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Description and reproducibility metrics of a procedure for measuring the obliquus capitis inferior muscle stiffness using shear-wave elastography in patients with chronic neck pain

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ABSTRACT

Objective: Reliable quantification of deep-cervical muscle stiffness is clinically relevant, yet the reproducibility of shear-wave elastography (SWE) for the obliquus capitis inferior (OCI) has not been established. This study aimed to describe a standardized SWE procedure for measuring OCI stiffness and to determine its intra- and inter-examiner reliability in patients with chronic mechanical neck pain.

Materials and methods: We conducted a longitudinal reliability study in 17 adults with neck pain ≥6 months (mean age: 19.7 years; mean symptoms duration: 13.4 months). Seventeen participants underwent bilateral OCI imaging by two examiners with different ultrasound experience using fixed scanner presets. For each acquisition, a longitudinal region of interest encompassing the full muscle thickness was analyzed to obtain shear-wave velocity (SWV) and shear modulus (SM). Intraclass correlation coefficients (ICCs), standard error of measurement (SEM), and minimal detectable change (MDC) were calculated.

Results: Inter-examiner reliability was good-to-excellent (single measurements $ICC_{3,2} = 0.897\text{-}0.938$; mean of two measurements $ICC_{3,2} = 0.951\text{-}0.968$), with negligible between-examiner bias and lower SEM/MDC when two acquisitions were averaged. Test-retest reliability was excellent for both examiners ($ICC_{3,1} = 0.925\text{-}0.947$), with small SEM/MDC relative to the stiffness magnitude.

Conclusions: A rigorously standardized long-axis SWE protocol provides highly reproducible OCI stiffness measurements in young adults with mechanical neck pain. For longitudinal monitoring, assessments should preferentially be performed by the same examiner, and in multi-operator settings, decisions should be based on the mean of at least two acquisitions per side to improve sensitivity to true change.

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Implications for practice

- SWE provides objective stiffness values in absolute units and, according to our results, these values can be measured reproducibly using a standardized protocol.
- A standardized long-axis SWE protocol can be used to quantify OCI stiffness in patients with neck pain.
- Keeping the same examiner for follow-ups (whenever possible) reduces measurement variability.
- Averaging $\geq$2 acquisitions per side improves precision and accuracy to detect real changes.
- The SEM/MDC thresholds provided in this study are a good reference to interpret changes rather than raw values alone.
- A brief protocol training and calibration can deliver repeatable operator results.

1. Introduction

The Obliquus Capitis Inferior muscle (OCI) is a deep cervical muscle located on the posterior aspect of the cranio-cervical region, extending from the apex of the spinous process of C2/axis to the interior and dorsal part of the transverse process of C1/atlas. The OCI receives its motor supply primarily from the suboccipital nerve, the dorsal ramus of C1 (which runs within the suboccipital triangle together with the vertebral artery and venous plexus [1]), but anatomical studies showed that may also receive additional branches from the greater occipital nerve (GON), the dorsal ramus of C2 [2].

This muscle plays a role in cranio-cervical motion and motor control. Its exceptionally high spindle density makes the OCI a key source of cervical proprioceptive input, supporting fine regulation of head orientation, postural control, and upper cervical joint stability, in line with the high concentration of muscle spindles described in the suboccipital region [3]. When pain, hypertonicity, or segmental dysfunction alter normal OCI activation, the resulting changes in afferent signaling can disturb cervical sensorimotor integration and favor maladaptive motor strategies (i.e., inhibition of deep neck muscles, compensatory overactivity of superficial musculature, and reduced joint control), patterns repeatedly reported in individuals with musculoskeletal pain and neck pain [4].

These disturbances, which include increased joint position error and impaired proprioceptive acuity, may be further aggravated when OCI tension restricts atlanto-axial rotation or generates nociceptive input, mechanisms that have been linked to reduced C1-C2 mobility, altered proprioception, and cervicogenic symptoms in experimental and clinical studies on OCI-targeted interventions such as dry needling [5]. Additionally, the involvement of the OCI in the myodural bridge complex suggests a potential role in modulating dural tension and contributing to cervico-cephalic symptomatology when motor control of this muscle is compromised [6].

From a clinical point of view, dysfunction of the OCI have been reported in several cervical pain conditions, especially those characterized by proprioceptive disturbances and abnormal sensorimotor control. Patients with chronic neck pain often show impaired cervical joint position sense and atypical activation of deep cervical muscles, indicating disruption of the proprioceptive system in which the OCI is particularly important because of its high spindle density and its contribution to cranio-cervical control [3]. These changes are consistent with evidence that musculoskeletal pain can alter motor output, leading to reflex inhibition of painful muscles and increased dependence on superficial musculature, mechanisms that are compatible with altered recruitment or inhibition of the OCI in cervical pain syndromes [7]. Furthermore, individuals with cervicogenic headache display heightened mechanical sensitivity and reduced pressure pain thresholds in the suboccipital region, suggesting nociceptive involvement of structures such as the OCI in the pathophysiology of these headaches [8].

The importance of tissue stiffness becomes particularly evident in the setting of myofascial pain syndrome and myofascial trigger points (MTrPs), which often coexist with the conditions described above (although not necessarily present in all patients with chronic neck pain) [9,10]. Current diagnostic criteria place strong emphasis on detecting a palpable taut band and a localized increase in stiffness at the MTrP site as key major features [11]. MTrPs are classically defined as “hyperirritable, palpable nodules within a taut band that can provoke local and referred pain, contribute to the patient's symptoms, and, when stimulated, partially or completely reproduce their usual pain pattern” [12]. In practice, manual palpation remains the primary method for identifying taut bands and focal firmness (essentially functioning as a de facto reference standard in the absence of a true Gold Standard [12,13]). However, palpation is inherently subjective, shows only modest concordance with objective measures of stiffness, and is limited to muscles that can be readily accessed clinically [14,15]. These drawbacks are particularly critical for deeper musculature [16], where direct palpation is not possible.

Consequently, there is a clear methodological imperative to complement neuromuscular examination with objective, reproducible stiffness metrics in the deep cervical muscles [17]. However, whether OCI stiffness is altered or clinically implicated in chronic neck pain remains unknown. Before this question can be properly addressed in case-control, prognostic, or intervention studies, it is first necessary to determine whether OCI stiffness can be measured reproducibly using a standardized imaging protocol. Developing and validating imaging-based or instrumented techniques to quantify stiffness in the OCI could decrease reliance on subjective palpation [18], allow assessment of muscles that cannot be examined manually, and yield mechanistic and prognostic biomarkers that can be monitored over time and in response to treatment. Such measures may help close the current gap between documented EMG/morphological alterations and the mechanical environment thought to underlie pain, dysfunction, and recovery in cervical disorders [19]. On this basis, the present study aimed to determine the intra- and inter-examiner reliability of OCI stiffness measurements obtained with shear-wave elastography (SWE) in a sample of individuals with chronic mechanical neck pain.

2. Material and methods

2.1. Study design

From September to November 2025, the research team carried out a longitudinal observational study on intra- and inter-examiner reliability in the *BLINDED*. The preparation of this manuscript followed the Guidelines for Reporting Reliability and Agreement Studies (GRRAS) to organize and present the information [20]. The study protocol received prior approval from the local ethics committee before the start of data collection.

2.2. Participants

A consecutive sample of patients was drawn from the *BLINDED*, selecting individuals who contacted the service with neck pain as their main reason for consultation. The decision to recruit participants with

chronic mechanical neck pain was based on the intended clinical application of the protocol. Chronic neck pain may be associated with changes in cervical muscle morphology and tissue composition, including fatty infiltration, atrophy, altered fiber characteristics, and changes in tissue stiffness, which may complicate the visualization of fascial planes and deep muscle borders [21]. Therefore, assessing reliability only in asymptomatic individuals could overestimate the reproducibility of the procedure under clinical conditions.

All potential participants received detailed written and oral information about the study and signed informed consent before any study-related procedure was initiated. Their participation did not modify the content or timing of their routine clinical management.

Patients were initially pre-screened based on their primary reason for seeking care and were subsequently screened against the predefined eligibility criteria. As inclusion criteria, we targeted adults aged 18-65 years, current neck pain lasting at least six months, a Neck Disability Index (NDI, 0-100) score greater than 8%, and a Numeric Pain Rating Scale (NPRS, 0-10) score greater than 3/10, as these cut-offs are commonly applied to distinguish symptomatic patients from asymptomatic controls [22,23]. Those participants declaring any medical, pharmacological, or physiotherapy interventions within the previous six months that could alter pain perception or muscle tone; traumatic causes of neck pain (e.g., whiplash-associated disorders, fractures, fissures); history of cervical spine surgery; clinical signs compatible with radiculopathy or myelopathy; and other significant comorbid conditions such as fibromyalgia or active oncologic disease were systematically excluded. Those who met all criteria attended a dedicated data-collection visit scheduled immediately before their routine treatment appointment, ensuring that neither the measurements nor their usual clinical care were affected or interfered with.

The minimum sample size was determined using the approach described by Walter et al. [24] for intraclass correlation coefficients (ICCs). We assumed a minimum acceptable reliability of 0.70 (commonly considered the lower limit for good reliability [25]), and an expected ICC of 0.90. Because no prior reliability data were available for this specific protocol, we used previously reported ICCs for assessing OCI with B-mode ultrasonography as a reference [26]. The calculation was performed for 80% power, a two-sided α of 0.05, and an anticipated 10% dropout rate due to the longitudinal nature of the study. Under these assumptions, a minimum of 26 side-specific OCI measurements was required because the SWE protocol was applied separately to the right and left OCI (both sides were assessed as side-specific measurement targets). Consequently, the target sample comprised 13 participants for intra-examiner reliability (n = 26 SWE images in Trial 1 and n = 26 in Trial 2) and 26 images for inter-examiner reliability (n = 26 images for Examiner 1 and n = 26 for Examiner 2).

2.3. Examiners

All ultrasound images were acquired and processed by two examiners with different levels of expertise. The senior examiner had over 10 years of experience in musculoskeletal ultrasound and clinical assessment, whereas the junior examiner had less than 1 year of experience.

Prior to participant recruitment, both examiners completed a structured 2-h training session on the study equipment to harmonize acquisition and analysis procedures. This session covered 1) key anatomical landmarks and standardized participant positioning; console setup with fixed presets; 2) probe-handling practice (minimal transducer pressure, sufficient gel, longitudinal B-mode alignment parallel to muscle fibers, and perpendicular insonation to minimize anisotropy); 3) SWE acquisition steps (activating the mode, placing a region of interest -ROI- that included the full muscle thickness while excluding fascial layers, and maintaining a steady image for approximately 10 s before freezing); 4) and recording both shear modulus (SM) and shear-wave velocity (SWV). Frames were only accepted when the elastography/quality map was stable and free from motion or visible artifacts.

Each examiner then rehearsed the complete protocol on a pilot asymptomatic volunteer, obtaining three repeated measurements per site and entering the values into the study datasheet to practice blinding procedures and data recording. Any discrepancies in technique or decision-making were reviewed immediately, with specific feedback and a checklist used to standardize ROI placement and criteria for rejecting artifacts before starting formal data collection.

Ultrasound assessments were performed in a dedicated, fully equipped examination room. For each participant, the workflow consisted of four consecutive acquisitions per side: first, the experienced examiner obtained one image on each side; second, the novice examiner repeated the measurements; third, the experienced examiner performed a second series; and finally, the novice examiner completed a final pass. This back-to-back sequence was designed to minimize temporal variation. Between examiners, participants briefly stood up from the table and walked. The initial side (right or left) for each measurement sequence was randomized.

2.4. Shear wave elastography: imaging acquisition and measurement

The US device used for collecting all the images was a Canon Aplio a device, using a linear transducer PLT-1005-BT 14L5 (5-14 MHz) transducer (Canon Medical Corp, 1385 Shimoishigami, Otawara, Tochigi 324-8550, Japan). The console settings were also standard for all the acquisitions (gain = 78 dB; dynamic range = 70; frequency = 11 MHz and depth = 40 mm).

Participants lay in a prone position with their shoulders abducted to 90° and elbows flexed to 90°. The head and neck were passively aligned in neutral and stabilized using the table's face opening. All volunteers were instructed to fully relax their neck muscles to minimize variability in stiffness measurements due to residual contraction. The C2 spinous process was first palpated and used as the landmark for placing the transducer in a transverse orientation. The cervical level was verified by sliding the probe in a craniocaudal direction to visualize the adjacent vertebrae, confirming C2 as the segment with the most prominent spinous process. From this starting point, the transducer was moved

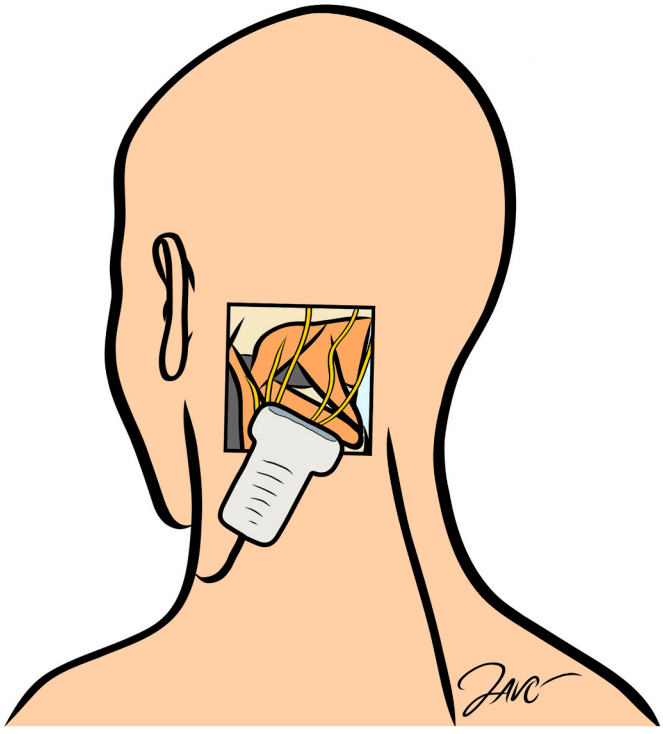


Fig. 1. Schematic representation of the ultrasound probe placement along the longitudinal axis of the obliquus capitis inferior muscle.

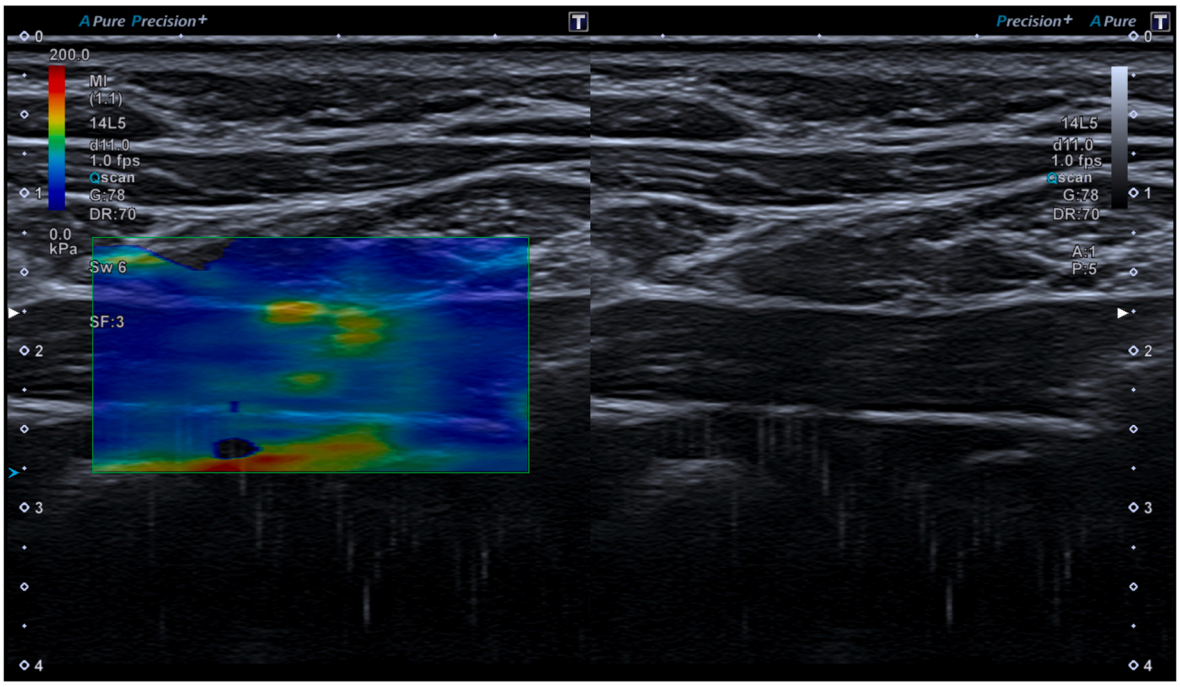


Fig. 2. Representative ultrasound of the obliquus capitis inferior (OCI) muscle. The left panel shows the B-mode image, and the right panel overlays shear-wave elastography, with a color-coded ROI depicting the tissue stiffness distribution.

laterally toward the side being assessed until the spinous process appeared in the lateral quarter of the screen. The probe was then rotated (clockwise when examining the left side and counterclockwise on the right) so that its outer edge projected over the C1 transverse process. At this depth, the OCI is seen as the only longitudinal structure. Particular attention was paid to aligning the muscle belly strictly perpendicular to the insonation plane, as any deviation could generate artifacts and distort stiffness measurements (Fig. 1).

To quantify OCI stiffness, the examiners switched on the SWE mode and allowed the elastography image to stabilize for approximately 10 s before capturing the frame. For the measurement, they positioned the region of interest (ROI) so that it encompassed the entire muscle thickness (carefully excluding adjacent connective tissue and bone) and at least 2 cm of fiber length. Once the ROI was delineated, the system automatically generated values for SM and SWV (Fig. 2).

2.5. Statistical analysis

All data were processed and analyzed using SPSS v.29 for macOS (Armonk, NY, USA). Two-sided tests were applied, and the level of statistical significance was set at $p < 0.05$. Distributional assumptions were checked through visual inspection of histograms and the Shapiro-Wilk test (variables were treated as normally distributed when $p > 0.05$). Sample characteristics were described using standard descriptive statistics.

As an exploratory descriptive analysis, a multivariate general linear model was used to examine whether OCI stiffness values differed according to sex, anatomical side, or their interaction. SWE metrics were entered as dependent variables, while sex and side were included as fixed factors, together with the sex-by-side interaction. This analysis was performed only to provide descriptive context for the reliability estimates and was not considered part of the primary reliability objective. To account for the assessment of two stiffness outcomes, Bonferroni correction was applied and the statistical significance threshold was adjusted to $p < 0.025$. For each effect, F values, partial eta squared (ηp^2), and adjusted p-values were reported.

Reliability metrics were calculated in line with the framework proposed by Koo and Li [25]. For test-retest (intra-examiner) reliability, we derived: (1) the mean of the two repeated measurements; (2) the absolute difference between both trials; (3) intraclass correlation coefficients using a two-way mixed-effects model for consistency, applying $ICC_{3,1}$ for single measurements reliability and $ICC_{3,2}$ for the reliability using the mean average of 2 measurements (values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.90 were interpreted as poor, moderate, good, and excellent reliability, respectively [25]); (4) the standard error of measurement (SEM), computed as SEM=SD_{mean

Table 1
Descriptive analyses of demographic and clinical characteristics of the sample.

Variables	Males (n = 8)	Females (n = 9)	Sex Difference (95% CI)
Demographics			
Age, years	18.9 ± 1.0	20.4 ± 1.3	1.6 (0.3; 2.8) p = 0.016
Weight, kg	75.5 ± 7.7	58.2 ± 10.4	17.3 (7.7; 26.9) p < 0.001
Height, m	1.80 ± 0.09	1.68 ± 0.04	0.11 (0.04; 0.19) p = 0.003
BMI, kg/m^2	23.3 ± 1.5	20.6 ± 3.9	2.6 (−0.5; 5.8) p = 0.097
Clinical Characteristics			
Neck Disability Index, 0-100	34.8 ± 15.5	43.8 ± 9.2	9.0 (−4.0; 22.0) p = 0.160
Numeric Pain Rating Scale, 0-10	5.9 ± 0.7	7.2 ± 1.2	1.3 (0.25; 2.3) p = 0.017

Abbreviatures: BMI, Body Mass Index; CI: Confidence Interval.

Table 2
Obliquus capitis inferior stiffness: Descriptive analysis and differences between genders and sides.

Sex	Side	Shear Wave Velocity (m/s)[a]	Shear modulus (kPa)[a]
Males	Mean	3.36 ± 0.82	36.0 ± 21.9
	Left (n = 8)	3.05 ± 0.39	28.4 ± 7.0
	Right (n = 8)	3.67 ± 1.03	43.6 ± 29.1
Females	Mean	3.40 ± 0.48	35.6 ± 10.8
	Left (n = 9)	3.26 ± 0.33	32.4 ± 6.4
	Right (n = 9)	3.55 ± 0.57	38.8 ± 13.6
Differences			
Sex	F	0.035	0.005
	P Value	0.852	0.947
	η_p^2	0.001	0.000
Side	F	0.570	0.605
	P Value	0.456	0.443
	η_p^2	0.019	0.020
Sex*Side	F	4.290	3.628
	P Value	0.047	0.066
	η_p^2	0.125	0.108

[a] Reported values are calculated as the mean average from four measurements (both trials and both examiners).

of trials}×√(1-ICC); and (5) the minimal detectable change (MDC), obtained as MDC=SEM ×1.96×√2. Because right and left OCI measurements were obtained from the same participants, side-specific observations were considered nested within participants. Therefore, the clustered structure of the data was addressed by including participant as a random effect in the reliability analysis to preserve the side-specific clinical interpretation of the measurements while accounting for the non-independence of bilateral data.

3. Results

From a total of 20 volunteers interested in collaborating with the study, 3 were excluded since they did not reach the minimum NDI or NPRS cut-off required to participate (n = 3). Therefore, n = 17 volunteers with neck pain were included and analyzed, with no data losses (n = 0).

Table 1 summarizes the sociodemographic and clinical characteristics of the sample by sex. Clinically, women presented with higher neck disability and pain scores. NDI values were greater in females, but this difference was not statistically significant (p = 0.160). In contrast, pain intensity on the NPRS was significantly higher in women (p = 0.017).

As an exploratory descriptive analysis, Table 2 summarizes OCI stiffness values by sex and anatomical side, together with the effects obtained from the multivariate general linear model. Overall mean stiffness was very similar between males and females (p > 0.05). In both groups, the right OCI tended to be stiffer than the left. However, neither the main effect of sex nor side reached statistical significance for SWV (p > 0.05) or SM (p > 0.05), with trivial effect sizes.

By contrast, the sex*side interaction was statistically significant for SWV (p = 0.047) and showed a similar trend for SM (p = 0.066), indicating that right-left asymmetry was somewhat more pronounced in males than in females. Nonetheless, absolute differences were modest relative to within-group variability and stiffness values clustered within a narrow range. It should be noted that the sample size calculation was based on reliability outcomes and all participants were symptomatic. Therefore, stiffness values should be interpreted as descriptive values for this clinical sample and not as normative reference values.

Inter-examiner reliability for global OCI stiffness is presented in Table 3. Agreement between the experienced and novice examiner was good-to-excellent for both SWV and SM. For single measurements, $ICC_{3,2}$ was 0.897 for SWV and 0.938 for SM; when the mean of two measurements was used, ICCs increased to 0.951 and 0.968, respectively. Between-examiner biases were minimal as reflected in mean differences. For single measurements, the mean difference was 0.10 m/s (p = 0.536) SWV and 2.0 kPa (p = 0.661) for SM; when averaging two measurements, differences further decreased to 0.07 m/s (p = 0.653) and 0.9 kPa (p = 0.834), all non-significant. In addition, precision improved with averaging as indicated by SEM and MDCs. For SWV, SEM declined from 0.22 to 0.15 m/s and MDC from 0.60 to 0.40 m/s; for SM SEM decreased from 4.7 to 3.0 kPa and MDC from 13.0 to 8.3 kPa. Expressed relative to the grand means, SEM was ~4-6% for SWV and ~8-13% for SM, with MDCs of ~12-18% and ~23-36%, respectively.

Test-retest reliability for SWV and SM is presented in Table 4 for both the novice and experienced examiner. Across trials, mean values were highly consistent. Between-trial differences were trivial and non-significant for both examiners (novice: Δspeed p = 0.717; Δmodulus p = 0.537; experienced: Δspeed p = 0.997; Δmodulus p = 0.947), indicating absence of systematic bias. Test-retest agreement was excellent for both operators. For the novice examiner, $ICC_{3,1}$ was 0.925 for SWV and 0.946 for SM; for the experienced examiner, $ICC_{3,1}$ values were 0.926 and 0.947, respectively. SEM was small in absolute terms, yielding MDCs of 0.52-0.51 m/s for SWV and 11.0-11.1 kPa for SM. Relative to the corresponding grand means, SEM was approximately 5-6% for speed and 11% for SM, with MDCs around 15-16% and 30-31%, respectively.

Table 3
Inter-examiner reliability analyses to determine general obliquus capitis inferior muscle stiffness.

Reliability estimates	Shear Wave Velocity (m/s)		Shear Modulus (kPa)	
	Experienced Examiner (n = 34 images*)	Novice Examiner (n = 34 images*)	Experienced Examiner (n = 34 images*)	Novice Examiner (n = 34 images*)
Single Measurements				
Scores	3.35 ± 0.63	3.45 ± 0.73	35.5 ± 19.1	37.5 ± 18.7
Mean	3.40 ± 0.68		36.5 ± 18.8	
Difference	0.10 (−0.22; 0.43) p = 0.536		2.0 (−7.1; 11.2) p = 0.661	
$ICC_{3,2}$, 0-1	0.897 (0.795; 0.949)		0.938 (0.876; 0.969)	
SEM	0.22		4.7	
MDC	0.60		13.0	
Mean Average of 2 Measurements				
Scores	3.35 ± 0.66	3.42 ± 0.67	35.4 ± 17.1	36.2 ± 16.8
Mean	3.38 ± 0.66		35.8 ± 16.8	
Difference	0.07 (−0.25; 0.39) p = 0.653		0.9 (−7.3; 9.1) p = 0.834	
$ICC_{3,2}$, 0-1	0.951 (0.901; 0.975)		0.968 (0.936; 0.984)	
SEM	0.15		3.0	
MDC	0.40		8.3	

Abbreviations: ICC: Intraclass correlation coefficients; MDC: Minimal Detectable Changes; SEM: Standard Error of Measurement.
*n = 34 side-specific measurements from 17 participants.

4. Discussion

SWE is an ultrasound-based method in which focused acoustic radiation force is used to induce transverse shear waves within tissues; by tracking their propagation speed, the system generates quantitative stiffness maps (SM) that are automatically aligned with the B-mode image and visualized as color-coded elastograms in m/s or kPa [27]. Compared with strain or compression elastography, SWE offers greater objectivity and reproducibility because it provides a direct, largely operator-independent estimate of tissue mechanical properties [28]. In musculoskeletal applications, shear waves propagate more rapidly in stiffer or contracted tissue and along the main axis of tendons or muscle fibers. As a result, SWE complements conventional grayscale and Doppler imaging for early detection, grading, and follow-up of pathology in tendons, muscles, peripheral nerves, ligaments, and selected soft-tissue lesions. Recent work suggests that SWE may be useful for monitoring tissue healing, staging chronic disorders, and even predicting tendon failure risk, while novel developments (such as continuous-wave and 3D SWE) are expanding its clinical scope [27–30].

Table 4
Test-retest reliability analyses to determine general obliquus capitis inferior muscle stiffness.

Reliability metrics	Novice Examiner				Experienced Examiner			
	Shear Wave Speed (m/s)		Shear Modulus (kPa)		Shear Wave Speed (m/s)		Shear Modulus (kPa)	
	Trial 1 (n = 34)	Trial 2 (n = 34)	Trial 1 (n = 34)	Trial 2 (n = 34)	Trial 1 (n = 34)	Trial 2 (n = 34)	Trial 1 (n = 34)	Trial 2 (n = 34)
Scores	3.45 ± 0.73	3.39 ± 0.66	37.5 ± 18.7	34.9 ± 15.6	3.35 ± 0.63	3.35 ± 0.73	35.5 ± 19.1	35.2 ± 15.8
Mean	3.42 ± 0.69		36.2 ± 17.1		3.35 ± 0.68		35.4 ± 17.4	
Difference	0.06 (−0.27; 0.39) p = 0.717		2.6 (−5.7; 10.9) p = 0.537		0.00 (−0.33; 0.33) p = 0.997		0.3 (−8.2; 8.7) p = 0.947	
$ICC_{3,1}$, 0-1	0.925 (0.850; 0.963)		0.946 (0.891; 0.973)		0.926 (0.853; 0.963)		0.947 (0.893; 0.973)	
SEM	0.19		4.0		0.18		4.0	
MDC	0.52		11.0		0.51		11.1	

Abbreviations: ICC: Intraclass correlation coefficients; MDC: Minimal Detectable Changes; SEM: Standard Error of Measurement.

Despite these advantages, SWE has several practical constraints. Its penetration is reduced in deeper structures, sometimes necessitating a gel standoff. Measurements are highly sensitive to transducer pressure, insonation angle, and tissue anisotropy, so even subtle misalignment can alter wave speed. In some devices, the ROI is limited in size or geometry and acquisitions must be separated by short pauses, which complicates dynamic or continuous assessments. Image quality may deteriorate in fluid-rich regions, generating voids or unreliable areas in the elastogram, and outcomes depend on manufacturer-specific presets and algorithms, which can yield misleading values if scales are misconfigured and limit cross-platform comparability [27–30].

Because these sources of variability influence reproducibility and clinical interpretation, a prerequisite for using SWE to evaluate the OCI is to determine how reliable its measurements are under standardized conditions [31]. It is also crucial to identify the smallest detectable changes that differentiate true shifts in muscle stiffness (whether over time due to treatment or natural progression, or between groups) from random measurement error. Defining these parameters is fundamental for interpreting previous research (many studies have been published without such reliability analyses) and for planning future work, including cross-sectional case-control comparisons and longitudinal intervention trials examining OCI stiffness [32]. In this context, the present study set out to describe a detailed, replicable SWE protocol for quantifying OCI stiffness and to report intra- and inter-examiner reliability indices that can underpin subsequent cross-sectional and longitudinal investigations.

When focusing on reliability, our intra- and inter-examiner analyses revealed consistently high stability of OCI stiffness measurements. Test-retest data revealed excellent within-examiner agreement for SWV and SM for both the novice and experienced examiner, with intraclass correlation coefficients above 0.90 and virtually no systematic change between trials, whether in SWV or SM. Between-examiner comparisons likewise demonstrated very good concordance, and reliability improved further when the mean of two acquisitions was used instead of a single measurement, as reflected by higher ICCs and noticeable reductions in SEM and MDC. Clinically, this pattern is important because the SEM quantifies the absolute measurement error associated with repeated measurements, while the MDC represents the smallest change that must be exceeded for a change to be interpreted as real rather than attributable to measurement error. The high inter-examiner ICCs indicate that the standardized protocol can be applied reliably by different trained examiners, particularly when the mean of two acquisitions is used. However, ICCs reflect relative reliability, that is, the degree to which participants maintain their ranking across repeated measurements or examiners, whereas SEM and MDC quantify absolute measurement error. This distinction is clinically important for longitudinal follow-up, where the main question is whether the change observed in an individual patient exceeds measurement error and can therefore be interpreted as a real change. For this reason, although our results support the use of the protocol in multi-operator settings, follow-up assessments should preferably be performed by the same examiner when feasible and based on the mean of at least two acquisitions per side, in order to minimize avoidable measurement variability and improve sensitivity to true change. The similarity of test-retest indices between the novice and experienced examiner suggests that, once a rigorous standardized protocol and brief calibration are in place, operator experience has only a modest impact on repeatability.

However, the extrapolation of these findings is constrained by the characteristics of our sample, which consisted almost exclusively of young adults with mechanical neck pain, relatively preserved BMI, and moderate disability and pain levels. Although the eligibility criteria considered a wide range of sociodemographic characteristics, the final sample showed a narrow distribution. This is clinically relevant because muscle stiffness [33], cervical muscle morphology [34], and degenerative changes of the cervical spine [35] may vary substantially with these factors and may influence both SWE values and measurement error [36]. Therefore, the reliability metrics reported here should be interpreted as primarily applicable to young adults with chronic mechanical neck pain. In individuals with different demographic or clinical severity characteristics, reliability metrics may vary because the quality and consistency of SWE acquisition can be influenced by factors affecting tissue visualization and anatomical landmark identification. For instance, in older adults or individuals with overweight, increased tissue depth and acoustic attenuation may reduce ultrasound image quality and increase artifacts, making anatomical landmarks and muscle borders less consistently visible [37,38]. Similarly, cervical muscle changes associated with greater pain or disability reported in previous studies such as increased fatty infiltration and reduced muscle size, may further obscure fascial planes and complicate ROI delineation, potentially increasing measurement error [21,39]. Conversely, reliability may be favored in young, asymptomatic individuals with clearer tissue interfaces and fewer morphology-related imaging constraints. Future studies should determine whether comparable reliability estimates and SEM/MDC thresholds are obtained in populations with different age, body composition, muscle morphology, and clinical presentations.

Although direct comparison with prior SWE reliability data for the OCI is not feasible (since this is the first study evaluating the reproducibility for assessing the OCI stiffness) indirect benchmarks can be derived from B-mode morphological assessments. Masoudi et al. [40] evaluated the cross-sectional area of the deep suboccipital muscles (including the OCI) in healthy volunteers and reported good intra- and inter-rater reliability overall, but with noticeably lower ICCs (0.61 for inter-examiner reliability and 0.61 to 0.86 for intra-examiner) and higher SEM/MDC values for the OCI than for the rectus capitis muscles, highlighting how challenging it is to reproduce measurements in this region even under controlled conditions. Likewise, Cho et al. [26] measured the cross-sectional area of the OCI and the adjacent greater occipital nerve in pain-free young adults and found excellent inter-examiner reliability for nerve size but only moderate-to-good agreement for OCI cross-sectional area (ICC ≈ 0.73), despite meticulous standardization of transducer orientation along and across the muscle.

Both studies agreed that the main source of variability lies in consistently relocating the same anatomical window with comparable

probe pressure and angle. Against this background, the good-to-excellent intra- and inter-examiner ICCs and relatively small SEM/MDC observed in our study suggest that, when probe positioning (using a long-axis view of the muscle rather than a transverse cross-section to facilitate perpendicular insonation and minimize anisotropy) and probe pressure and alignment standardization is provided, SWE-derived stiffness estimates for the OCI can achieve at least comparable, and in some respects superior, reproducibility to B-mode morphology in healthy samples.

In line with this hypothesis, Molaei et al. [41], who assessed the test-retest reliability of a long-axis scan procedure for measuring the thickness of the OCI in a sample of adults with non-specific chronic neck pain, reported reported excellent intrasession ICCs (0.89-0.95) and intersession ICCs (0.84-0.91), with low SEM (0.35-0.44 mm intrasession; 0.50-0.57 mm intersession) and corresponding MDC values of roughly 1.0-1.6 mm, alongside narrow limits of agreement and no systematic bias.

Finally, these findings also have implications for future clinical research. As discussed previously, the assessment of myofascial and upper cervical dysfunction still relies largely on subjective palpation, particularly when clinicians attempt to identify taut bands, focal stiffness, or somatic dysfunction in deep cervical muscles [14–16]. The standardized SWE protocol described in this study could provide a reproducible objective outcome for future longitudinal studies examining whether commonly used interventions (e.g., manual therapy, dry needling, or exercise) modify OCI stiffness over time [42]. Such studies could also explore whether changes in SWE-derived stiffness are associated with improvements in pain, disability, cervical mobility, proprioception, or the clinical resolution of upper cervical dysfunction.

4.1. Limitations

One important limitation is that the sample was restricted to participants with mechanical neck pain. Given that neck pain encompasses several clinical presentations (e.g., conditions characterized by mobility deficits, headache-related pain, radiating symptoms, or movement coordination impairments [42]), muscle visualization clarity may vary across different etiologies. Reliability is partially determined by the quality and consistency of image acquisition, anatomical landmark identification, and ROI delineation. This is relevant because muscle changes are not consistent across neck pain etiologies. For example, chronic idiopathic neck pain and whiplash-associated disorders may show different patterns of cervical muscle morphology, with fatty infiltration being more consistently reported in whiplash-associated disorders [21,43]. Therefore, clinical conditions that make muscle borders or fascial planes less clearly visible may increase measurement error and affect reproducibility. A further constraint is that all measurements were obtained at a single, relatively general anatomical site and using only one ultrasound device. Future studies should focus on more specific targets (such as myofascial trigger points as conducted in previous research [44,45]) and incorporate different ultrasound brands and models to enhance generalizability.

Another limitation is that bilateral OCI measurements were obtained from each participant. Although the right and left OCI were assessed as side-specific clinical measurement targets, these observations are not fully independent because they are nested within individuals. We addressed this clustered structure in the statistical analysis; however, the modest number of participants limits the precision of these estimates. In addition, stiffness values were reported according to anatomical side rather than symptom laterality, as the study was designed to evaluate the reproducibility of a standardized bilateral SWE protocol rather than to compare symptomatic and asymptomatic sides. Therefore, the present findings cannot determine whether OCI stiffness differs according to pain laterality. Future studies with larger samples should record unilateral or bilateral symptom presentation, compare symptomatic and asymptomatic sides when appropriate, and confirm these findings using statistical models specifically designed for bilateral or clustered reliability data.

Lastly, although we described and compared stiffness values by sex and side, the study was designed and powered for reliability outcomes rather than between-group comparisons. Therefore, it may not have adequate power to detect small-to-moderate differences or associations, and these exploratory results should be viewed with caution and replicated in larger, hypothesis-driven cohorts.

5. Conclusions

Using a rigorously standardized SWE protocol with a long-axis acquisition of the OCI, we found that SWV and SM can be measured with good-to-excellent reliability. Inter-examiner agreement between the experienced and novice operator was high, with ICCs close to or above 0.90 and negligible mean bias, and further improved when two acquisitions were averaged, as reflected by lower SEM and MDC values. Test-retest analyses also showed excellent within-examiner stability for both examiners.

These findings support the use of this protocol by different trained examiners for side-specific OCI stiffness assessment. Nevertheless, for longitudinal monitoring, SEM and MDC are more clinically informative than ICCs because they quantify the absolute measurement error that must be exceeded to identify true change. Therefore, follow-up assessments should preferably be performed by the same examiner when feasible, and in multi-operator settings, decisions should be based on the mean of at least two acquisitions per side. The stiffness values reported here should be interpreted as descriptive values from this specific symptomatic sample and not as normative reference values.

CRediT authorship contribution statement

Juan Antonio Valera-Calero: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jorge Buffet-García:** Writing – review & editing, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Sandra Sánchez-Jorge:** Writing – review & editing, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Umut Varol:** Writing – review & editing, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Dariusz Kosson:** Writing – review & editing, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Marcin Kołacz:** Writing – review & editing, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Germán Monclús-Díez:** Writing – review & editing, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation.

Informed consent statement

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

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 Hospital Universitario 12 de Octubre (CEIm 24/121; Approval date 25.06.2024).

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors contribution

- *Juan Antonio Valera-Calero:* Conceptualization; methodology; software; validation; formal analysis; investigation; data curation; writing-original draft preparation; writing-review and editing; visualization; supervision; project administration.
- *Jorge Buffet-García:* Methodology; software; validation; formal analysis; investigation; resources; data curation; writing-review and editing; visualization.
- *Sandra Sánchez-Jorge:* Methodology; software; validation; formal analysis; investigation; resources; data curation; writing-review and editing; visualization
- *Umut Varol:* Methodology; software; validation; formal analysis; investigation; data curation; writing-review and editing; visualization.
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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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