Shear wave elastography using ultrasound: effects of anisotropy and stretch stress on a tissue phantom and *in vivo* reactive lymph nodes in the neck

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Purpose: The purpose of this study was to evaluate how the anisotropy and the static stretch stress of the cervical musculature influence the measured shear modulus in a tissue-mimicking phantom and in cervical lymph nodes *in vivo* by using shear wave elastography (SWE).

Methods: SWE was performed on a phantom using a pig muscle and on the middle jugular cervical lymph nodes in six volunteers. Tissue elasticity was quantified using the shear modulus and a supersonic shear wave imaging technique. For the phantom study, first, the optimal depth for measurement was determined, and then, SWE was performed in parallel and perpendicular to the muscle fiber orientation with and without strain stress. For the *in vivo* study, SWE was performed on the cervical lymph nodes in parallel and perpendicular to the sternocleidomastoid muscle fiber direction with and without neck stretching. The mean values of the shear modulus (meanSM) were then analyzed.

Results: In the phantom study, the measured depth significantly influenced the meanSM with a sharp decrease at the depth of 1.5 cm ($P<0.001$). Strain stress increased the meanSM, irrespective of the muscle fiber orientation ($P<0.001$). In the *in vivo* study, the meanSM values obtained in parallel to the muscle fiber orientation were greater than those obtained perpendicular to the fiber orientation, irrespective of the stretch stress ($P<0.001$). However, meanSM was affected significantly by the stretch stress parallel to the muscle fiber orientation ($P<0.001$).

Conclusion: The anisotropic nature of the cervical musculature and the applied stretch stress explain the variability of the SWE measurements and should be identified before applying SWE for the interpretation of the measured shear modulus values.

Keywords: Neck; Lymph nodes; Elasticity imaging techniques; Shear wave imaging; Anisotropy; Elasticity imaging techniques

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Introduction

Lymph node evaluation is essential in patients with head and neck carcinoma because it is related to disease prognosis and aids treatment planning [1,2]. Gray-scale ultrasound (US) imaging is a widely accepted modality for the preoperative imaging of head and neck carcinomas. Morphological criteria, such as size, shape, and internal architecture, are the major features of gray-scale US that enable the differentiation between malignant and benign lymph nodes. The major features of malignant lymph nodes that show changes in the internal architecture include loss of fatty hilum, the presence of cortical heterogeneity or central necrosis, cystic change, and calcification [3,4]. However, although US and computed tomography (CT) have similar specificities, the sensitivity of US (77%) is slightly lower than that of CT (91%) or that of magnetic resonance imaging (93%) [5].

Recently, US elastography has emerged as a complementary imaging tool for the detection of metastatic cervical lymph nodes [6,7]. Two forms of US elastography are available these days, namely strain and shear wave elastography (SWE). SWE is based on the measurements of the velocity of the propagating shear waves. It combines the induction of a radiation force in tissues with a US beam and ultrafast imaging, which captures the propagation of shear waves in real time [8]. Tissue elastic properties are described using the shear modulus "G," defined as being equal to $\rho\upsilon_s^2$, where ρ denotes the tissue density and υ_s represents the velocity of the generated shear wave. SWE involves the application of a radiation force using a probe rather than a compressive force by an operator; this improves reproducibility. Furthermore, SWE provides quantitative elasticity measurements, whereas strain elastography does not. SWE has been used on several organs, including cervical lymph nodes [9,10], which suggests that it could be useful for detecting malignant lymph nodes.

Because cervical lymph nodes are mainly located posterior to the cervical musculature, particularly the sternocleidomastoid (SCM) muscle, attention should be paid to the effects of over tissue elasticity from the surrounding tissues. During the US examinations of the neck, patients are requested to extend their necks, which in turn increases the stretching stress. The SCM muscle is a highly ordered structure with a high anisotropy that depends on the fascicular direction; this could hinder the propagation of shear waves in a direction perpendicular to the muscle fiber orientation. Furthermore, although the mechanical properties of the surrounding tissues could influence tissue density and the velocity of the propagating shear waves, no study has yet evaluated the effects of the biomechanical parameters of the target environment on a cervical lymph node assessment performed using SWE. Therefore, the purpose of this study was to evaluate how the anisotropy and the static stretch stress of the background influence the measured shear modulus in a tissue-mimicking phantom and in the cervical reactive lymph nodes located behind the SCM muscle *in vivo* by using SWE. We also evaluated the effect of depth on the SWE measurements in the tissue phantom.

Materials and Methods

Gray-Scale US and SWE Measurements

All SWE measurements were conducted using the Aixplorer ultrasound system (SuperSonic Imagine, Aix en Provence, France) and a 50-mm linear probe with a frequency range of 4 to 15 MHz. All examinations were performed by a single investigator (H.Y.L.) with 8 years of head and neck US experience.

After gray-scale US, the system was changed to the SWE mode (SWE was performed using the same probe as that used for the gray-scale US). Depending on the level of signal gain, the acquisition mode was selected to be either the standard mode or the penetration mode. Image sets were simultaneously presented on two panels. Color-coded SWE maps were displayed on the box in real time by using a calculated elasticity value in kilopascal (kPa). We waited 10 seconds for each measurement to achieve complete mapping of the elasticity values within the SWE display window. After selecting a temporally stable static image containing the fewest artifacts, we placed a circular region of interest (ROI) having a fixed diameter of 3 mm in the target area. Within the ROI, the software generated three indices, namely the mean, maximum, and minimum shear modulus, and their standard deviations in kilopascals. We selected the mean values of the shear modulus (meanSM) for the SWE measurements. SWE was performed 10 times on each target to reduce the intraobserver variability in the phantom and the *in vivo* study. A measurement was defined as a failure when no signal was obtained in the SWE box, in spite of the fact that the SWE mode was changed to penetration. SWE measurements with a value of <0.1 were considered unreliable.

Phantom Study

An experimental in-house setup based on a tissue-mimicking phantom was used for characterizing the effect of the anisotropy and the stretch stress of the background tissue on the SWE measurements before the *in vivo* study. We chose a piece of porcine sirloin with muscle fibers running parallel to each other in one direction, and cut the muscle into a rectangular block (width, 10 cm; height, 5 cm; depth, 4 cm), which was used as a tissue phantom. The tissue phantom was submerged in a water tank for the US examination (Fig. 1). In order to minimize stress, the US probe

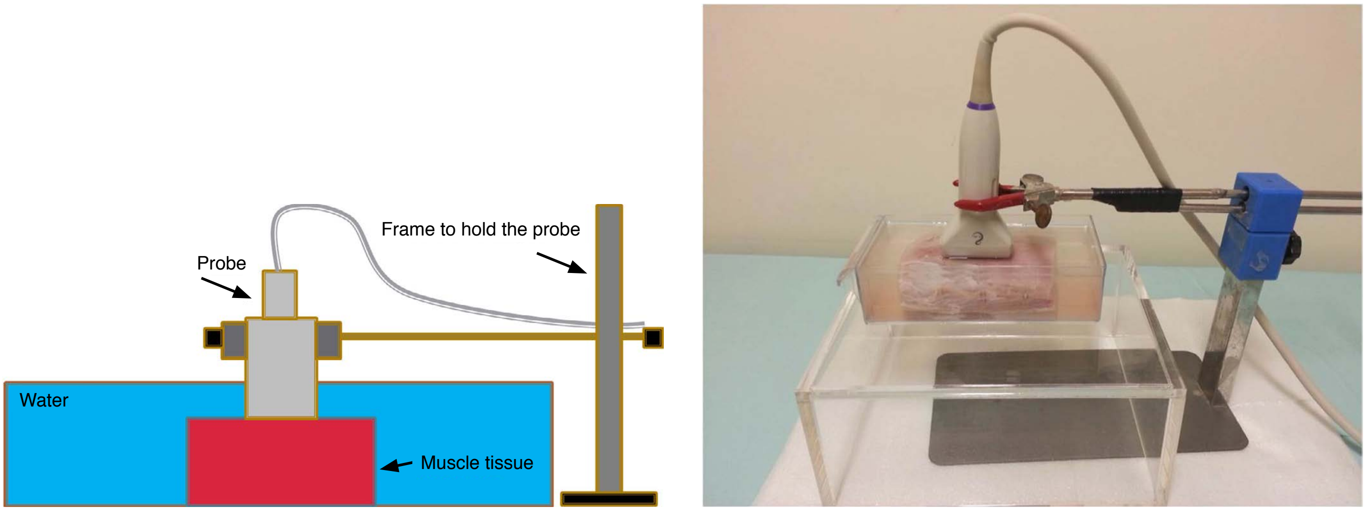

Fig. 1. Equipment used for the phantom study.
A, B. In the schematic drawing (A) and photograph (B) of the equipment used, a pig muscle phantom is submerged in the water tank. An ultrasound probe is anchored to a fixed support and placed such that it does not compress the surface of the phantom. Muscle fiber orientations were determined by gray-scale ultrasonography (US) imaging. Strain stress was applied to the tissue phantom by resting the US probe on its surface.

anchored on a fixed support was placed in such a way that it did not compress the surface of the phantom; then, it was rotated to examine the effects of tissue anisotropy. Strain stress was applied to represent the static stretch effect in the *in vivo* SCM muscle. To apply uniform strain stress, the muscle tissue was compressed using the weight of the probe.

First, we performed SWE at six different sites to find an optimal measurement depth with reliable meanSM values using different depths; the distance between the probe and the tissue of interest was varied between 0.5 cm and 3 cm with increments of 0.5 cm. Measurements were repeated 10 times at each depth, and the results were compared. A total of four sets of SWE measurements were performed in parallel and in perpendicular to the muscle fiber orientation in the presence and the absence of the strain stress at the same depth as that determined in the preceding experiment (Fig. 2). These sets of measurements were repeated 10 times.

In Vivo Study

Six young healthy volunteers (3 males and 3 females; age, 31–35 years) were recruited for this study. All subjects were informed of the nature of the study, and signed informed consent was obtained from them. The study protocol was approved by our institutional review board. We selected a reactive lymph node by using the following criteria: a cervical level III location, lying behind the SCM muscle, and an axial diameter of >3 mm in a transverse sonogram. With a subject in the supine position with head resting on a pillow in the neutral position without static stretch stress, we performed the SWE as described above to obtain the meanSM values in parallel or in perpendicular to the SCM muscle orientation without the stretch stress. The subject was then positioned with a pillow under his neck with the head rotated to the contralateral side as much as possible in order to apply the static stretch stress. Measurements were repeated for the parallel and perpendicular SCM muscle orientations (Fig. 3).

Statistical Analysis

The Kolmogorov-Smirnov test was performed to determine whether the measured values from the phantom and *in vivo* studies were normally distributed. According to the results of the Kolmogorov-Smirnov test, analysis of variance (ANOVA) was performed to assess the effect of the depth variation on the meanSM values as determined by SWE. Tukey's honestly significant difference test was used for the *post hoc* multiple comparisons. The mean values of meanSM were compared using a paired t test to evaluate the influence of the tissue anisotropy and the stretch stress of the background on the SWE measurements for the phantom and *in vivo* studies. All statistical analyses were performed using IBM SPSS ver. 19.0 (IBM Corp., Armonk, NY, USA). The P-values were considered significant when $P<0.05$.

Results

Phantom Study

All SWE measurements were performed using the penetration mode because of the poor signal gain at the deep portion of the phantom tissue. The shear modulus values measured at different depths are presented in Fig. 4. The meanSM values varied significantly with depth (P<0.001). On the *post hoc* multiple comparison test,

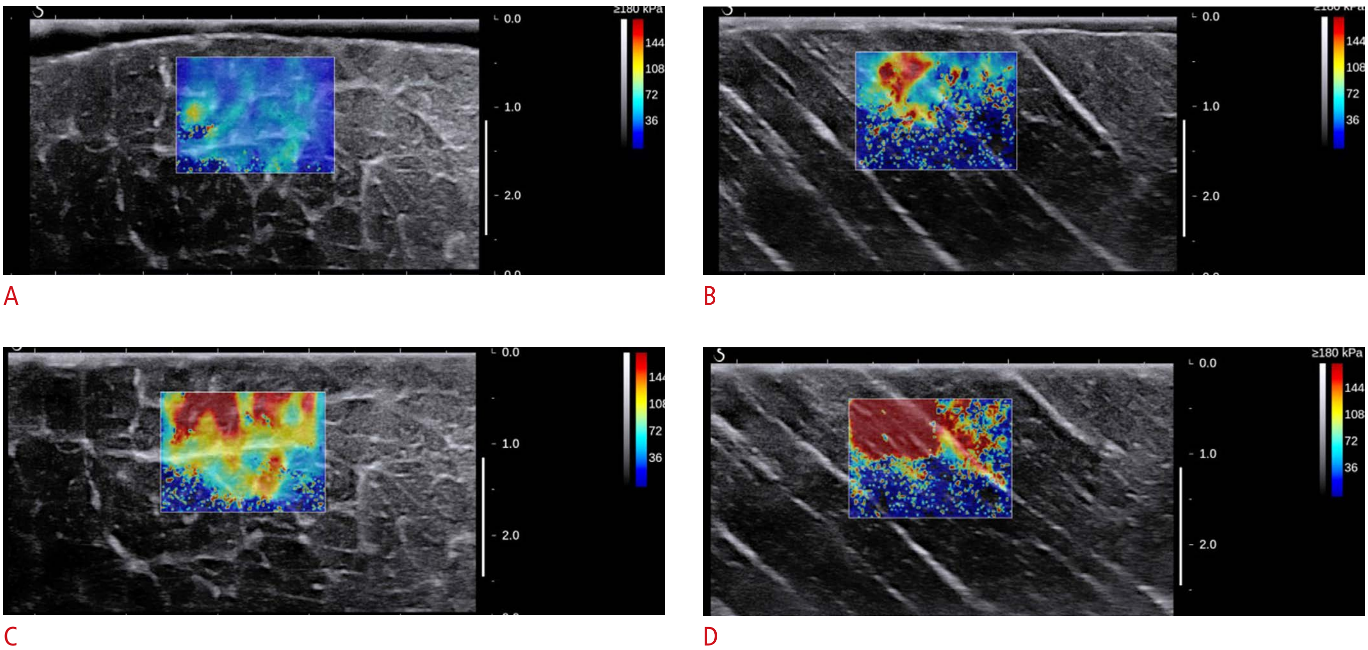

Fig. 2. Shear wave elastography images of a phantom study.
A, B. Tissue elasticity was measured in parallel (A) and perpendicular (B) to the fiber direction without strain stress. C, D. Tissue elasticity was measured in parallel (C) and perpendicular (D) to the fiber direction with strain stress.

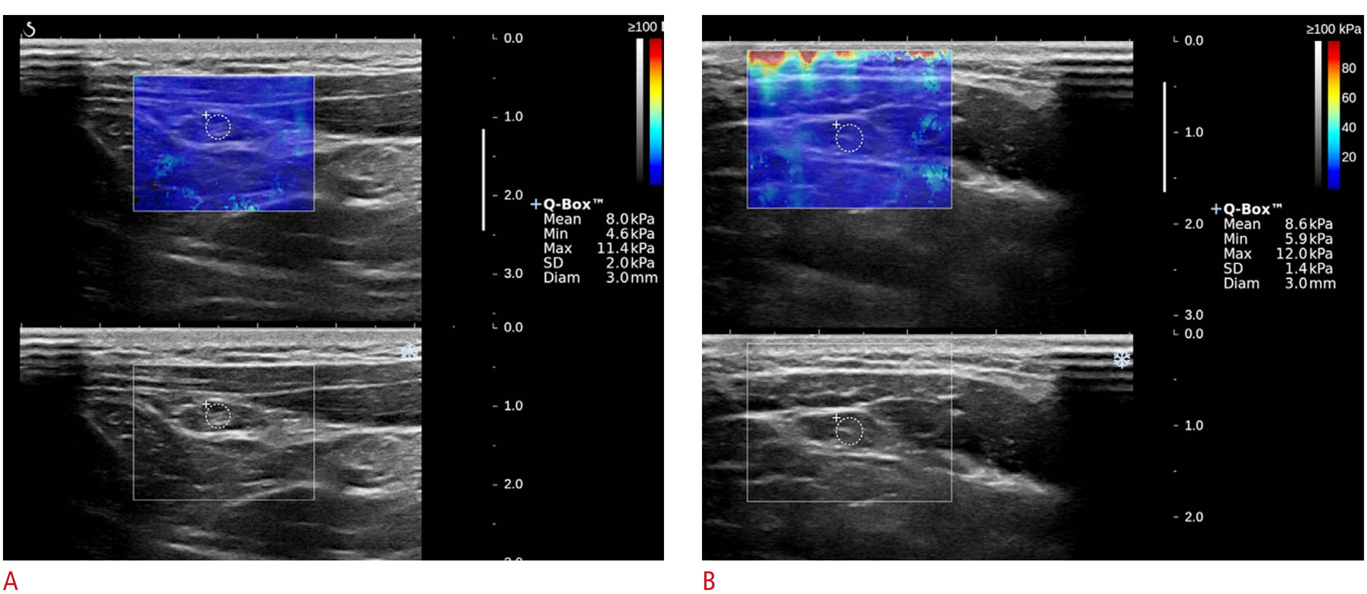

Fig. 3. Shear wave elastography (SWE) and corresponding gray-scale sonograms of a reactive lymph node on the left of cervical level III in subject 2.
A, B. During the measurement in parallel (A) and perpendicular (B) to the sternocleidomastoid muscle fiber direction, a circular region of interest having a diameter of 3 mm was placed at the center of the lymph node after applying the SWE mode.

the meanSM values were significantly different between depths (P<0.05), except for 1.5 cm and 2 cm (P=0.107). Below 1.5 cm from the surface, the meanSM values were markedly decreased and were non-reliable because of their marked variation. At a depth of 3 cm, the signals were slight or absent. Based on the results obtained, the depth for the SWE measurements in the subsequent experiment was set as 1 cm.

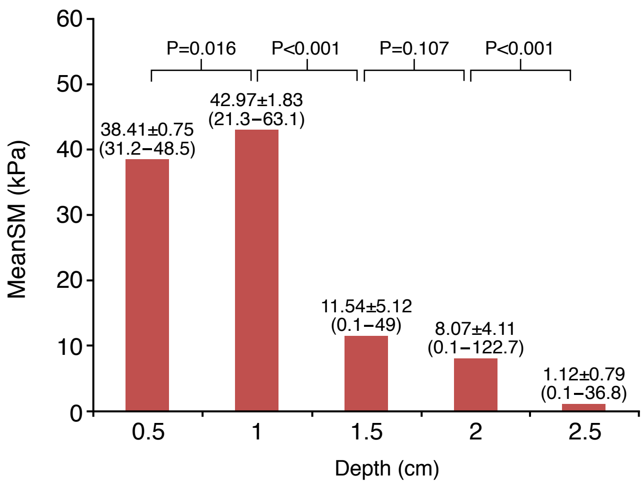

Fig. 4. **Shear moduli measurements at different depths in the phantom study.** Numbers above the bars denote mean values of the shear modulus (meanSM)±standard deviation with ranges in the parentheses. The P-values are the results of *post hoc* comparisons and were considered significant when P<0.05.

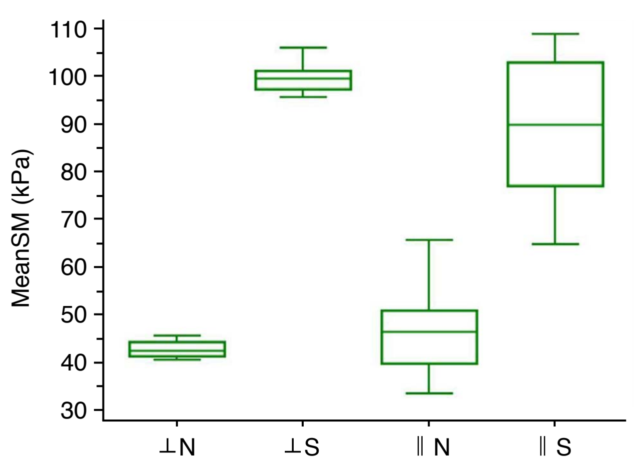

Fig. 5. **Box-and-whisker plots of mean values of the shear modulus (meanSM) values measured at different muscle fiber orientations with or without the strain stress in the phantom study.** The upper and lower lines of the boxes represent the values of the lower and upper quartiles of meanSM. The center lines denote the median meanSM values. Whiskers extend from the minimum to the maximum values. The strain stress significantly increases the measured meanSM irrespective of the fiber direction. ⊥, perpendicular axis; ∥, parallel axis; N, neutral condition; S, strain stress.

The strain stress significantly increased the meanSM values in directions parallel and perpendicular to the fiber orientation (89.23±15.36 vs. 47.54±10.06 in the parallel orientation, 99.94±3.47 vs. 42.87±1.75 kPa in the perpendicular orientation; P<0.001) (Fig. 5). The measured shear modulus values are presented in Table 1.

In Vivo Study

All SWE measurements were performed using the standard mode. A total of seven reactive lymph nodes meeting the criteria described above were selected at cervical level III in the six subjects. Lymph nodes close to the common carotid artery were not chosen in order to avoid the effects of arterial pulsation. The SWE measurements in the three subjects were averaged to minimize individual variations in muscle thickness and body mass index. The measured meanSM values are presented in Table 2.

Both anisotropy and static stretch stress significantly influenced the measured meanSM values (Fig. 6). The meanSM values obtained in parallel to the muscle fiber orientation were significantly greater

Table 1. Measured shear modulus values for two muscle fiber orientations with or without the application of strain stress-phantom study

Scan direction	Strain	Shear modulus (kPa)	
		Mean±SD	Range
Perpendicular	No	42.87±1.75	28.9–65.4
	Yes	99.94±3.47	61.1–138.1
Parallel	No	47.54±10.06	1.9–133.3
	Yes	89.23±15.36	0.1–297.2

Table 2. Combined shear modulus values of the volunteers for measurements conducted in parallel and in perpendicular to the muscle fiber orientation with or without the stretch stress–*in vivo* study

Scan direction	Strain	Shear modulus (kPa)	
		Mean±SD	Range
Perpendicular	No	10.99±4.43	0.1–48.8
	Yes	13.24±4.36	0.2–44.3
Parallel	No	17.49±5.93	0.8–39.8
	Yes	37.11±21.62	3.6–106.6

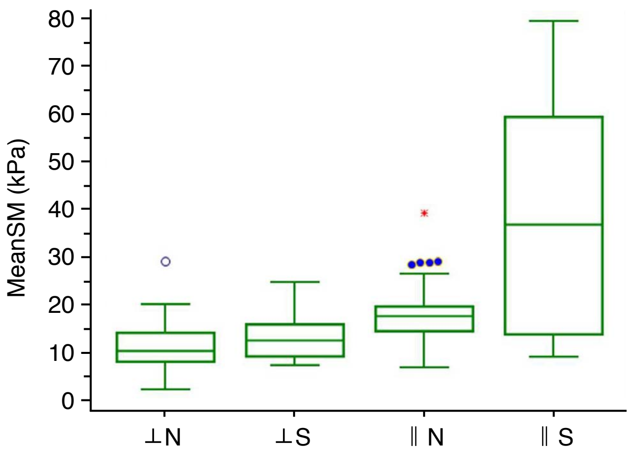

Fig. 6. Box-and-whisker plot of the mean values of the shear modulus (meanSM) measured in the seven lymph nodes from the six volunteers. The upper and lower lines of the boxes represent the lower to upper quartiles of meanSM. The center lines denote the median meanSM values. Whiskers extend from the minimum to the maximum values. The meanSM measured in parallel to the muscle fiber orientation axis is significantly greater than that measured in the perpendicular orientation, irrespective of the applied stress. ⊥, perpendicular axis; ∥, parallel axis; N, neutral condition; S, stretch stress.

than those obtained in perpendicular to the muscle fiber orientation, irrespective of the stretch stress (17.49±5.93 vs. 10.99±4.43 under the neutral condition, 37.11±21.62 vs. 13.24±4.34 with stretch stress; P<0.001). The measured meanSM values significantly increased with the static stretch stress when measured in parallel to the muscle fiber orientation, but not in the perpendicular orientation (37.11±21.62 vs. 17.49±5.93 in the parallel orientation, 13.24±4.34 vs. 10.99±4.43 in the perpendicular orientation; P<0.001, P=0.100).

Discussion

This study shows that elasticity measurements can be influenced by the biomechanical status of the background, such as by tissue anisotropy and static stretch stress. Prior to the *in vivo* study, the effects of biomechanical parameters, including tissue anisotropy and stretch stress, were investigated using a pig muscle phantom and both tissue anisotropy and static strain were found to significantly affect the measured meanSM values. In the *in vivo* study, the meanSM values of the cervical lymph nodes were significantly higher when the US probe was placed parallel to the SCM fiber orientation, but stretch stress had a significant effect on the measured meanSM values only in parallel to the fiber orientation. The present study is the first to demonstrate that biomechanical factors of the measurement environment influence the quality of SWE measurements of the cervical lymph nodes. This finding is important in terms of decreasing the intra- and inter-observer variability of *in vivo* measurements. In particular, this finding implies that to increase the reproducibility and reliability of SWE measurements, it is important to minimize the effects of these factors on the quality of SWE.

Several reports have been issued on the effects of tissue anisotropy on the mechanical properties of biological media, including muscles, tendons, and kidneys [9–18]. In muscle tissues, which have high levels of transverse anisotropy, the speed of a shear wave is higher in a direction parallel to the fiber orientation, which, in turn, results in a higher shear modulus [15]. On the other hand, when the shear propagates perpendicularly to the fiber orientation, in the presence of multiple intervening tissue interfaces, its propagation speed is lower, and thus, the shear modulus is reduced. In the present study, the shear moduli of *in vivo* cervical lymph nodes measured in parallel to the SCM fiber orientation was greater than those measured in the perpendicular direction; this implies that the direction of US scanning during an SWE measurement in cervical lymph nodes should always be taken into consideration.

In a previous *in vivo* study, both contraction and passive extension increased the shear wave velocity of the biceps muscle along the fibers more significantly than perpendicularly to the fibers [15]. The stretching effects have been reported in *in vivo* gastrocnemius by using SWE [18]. In the present study, the meanSM values measured in reactive cervical lymph nodes significantly increased with the static stretch of the SCM muscle along the fiber orientation. Similarly, compression of the tissue phantom, which was conducted to represent passive muscle stretching, increased the meanSM values. The application of strain or stretch stress increases tissue hardness and the shear modulus [16,18]. Similar to the previous *in vivo* studies, the present study demonstrated that the stretching stress influences elasticity, predominantly along the fibers [15]. A muscle is made up of hundreds of aligning fibers, and there are no specific connections transversally between fibers [19]. The changes in the muscle hardness during stretching can be mainly attributed to the longitudinal connections between fibers. In other words, the anisotropic property influences the effect of muscle stretching on elasticity. In addition, the meanSM values showed a marked dispersion when static stretch was applied and measured in parallel to the fiber direction. On the other hand, the applied stress could have been the main factor in a non-viable muscle, which is relatively soft and easily compressed. Recently, the results of the SWE evaluations of cervical lymph nodes for the differentiation of metastasis were published [9,10]. According to these studies, the shear moduli of metastatic cervical lymph nodes were higher than those of benign lymph nodes, which implies that metastatic nodes are stiffer. The present study shows that biomechanical properties, such as tissue anisotropy and muscle stretch, can variably influence SWE measurements and cause false-positive results. Therefore, when performing SWE examinations in the cervical region, the direction of any anisotropic structure, such as, the SCM muscle, in the vicinity of the target should be determined, and SWE should be performed perpendicular to the muscle fiber orientation in order to standardize the SWE results.

This study has several limitations. First, the methods used for generating the static stretch in the phantom and *in vivo* studies differed. Further, the shear modulus is affected by the presence of fractures or cracks in the perpendicular direction [20]. Next, strain and stretch strain have been known to cause similar biomechanical changes including muscle thinning and increased density of muscle fibers according to the literature [15,16,18]. Therefore, we can assume that strain can be applied to cause effects similar to those of stretch stress: increasing fiber density and consequently decreasing "cracks" between the fibers. Second, the results of the phantom study showed a remarkable dispersion and the measured meanSM values tended to be higher in the phantom tissue than in the *in vivo* lymph nodes. We assumed that the presence of a dense fascial interface between relatively soft muscle bundles within the ROIs resulted in a wide variation of the repeatedly measured shear moduli, particularly in the parallel direction. The fascial interface could result in a marked attenuation of the sonic beam penetration or accelerate the shear wave transmission according to its direction or plane. Gravity might have an effect particularly in the non-viable, less-elastic phantom tissue given the higher meanSM values in the phantom tissue than in the *in vivo* lymph nodes. These should be considered in future studies on SWE using a muscle phantom. Third, potential confounders that might have influenced the SWE measurements, such as arterial pulsation, were not evaluated, and as mentioned above, this may have caused the difference between the results of the phantom and *in vivo* studies with respect to the effects of the stretch stress. This issue requires further investigation. Lastly, we did not apply strain in a quantitative manner in the phantom study. However, given that the purpose of this study was to evaluate the variations in the measured SWE values caused by the target environment, this is unlikely to have affected our results.

In conclusion, this experimental study documents the effect of the anisotropy and the stretch stress of the cervical musculature on the SWE results of the cervical lymph nodes. The study shows that the anisotropic nature and the static stretch of the SCM muscle are responsible for the SWE measurements, and indicate that both sources of anisotropy and stretch stress need to be identified and considered before applying SWE and before interpreting the measured shear modulus values.

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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