reliability for the anterior scalene US metrics

Variables	Mean ± SD	Absolute Error	$ICC_{3,2}$ (95% CI)	SEM	MDC_{95}
One Trial					
Cross-sectional area (mm^2)	166.3 ± 40.9	22.8 ± 22.7	0.841 (0.723;0.909)	16.3	45.2
Muscle Perimeter (mm)	49.9 ± 6.4	4.5 ± 3.6	0.795 (0.643;0.882)	2.9	8.0
Circularity (0-1)	0.83 ± 0.06	0.08 ± 0.07	0.561 (0.236;0.748)	0.04	0.11
Aspect Ratio	1.49 ± 0.26	0.21 ± 0.16	0.745 (0.555;0.853)	0.13	0.36
Roundness	0.69 ± 0.10	0.09 ± 0.07	0.709 (0.492;0.833)	0.05	0.15
Solidity	0.97 ± 0.02	0.02 ± 0.02	0.532 (0.184;0.731)	0.01	0.04
Mean echo-intensity (0-255)	50.1 ± 8.9	4.4 ± 4.4	0.907 (0.838;0.946)	2.7	7.5
Mean Average of Two Trials					
Cross-sectional area (mm^2)	165.0 ± 41.7	20.9 ± 18.9	0.880 (0.791;0.931)	14.4	40.0
Muscle Perimeter (mm)	49.8 ± 6.7	3.8 ± 3.2	0.836 (0.714;0.906)	2.7	7.5
Circularity (0-1)	0.83 ± 0.07	0.06 ± 0.06	0.550 (0.217;0.742)	0.05	0.13
Aspect Ratio	1.49 ± 0.28	0.20 ± 0.17	0.745 (0.556;0.854)	0.14	0.39
Roundness	0.69 ± 0.11	0.08 ± 0.07	0.726 (0.522;0.843)	0.06	0.15
Solidity	0.97 ± 0.03	0.02 ± 0.02	0.524 (0.171;0.727)	0.02	0.05
Mean echo-intensity (0-255)	49.3 ± 8.7	4.4 ± 3.7	0.925 (0.869;0.957)	2.4	6.6

SEM and MDC_{95} are expressed in the units described for each parameter

Figure 1

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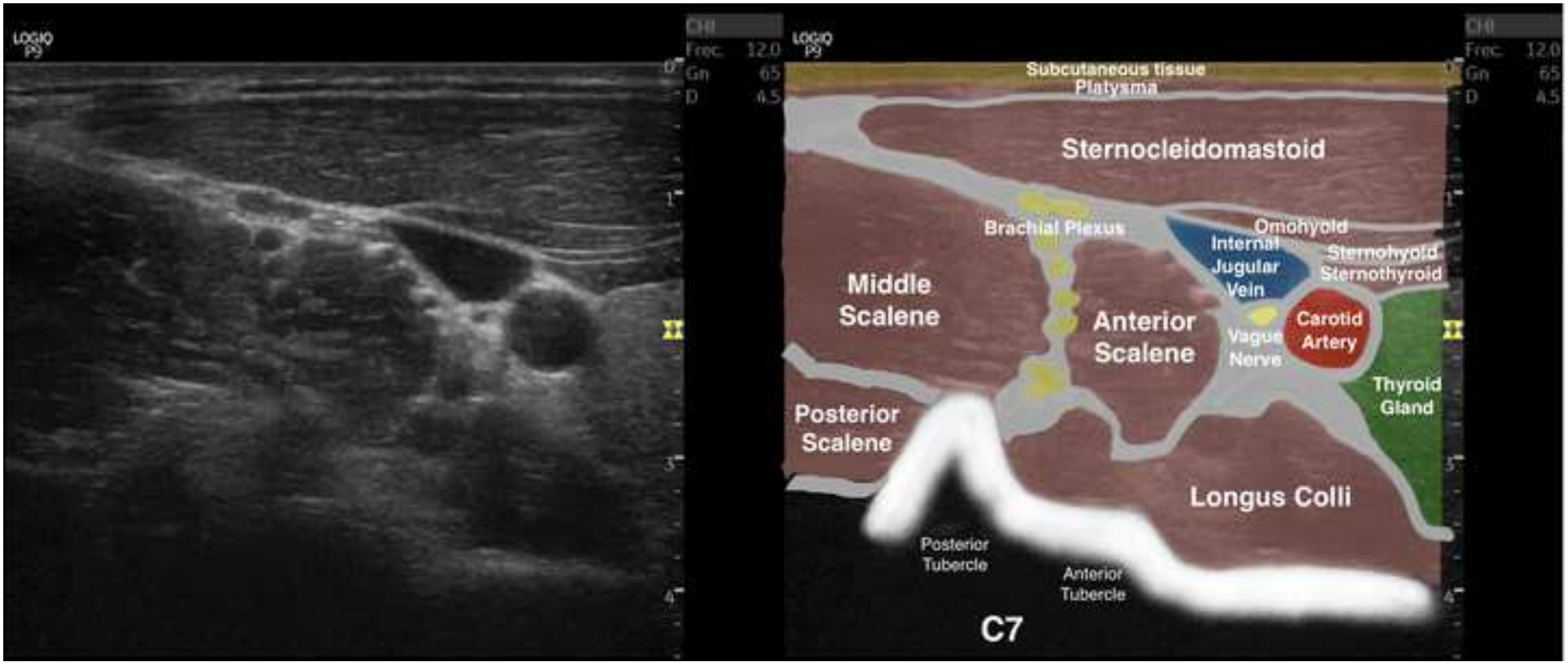


Figure 2

Click here to access/download;Figure;Figure 2.png

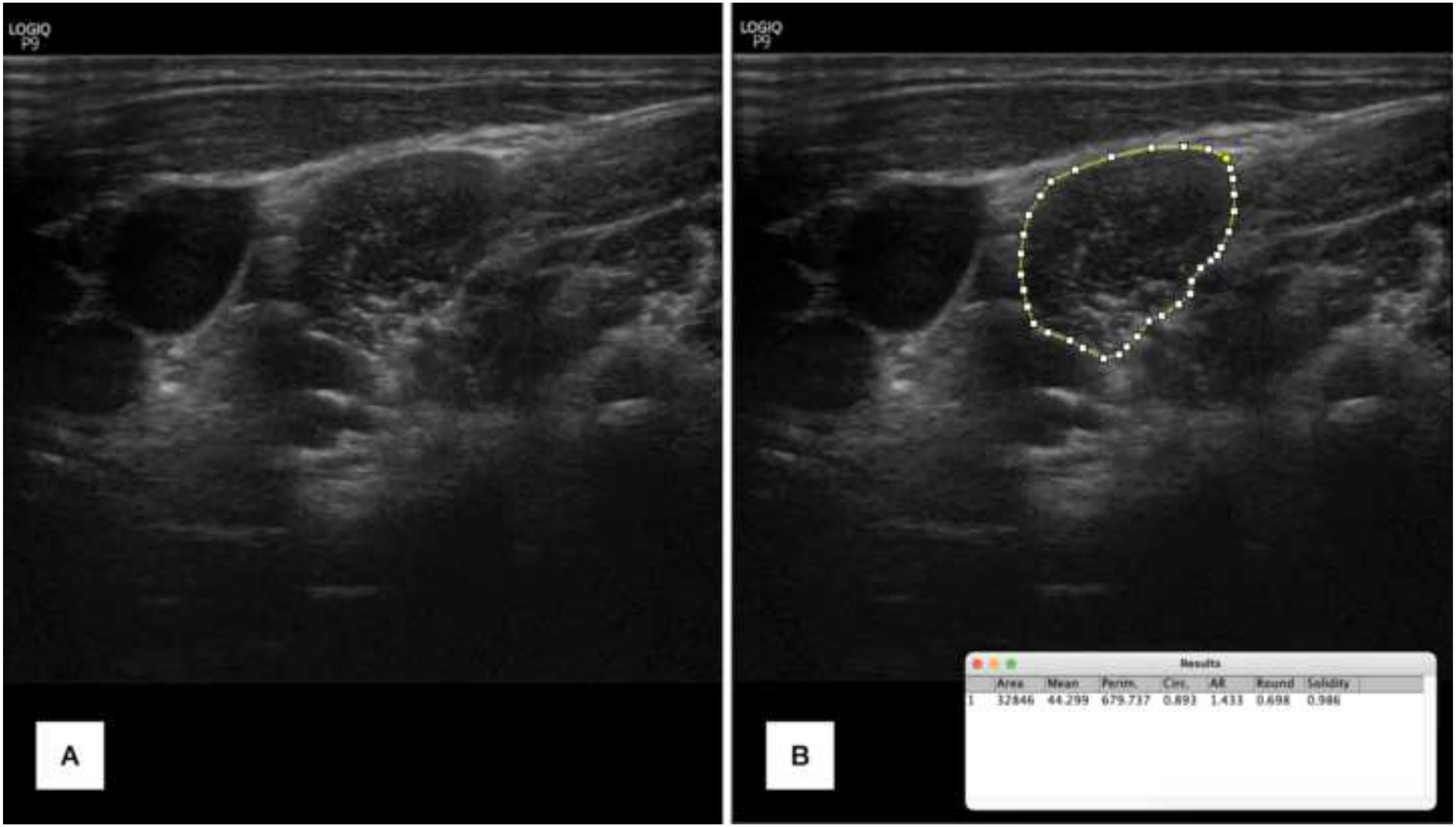
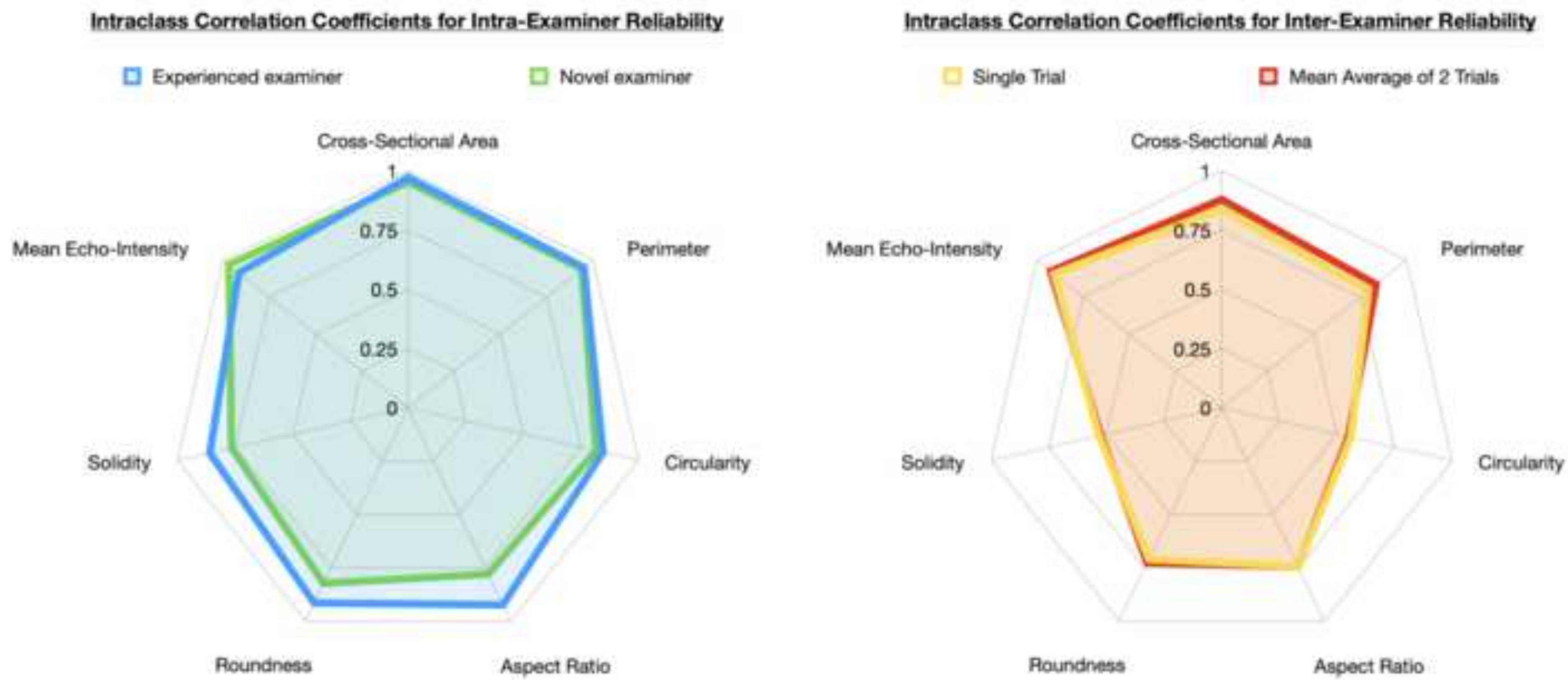


Figure 3



Intraclass Correlation Coefficients for Intra-Examiner Reliability
Experienced examiner
Novel examiner
Cross-Sectional Area
Perimeter
Circularity
Aspect Ratio
Roundness
Solidity
Mean Echo-Intensity
1
0.75
0.5
0.25
0
Intraclass Correlation Coefficients for Inter-Examiner Reliability
Single Trial
Mean Average of 2 Trials
Cross-Sectional Area
Perimeter
Circularity
Aspect Ratio
Roundness
Solidity
Mean Echo-Intensity
1
0.75
0.5
0.25
0