Journal of Visualized Experiments

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--Manuscript Draft--

Article Type:	Invited Methods Collection - JoVE Produced Video
Manuscript Number:	JoVE62900R2
Full Title:	Nerve Ultrasound Protocol to Detect Dysimmune Neuropathies
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TITLE:

Nerve Ultrasound Protocol to Detect Dysimmune Neuropathies

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SUMMARY:

This article presents a protocol for nerve ultrasound in polyneuropathies to aid the diagnosis of inflammatory neuropathies.

ABSTRACT:

Nerve ultrasound is increasingly used in the differential diagnosis of polyneuropathy as a complementary tool to nerve conduction studies. Morphological alterations of the peripheral nerves, such as increasing the cross-sectional area (CSA), have been described in various immune-mediated polyneuropathies. The most prominent morphological changes in nerve ultrasound have been described for the chronic inflammatory demyelinating polyneuropathy (CIDP)-spectrum disease. CIDP may be distinguished from hereditary and other polyneuropathies by measuring the extent and pattern of nerve swellings (CSA increase). Typical findings in demyelinating inflammatory neuropathies are multifocal nerve swellings with inhomogeneous fascicular structure, while CSA increase in demyelinating hereditary neuropathies occurs in a more generalized and homogenous manner. In other non-inflammatory axonal neuropathies, nerves can appear with normal or slight CSA increases, especially in typical entrapment sites. This article presents technical requirements for nerve ultrasound, an examination procedure using a standardized examination protocol, current reference values for the CSA, and typical sonographic pathological findings in patients with inflammatory neuropathies.

INTRODUCTION:

Next to clinical examination, evaluating any large-fiber polyneuropathy includes an electrophysiological examination to characterize the motor or sensory system's involvement and differentiate axonal from demyelinating damage[1]. In axonal polyneuropathy, toxic and diabetic neuropathy are the leading causes, while in demyelinating polyneuropathies, hereditary or inflammatory neuropathies such as CIDP should be considered[2–4]. Commonly used diagnostic criteria for CIDP are the European Federation of Neurological Societies/Peripheral Nerve Society

(EFNS/PNS) criteria established in 2005 and revised in 2010 and 2021[5]. These define clinical and electrophysiological criteria to diagnose CIDP and describe additional criteria such as nerve biopsy to detect demyelination or inflammation. However, in some cases, despite a thorough diagnostic workup, the cause of neuropathy remains ambiguous. In these cases, nerve ultrasound offers a complementary method to examine the nerves not functionally but morphologically[6]. Several studies proved the use of nerve ultrasound as an additional tool in diagnosing CIDP, so that the 2021 revised EFNS/PNS criteria implemented nerve ultrasound in the guideline[5]. The advantage of nerve ultrasound compared to other imaging methods such as magnet resonance neurography (MRN) is that it can be used directly by the treating neurologists as a bedside tool; it is relatively cost-efficient. It can be used repeatedly, as it is noninvasive and not painful.

Typical characteristics of CIDP observed in nerve ultrasound are cross-sectional-area (CSA) increase enlarged nerves[7,8], also found in hereditary polyneuropathies. In CIDP, this affects individual nerve segments heterogeneously[7,9].

A variety of examination protocols have been published[10–15] trying to clarify normal CSA values and determine the adequate anatomical positions of ultrasound examination. Some of these positions are similar in most examination protocols. However, a widely accepted protocol to standardize the examination process and simplify the interpretation of the measurements does not exist.

This article demonstrates the nerve ultrasound examination using a standardized protocol for polyneuropathies, presents various reference values for the CSA, and shows typical pathological findings in patients with inflammatory neuropathies.

Technical requirements for nerve ultrasound

The neuromuscular ultrasound is performed in B-mode (Brightness mode, two-dimensional image with gray levels) using the compound imaging of the corresponding sonographic device[6,16]. Compound imaging enables electronic control of the piezoelectric elements in the sonic probe (transducer) to illuminate the target structure from different angles[17]. The ultrasound waves are reflected in several directions due to the histological structure of the peripheral nerves. As a result of the sound coming from different angles, a more significant part of the otherwise lost reflections gets back to the sound probe (receiver) and can generate images. For neuromuscular ultrasound, a high-resolution ultrasound probe with 18 MHz linear array transducer, for deeper nerves, an additional 12 MHz linear array probe (e.g., to display tibial and fibular nerve in the popliteal fossa) is used[6,16]. Transducers with lower frequencies result in reduced spatial and lateral resolution so that the differentiation of the nerve boundaries from the surrounding structures is less precise. The optimal settings can be kept constant using a preset for neuromuscular imaging provided by the manufacturer. During the examination, the image depth and the focus position must be adjusted to the structure to be examined and constantly adapted to the position of the nerve. The B-image gain and the depth-dependent gain can be adjusted for image optimization with uniform brightness. Blood vessels are often close to neural structures and are often used as landmarks to make the measurements at the same position. To depict their anatomical interaction and distinguish between nerves and vessels, it is also necessary to display

the flow velocity and direction using pulsed Doppler and color-coded duplex sonography[16,18]. The pulse repetition frequency must be adapted to the expected low flow velocities in the primarily venous blood vessels of the extremities, or the power Doppler must be selected for color-coding[16].

Nerves reflect the ultrasound waves differently from different angles of incidence so that the sonographic image varies in echogenicity (anisotropy)[16,19]. The best image is achieved from an orthograde angle since the ultrasonic waves are reflected most strongly by the nerves in this angle. For avoiding artificial anisotropy or nerve deformity, the probe must therefore be held in a neutral position during the examination without applying additional pressure perpendicular to the nerves (**Figure 1**). The cross-sectional area (CSA) is measured within the thin, hyperechoic epineurium (**Figure 2**) to avoid alterations of the epinerval tissue in the measurement[19]. More details on technical ultrasound can be found in References[6, 16–21].

PROTOCOL:

All examinations for this work were performed in compliance with institutional guidelines of the Ruhr-University Bochum, Germany.

1. Experimental preparations

1.1. Patient preparation

1.1.1. Check the patient inclusion criteria: examine adult patients diagnosed with polyneuropathy, suspicious of inflammatory origin.

1.1.2. Check the patient exclusion criteria: do not examine patients with open wounds or infections in the regions to be examined.

1.2. Instrumental checkpoints

1.2.1. Check the integrity of the ultrasound machine and all the materials used (see **Table of Materials**).

1.2.2. Enter patient name and details in the ultrasound machine before starting the ultrasound examination (depending on the machine).

1.2.3. Choose an appropriate ultrasound probe (preferred 14–18 MHz) (see **Table of Materials**) and preset for the neuromuscular ultrasound.

1.2.4. During the whole examination, adjust the depth and focus on obtaining optimal image quality.

1.2.5. Whenever possible, examine the complete course of each nerve in a cross-sectional view.

NOTE: The nerves recommended for examination are: median, ulnar, radial nerve, cervical roots, brachial plexus, and vagal nerve, as well as tibial, fibular, and sural nerve (**Figure 3**). The examination of each of these nerves is shown in the next section and the video. The entire ultrasound examination according to the following protocol will take ~30–45 min.

2. Ultrasound examination

2.1. Start examining the arm nerves with the patient sitting in a neutral position with the arm resting supinated on a surface, e.g., the leg.

2.2. Place some ultrasound gel over the transducer probe, the wrist, the forearm, the elbow, and the upper arm.

2.3. For examination of the median nerve, start by performing a transverse scan at the wrist level.

2.4. Move proximally to follow the anatomical course of the median nerve to the upper arm.

2.5. Measure the CSA of the median nerve at the following sites: at the entrance to the carpal tunnel (retinaculum flexorum); at the forearm (10–15 cm proximal to retinaculum flexorum; at the elbow (crook of the elbow); at the upper arm next to the brachial artery (at the middle of the distance between the medial epicondyle and axillary fossa).

2.6. For examination of the ulnar nerve, start by performing a transverse scan at the level of the wrist ulnar to the median nerve.

2.7. Move proximally to follow the anatomical course of the ulnar nerve along the sulcus to the upper arm.

NOTE: Moving toward the upper arm, let the patient raise the arm bent at the elbow to examine the sulcus and upper arm.

2.8. Measure the CSA of the ulnar nerve at the following sites: at the entrance to Guyon's canal; at the forearm (10–15 cm proximal to Guyon's canal); at the elbow (between the medial epicondyle and olecranon); at the upper arm (at the middle of the distance between the medial epicondyle and axillary fossa).

2.9. To examine the radial nerve, let the patient hold the arm in front of the stomach bent in the elbow and scan the radial nerve directly next to the humerus.

2.10. Use color doppler mode to avoid confusion with the accompanying artery and vein.

NOTE: Color doppler mode shows blood flow in arteria profunda brachii, and low flow in the corresponding vein, while no flow occurs in the radial nerve. Additionally, the vein can be

compressed by exerting external pressure, and the nerve cannot.

2.11. Measure the CSA of the radial nerve at the following site: radial nerve in the spiral groove.

2.12. Carry on with the examination of the vagal nerve, cervical nerve roots, and the brachial plexus.

2.13. Place ultrasound gel at the middle of the neck.

2.14. To examine the vagal nerve, perform a transverse scan at the middle of the neck and find the carotid artery.

NOTE: The vagal nerve can be found directly next to the carotid artery and jugular vein.

2.15. Measure the CSA of the vagal nerve at the following site: at carotid sheath at the level of the carotid bifurcation.

2.16. For the examination of cervical nerve roots, C5, C6, C7 move the probe dorsal and a little up and down.

NOTE: The cervical nerve roots appear between the anterior and posterior tubercle of the transverse process. C7 can be recognized by the absence of the anterior tubercle from its transverse process, while both anterior and posterior tubercles are found with the other cervical nerve roots.

2.17. Measure either the CSA or the diameter of the cervical nerve roots at the most proximal location possible, where the nerve root exits over the transverse process: C5; C6; C7.

2.18. To examine the brachial plexus, follow the anatomical course of the cervical nerve roots distally and find them perform trunks and cords.

2.19. Measure the CSA of the plexus at the following sites: Intrascalene space (between anterior and medial scalene muscle); Supraclavicular space (next to A. subclavia).

2.20. Carry on with the examination of the leg nerves.

2.21. Let the patient lie down to one side with the legs slightly bent. Place some ultrasound gel over the transducer probe, the popliteal fossa, the fibula, the malleolus, and the lower leg.

2.22. For examination of the fibular nerve, feel the fibular head, place the transducer directly behind it, and then follow the course of the nerve to the popliteal fossa.

2.23. Measure the CSA of the fibular nerve at the following sites: at the beginning of the fibular head; in the popliteal fossa.

2.24. To examine the tibial nerve in the popliteal fossa, find the fibular nerve and the popliteal artery in the popliteal fossa.

NOTE: The tibial nerve can be found just above the popliteal artery in most cases.

2.25. Measure the CSA of the tibial nerve at the following site: in the popliteal fossa.

2.26. For examination of the tibial nerve at the ankle, place the probe directly behind the medial malleolus.

NOTE: The tibial nerve can be found just next to the posterior tibial artery in most cases.

2.27. Measure the CSA of the tibial nerve at the following site: at the level of the medial ankle.

2.28. For examination of the sural nerve, place the probe at the lateral ankle.

NOTE: The sural nerve can be found next to a superficial vein in most cases.

2.29. Follow the anatomical course of the sural nerve proximally to the lower leg.

2.30. Measure the CSA of the sural nerve at the following site: between the lateral and medial head of the gastrocnemius muscle.

2.31. Perform all measurements on both sides.

2.32. Save the results of all the measurements (depending on the ultrasound machine) and end the examination.

NOTE: **Figure 3** gives an overview of all measuring sites for CSA.

REPRESENTATIVE RESULTS:

Each ultrasound laboratory should establish its CSA reference values by collecting data from the healthy local population, as specific ultrasound machines and examiner or population-dependent variables can lead to slightly different results in each laboratory. However, to indicate which CSA values can be considered normal, data from two leading German nerve ultrasound groups and a recent meta-analysis of all published reference values so far[13–15,22,23] are summarized in **Table 1**. Reference values for patients studied under this protocol in our department are those by Kerasnoudis et al.[22] (**Table 1**).

Typical findings in demyelinating inflammatory neuropathies are multifocal nerve swellings with inhomogeneous fascicles, while nerve swellings in demyelinating hereditary neuropathies occur more generalized and homogenous[12,24]. The histologic correlate of increased CSA is assumed to be acute inflammation and repeated de- and remyelination; however, this remains to be

investigated[7]. In other non-inflammatory axonal neuropathies, nerves can appear normal or slightly increased in size, especially in typical entrapment sites[25–28].

To simplify the interpretation of the results, the adjusted Bochum Ultrasound Score is suggested as a scoring system, which helps to distinguish chronic inflammatory neuropathies such as CIDP from non-inflammatory neuropathies.

The adjusted Bochum Ultrasound Score is calculated from the number of sites with significantly enlarged CSA of six of the above-described measurement sites: median nerve at the forearm, the median nerve at the upper arm, ulnar nerve at the forearm, ulnar nerve at the upper arm, the radial nerve at the upper arm and sural nerve at the calf. Examination of only these six sites will take ~15 min. Each of these six sites is scored with 1 point if the nerve shows pathological CSA enlargement on one or both sides of the body. Thus, the minimum score is 0 points, and the maximum score is 6 points. With this scoring system, if ≥2 points are assigned, the diagnosis of CIDP is possible with a sensitivity of ~53% and a specificity of ~83%, even if additional axonal damage in nerve conduction studies results in difficult detection by electrophysiological criteria.

Different groups have proposed other scoring systems to differentiate between neuropathies[10,11,18,29,30]. None of these scores is widely used. The adjusted Bochum Ultrasound Score is based on earlier publications which describe the Bochum Ultrasound Score[10] derived from four measurement sites to distinguish CIDP from Guillain-Barré Syndrome and the Nerve ultrasound protocol[30] derived from nine measurement sites to differentiate CIDP from MMN, MADSAM and vasculitic or paraproteinemic neuropathy. These different scores should be used according to the exact question. The adjusted Bochum Ultrasound Score was developed to diagnose CIDP if nerve conduction studies show possible CIDP defined by electrophysiological EFNS/PNS criteria[5].

However, even if the adjusted Bochum Ultrasound Score only uses six nerve sites for calculation, still all other described nerve sites and the whole course of each nerve should be examined to detect focal lesions[31] or exclude homogenous enlargement. In the case of homogenous nerve enlargement, hereditary neuropathy should be considered[24]. Scoring systems for homogeneity and alterations of the fascicular structure were described before and may aid in evaluating homogeneity[8,24,32].

For ultrasound images of a healthy person, see **Figure 4**; for example, images from a CIDP patient, see **Figure 5**.

FIGURE AND TABLE LEGENDS:

Figure 1: Examination of the median nerve at the wrist. To avoid artificial anisotropy or nerve deformity, the probe must be held in a neutral position during the examination without applying additional pressure perpendicular to the nerves.

Figure 2: Measurement of the cross-sectional area (CSA). The cross-sectional area is measured

within the thin, hyperechoic epineurium.

Figure 3: Overview of measuring sites for CSA. Blue stars – median nerve, green stars – ulnar nerve, red star – radial nerve, pink star – vagal nerve, yellow stars – cervical roots and brachial plexus, white stars – fibular nerve, purple stars – tibial nerve, brown star – sural nerve.

Figure 4: Example images of a healthy person of the six nerve sites used in adjusted Bochum ultrasound score. A – median nerve at the forearm, B – median nerve at the upper arm, C – radial nerve at the upper arm, D – ulnar nerve at the forearm, E – ulnar nerve at the upper arm, F – sural nerve at the calf.

Figure 5: Example images of a patient with CIDP of the six nerve sites used in adjusted Bochum ultrasound score. A – median nerve at the forearm, B – median nerve at the upper arm, C – radial nerve at the upper arm, D – ulnar nerve at the forearm, E – ulnar nerve at the upper arm, F – sural nerve at the calf.

Table 1: Reference CSA values for patients. Proposed reference values are based on the publication of Kerasnoudis et al.[22], Grimm et al.[23,] and a recent meta-analysis by Fisse et al.[13–15]. Reference values for patients studied under this protocol in our department are those by Kerasnoudis et al.[22].

DISCUSSION:

Nerve ultrasound is a helpful additional diagnostic tool in polyneuropathies. It can give information on the possible causes of polyneuropathy depending on the extent and pattern of nerve enlargement. Moreover, CSA alterations in the longitudinal disease course of patients with CIDP were described to correlate to clinical disease course and treatment response[33–36].

Critical steps within the protocol

For obtaining reproducible results, consistent methodology and standardization of the examinations are essential[37,38]. Each examiner must consider deviations resulting from different ultrasound devices and local differences of demographics. To ensure the high quality and reproducibility of ultrasound measurements, specific training of the examiner is also necessary[13–15,21,39].

Modifications and troubleshooting of the technique

Typical findings in demyelinating inflammatory neuropathies are multifocal nerve swellings with inhomogeneous fascicles[33,40]. Therefore, the measurement of CSA of specific nerve sites is necessary, but the whole nerve must be scanned. Also, evaluation of fascicular structure and echogenicity can help in inconclusive cases, as not only CSA increase of the entire nerve but also intrafascicular swellings as well as hypoechoic and hyperechoic nerves are found in CIDP. Hypoechoic nerves are considered to result from acute edema, while hyperechoic nerve expressions result from remodeling[40,41].

Limitations of the technique

There are anatomical limitations for nerve ultrasound, i.e., examination of cervical nerve roots can be difficult to impossible in patients with obesity and short neck. Also, imaging of proximal nerve roots of the lower extremity nerves of the lumbosacral plexus is not possible due to the limited penetration depth of the ultrasonic rays. An alternative method, evaluating these nerves, is possible by MRN[42], but ultrasound is the more common method due to its spatial and temporal flexibility and cost-effective use[43].

Significance with respect to existing methods

Nerve ultrasound is recommended as an additional and complementary tool in diagnosing polyneuropathies to evaluate nerve morphology. Standard diagnostic workouts, including nerve conduction studies and other tools such as cerebrospinal fluid analysis, should still be performed.

Future applications of the technique

For experts in polyneuropathies, nerve ultrasound is of interest for diagnosis in clinical routine and as it can give insights into possible pathophysiologic aspects, i.e., represent inflammation. Therefore, nerve ultrasound is a promising method not only in clinical use but also in neuromuscular research. Also, with increasing progress in ultrasound technology, future ultrasound characteristics such as shear wave elastography or vascularization of peripheral nerves may add further aspects in assessing polyneuropathies.

ACKNOWLEDGMENTS:

We acknowledge the support from Ruhr-University Bochum for our research on neuromuscular ultrasound.

DISCLOSURES:

The authors declare no conflicts of interest related to this manuscript.

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Figure 1

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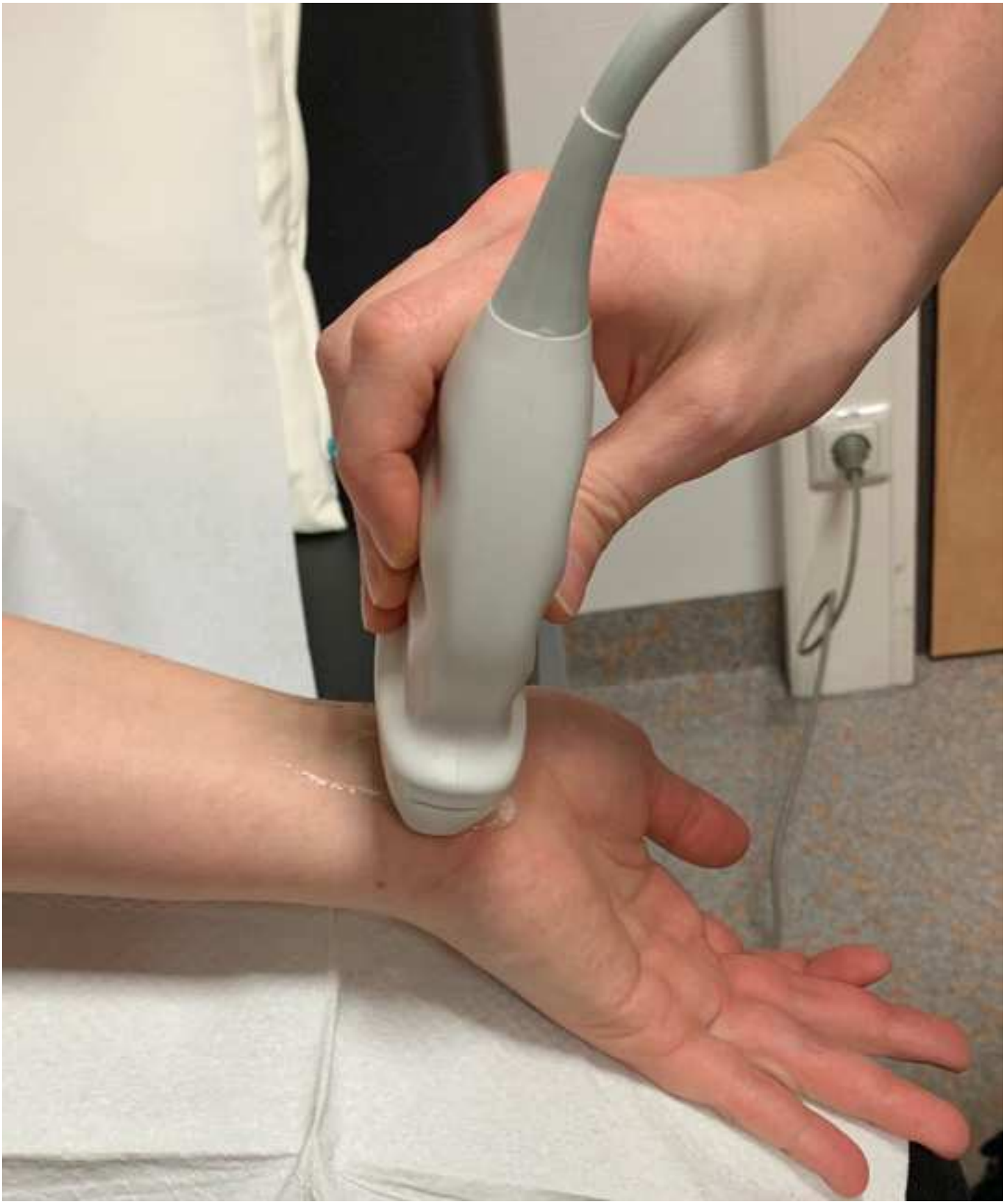

Figure 2

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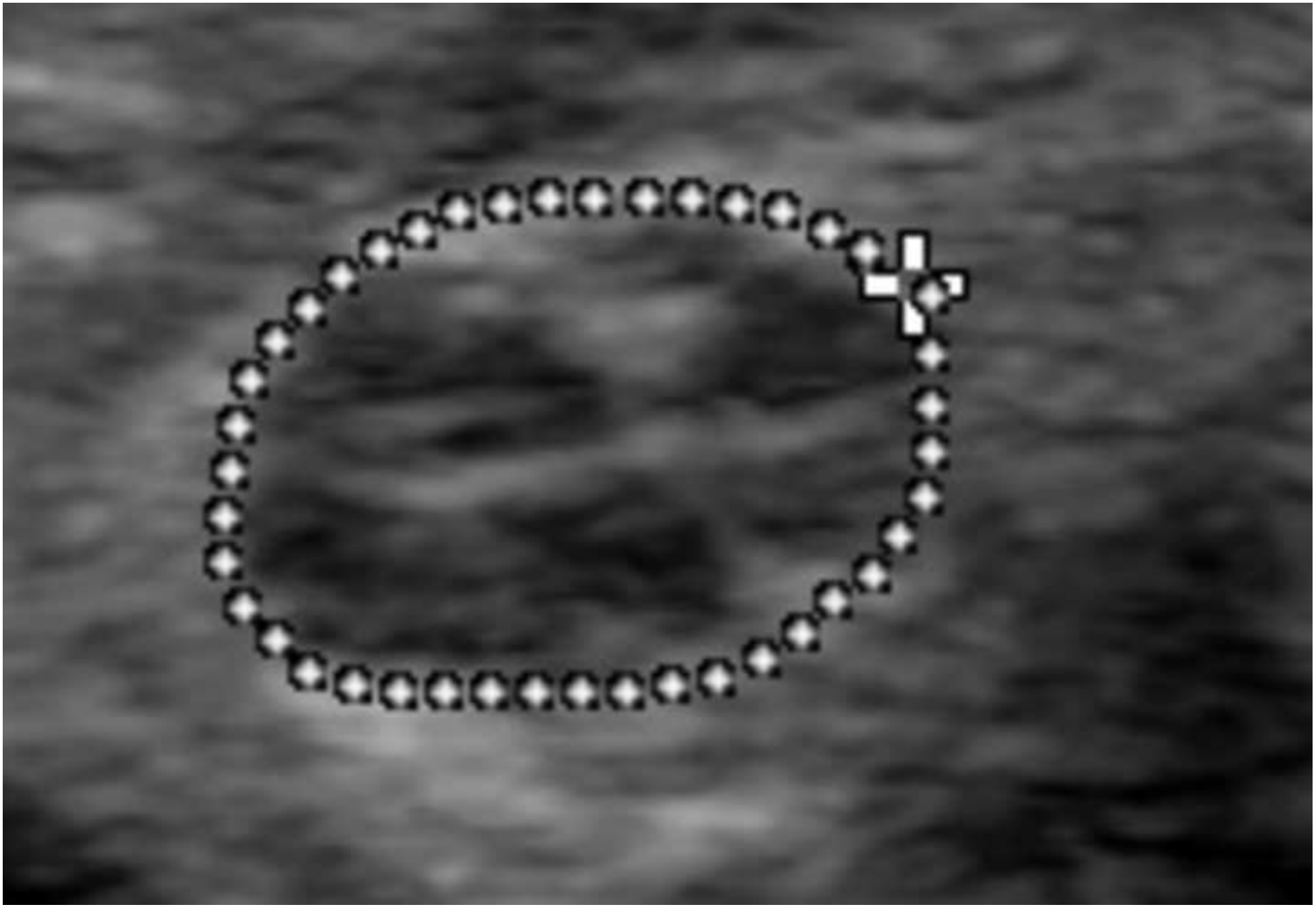

Figure 3

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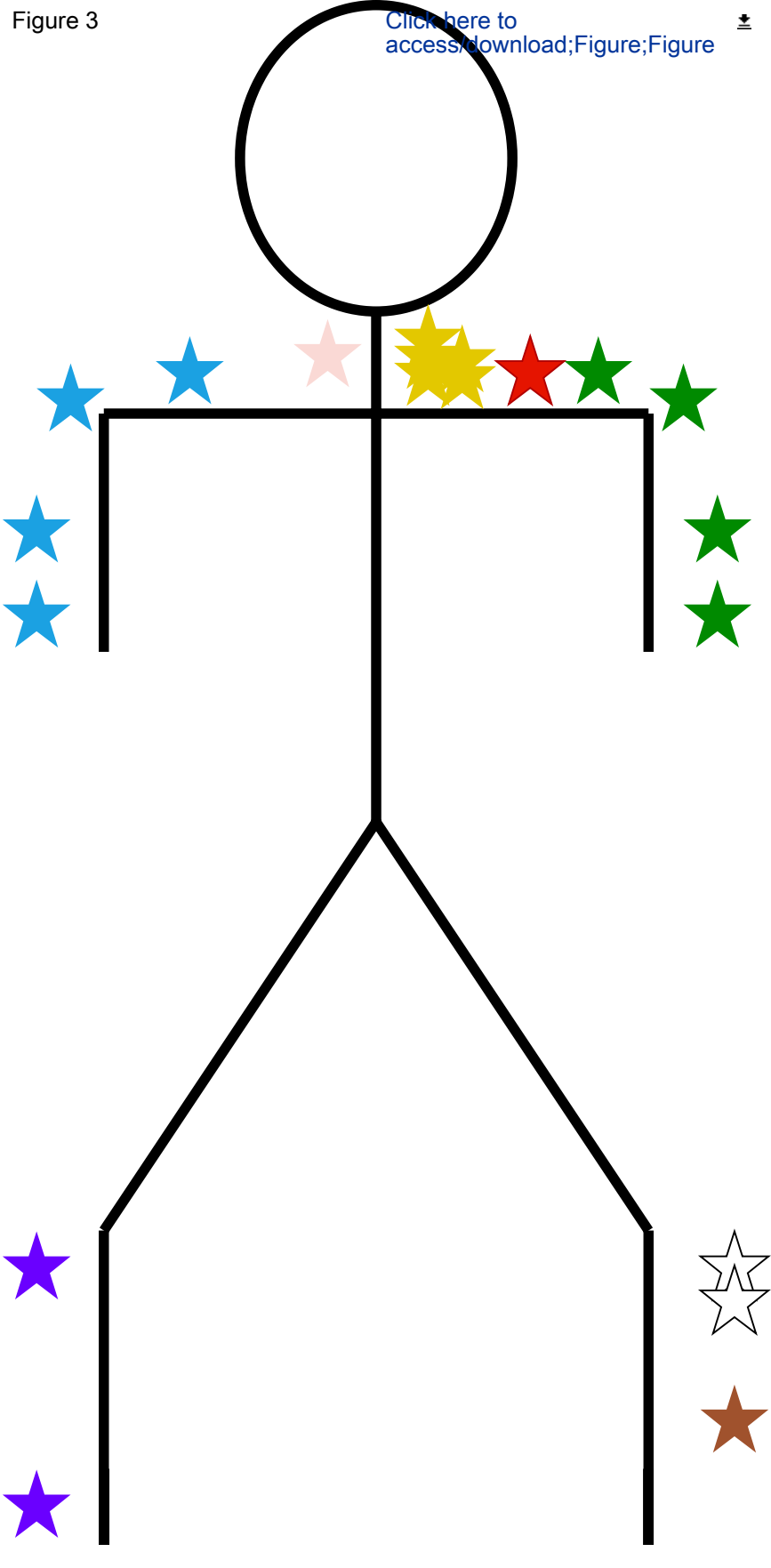

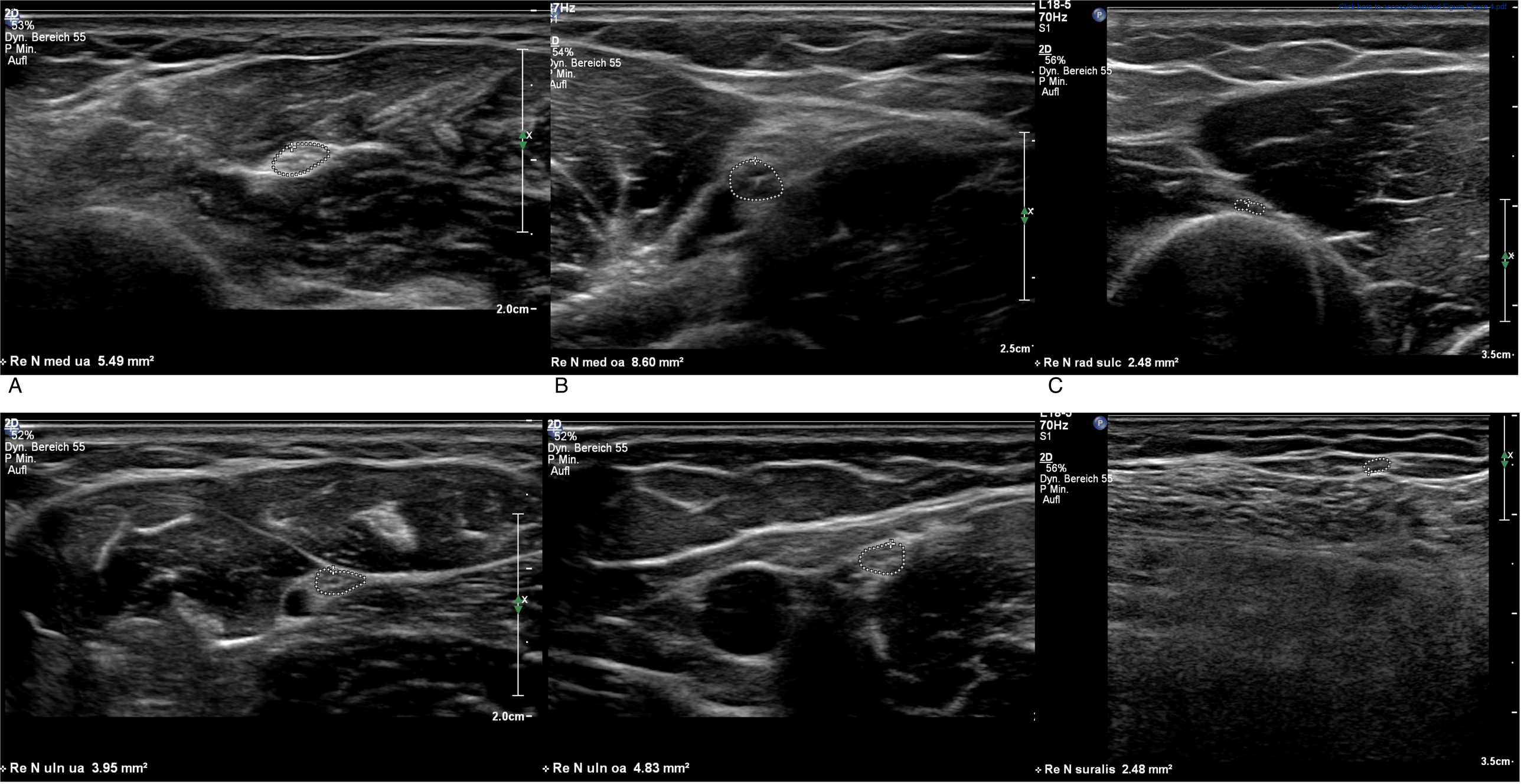
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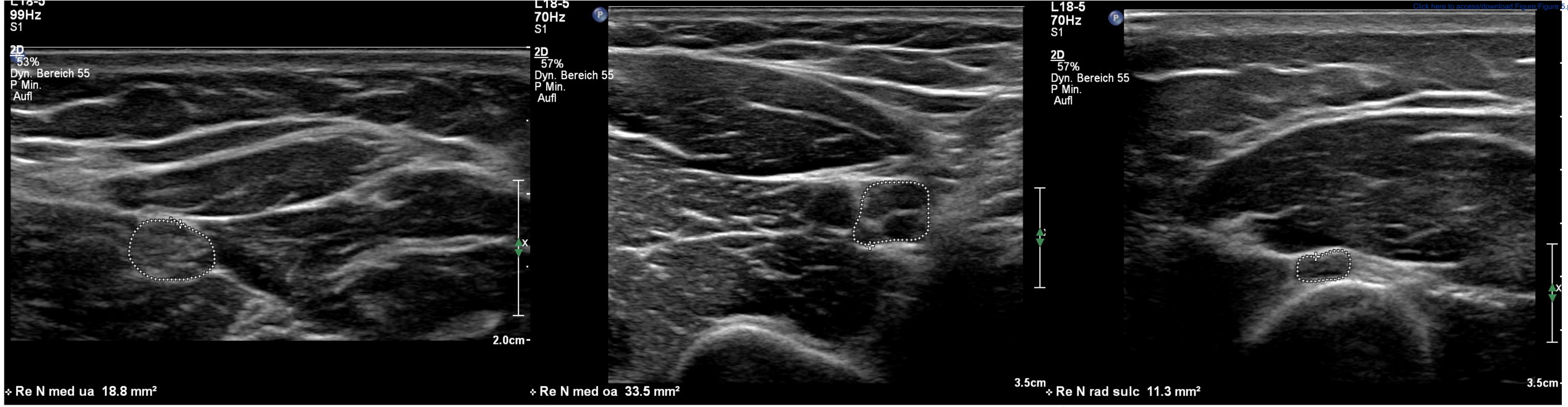

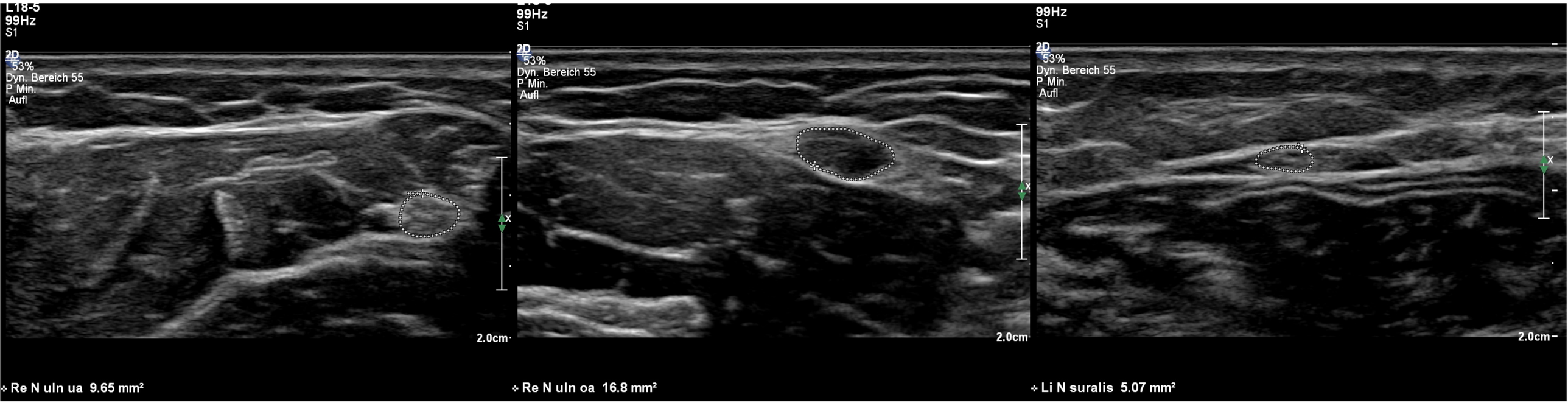

		Kerasnoudis et al.[22]		Grimm et al.[23]
Nerve	**Site**	**mean CSA (mm^2)**	**standard deviation**	**mean CSA (mm^2)**
Median nerve	Wrist	8.43	2.07	10.6
	Forearm	6.6	1.6	7.2
	Elbow	-	-	9.2
	Upper arm	8.4	2.87	9.1
Ulnar nerve	Guyon loge	5.16	1.03	-
	Forearm	5.46	1.26	5.9
	Elbow	5.33	1.4	8.7
	Upper arm	6.53	1.82	7
Radial nerve	Upper arm	3.26	1.52	-
Vagal nerve	Carotid sheath	-	-	2.2
C5		-	-	2.4*
C6		-	-	3.4*
C7		-	-	-
Brachial plexus	Intrascalene space	30.93	10.82	-
	Supraclavicular space	46.13	18.27	-
Fibular nerve	Fibula Head	7.1	2.3	-
	Popliteal fossa	8.60	1.77	8.4
Tibial nerve	Popliteal fossa	8.43	2.68	23.2
	Malleolus	6.36	1.45	10.2
Sural nerve	Heads of gastrocnemius muscle	1.82	0.64	2.2**

* Grimm et al.[23] measured diameter, not the CSA for C5 and C6 root.

** Grimm et al.[23] measured sural nerve at the lateral ankle.

Meta-Analysis by Fisse et al.[13-15]	
mean CSA (mm^2)	**95% CI (mm^2)**
8.3	7.9 - 8.7
6.4	5.9 - 6.9
-	-
8.3	7.5 - 9.0
4.1	3.6 - 4.6
5.2	4.8 - 5.7
5.9	5.4 - 6.5
6.6	5.1 - 6.1
5.1	4.0 - 6.2
2.2	1.5 – 2.9
5.6	4.6 – 6.7
8.8	7.4 – 10.3
9.5	8.0 – 10.9
-	-
-	-
8.4	6.8 – 9.9
7.9	6.6 – 9.2
25.9	17.5 – 34.4
10	7.7 – 12.4
2.4	1.7 – 3.1

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Editorial Office
JOVE

06.09.2021

Nerve ultrasound protocol to detect dysimmune neuropathies

Dear editors,

please find enclosed our revised manuscript entitled “Nerve ultrasound protocol to detect dysimmune neuropathies”. All comments are addressed in the manuscript file, as you suggested.

For further questions we remain at your disposal.

Yours sincerely,

Anna Lena Fisse

Point by point answer to the reviewers

Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

   Done.

2. Please revise the following lines to avoid previously published work: 133-139, 189-194.

   Done.

3. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

   Done.

4. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets or dashes.

   Done.

5. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step.

   Done.

6. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

   Done.

7. Please add more details to your protocol steps:
   Step 1: Please include any patient inclusion/exclusion criteria.
   Line 98: Please provide the ultrasound probe details in the Table of Materials.
   Line 103: Please provide the details of the ultrasound gel used in the Table of Materials.

   Done (see protocol no 1-2 and table of materials).

8. Please mention the duration for each ultrasound scan.

   Done (see protocol no 3).

9. Please include a one-line space between each protocol step and then highlight up to 3 pages of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Also, please ensure that it is in line with the title of the manuscript. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

   Done. Essential steps of the protocol for the video were highlighted in yellow.

10. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:
    a) Critical steps within the protocol
    b) Any modifications and troubleshooting of the technique

c) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique

The according sections were added in the discussion part.

11. Please provide reprint permissions for the reuse of the data for the Table.

    The table itself is not a reprint but was created for this article. Source of original data are cited. If, in addition, a permission for the use of the data is required, can the editorial office provide an appropriate template so that we can contact the authors of the cited data?

12. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al. Please include volume and issue numbers for all references.

    Done.

Reviewer #1:

Manuscript summary:
The manuscript by Fisse and colleagues is a methodological study about Nerve ultrasound protocol to detect morphological alterations of cross-sectional area in patients with inflammatory neuropathies. The main object of the study is to present technical requirements for nerve ultrasound, and a standardized examination protocol,
The paper itself is of interest and therefore potentially suitable for publication, provided it undergoes successful revision. The paper is adaequate in length.

Introduction:
Introduction is well written.
Please also include more recent references of CIDP and GBS

> We added some more recent references:
> - Lehmann, H.C., Wunderlich, G., Fink, G.R., Sommer, C. Diagnosis of peripheral neuropathy. Neurological Research and Practice. 2 (1), 20 (2020).
> - Shahrizaila, N., Lehmann, H.C., Kuwabara, S. Guillain-Barré syndrome. The Lancet. 397 (10280), 1214–1228 (2021).
> - Lehmann, H.C., Burke, D., Kuwabara, S. Chronic inflammatory demyelinating polyneuropathy: update on diagnosis, immunopathogenesis and treatment. Journal of neurology, neurosurgery, and psychiatry. 90 (9), 981–987 (2019).

Methods/Technical requreiments:
Methods are described sufficiently, but
„For neuromuscular ultrasound, a high-resolution ultrasound probe with 18 MHz linear array transducer, for deeper nerves, an additional 12 MHz linear array probe (e.g., to display tibial and fibular nerve in the popliteal fossa) is used" -> please indicate if this is mandatory i.e.what happens if you use ultrasound wih less than 18Mhz.

> Thank you for that suggestion. We added the following sentence to the technical requirements part:
> “Transducers with lower frequencies result in reduced spatial and lateral resolution, so that the differentiation of the nerve boundaries from surrounding structures is less precise.” (page 2)

„The "adjusted Bochum Ultrasound Score" is calculated from six of the above-described measurement sites, resulting from the number of sites with significantly enlarged CSA:"
The autors should indicate how long it takes to measure the nerves.

> We added to the manuscript: “Examination of only these six sites will take approx. 15 minutes.” (page 7)

Discussion:
Discusson is ok, but limitations of the methods should be more clearly indicated

> We added a subheading to the discussion part “Limitations of the technique” to be more specific. Also, the limitations were described more clearly: “There are anatomical limitations for nerve ultrasound i.e., examination of cervical nerve roots can be difficult to impossible in patients with obesity and short neck. Also, imaging of proximal nerve roots of the lower extremity nerves or the lumbosacral plexus is not possible due to limited penetration depth of the ultrasonic rays.” (page 8).

References:
Please also include more recent references of CIDP and GBS

> Please see first comment on added references above.

Figures are ok.

Reviewer #2:
Manuscript Summary:
The authors provide a brief reasoning for the application of high resolution ultrasound in patients with inflammatory neuropathies. The protocol established in Bochum is presented in a concise manner, some representative findings are provided as illustrative examples. The derivation of the "adjusted Bochum Ultrasound Score" is introduced.

The manuscript will serve as a well-written and concise basic introduction to a proven workflow for teams without any previous experience in the field.

I therefore recommend the acceptance of the article.

Major Concerns:
None

Minor Concerns:
None

Thank you for the review. No changes were made according to comments of reviewer #2.

Reviewer #3:
Manuscript Summary:
The use of nerve ultrasound has clinical relevance and utility in helping to diagnose and monitor certain peripheral neuropathies, particularly possible or probable CIDP.
As such this article is an important contribution.
They provide a clear protocol for systematically quantifying CSA in multiple nerves and also provide normal ranges.

Major Concerns:

1. What is the evidence to support the thesis that increased CSA is associated with demyelination/remyelination?

   Increased CSA similar to CIDP occurs in hereditary neuropathies, most prominent in patients with Charcot Marie Tooth 1a disease. In CMT 1a, inflammation may occur but is not key point in pathogenesis. The histopathological similarity to CIDP rather is the repeated de- and remyelination. This is why there is evidence that increased CSA can not only result from inflammation but also from de- and remyelination alone.
   To make this train of thought more understandable, we added the reference to hereditary polyneuropathies in this sentence: “Typical findings in demyelinating inflammatory neuropathies are multifocal nerve swellings with inhomogeneous fascicles, while nerve swellings in demyelinating hereditary neuropathies occur more generalized and homogenous 12, 18. Histologic correlate of increased CSA is assumed to be acute inflammation and repeated de- and remyelination, however this remains to be investigated 7.” (page 6)

2. The Bochum score relies on CSA. However, are there any scoring systems for the alterations in the fascicular structure and homogeneity?

   There is literature reporting on scoring systems for homogeneity and alterations of fascicular structure. First report on evaluation of homogeneity of nerve enlargement was by Padua et al. Further reports from Grimm et al. used the homogenity score and regional nerve enlargement index to stratify for inflammatory and hereditary polyneuropathies.
   We added these aspects to the manuscript text, but did not describe these scores in detail, as the main focus of the manuscript should be the ultrasound examination itself also shown in the videos being produced based on this manuscript, not the different scoring systems in detail.
   “Scoring systems for homogeneity and alterations of fascicular structure were described before and may aid in evaluating homogeneity.”

   - Padua, L. et al. Intra- and internerve cross- sectional area variability: New ultrasound measures. Muscle & Nerve. 45 (5), 730–733 (2012).
   - Padua, L. et al. Heterogeneity of root and nerve ultrasound pattern in CIDP patients. Clinical Neurophysiology. 125 (1), 160–165 (2014).
   - Grimm, A. et al. Ultrasound pattern sum score, homogeneity score and regional nerve enlargement index for differentiation of demyelinating inflammatory and hereditary neuropathies. Clinical Neurophysiology. 127 (7), 2618–2624 (2016).

3. The addition of an anatomical diagram identifying the different sites for ultrasound examination would be useful.

   We added figure 3 to give an overview of all described measuring sites for CSA.

4. Are there any studies showing the effect of therapy on ultrasound outcomes?

   There are some longitudinal studies with CIDP patients which showed correlation of CSA course with clinical course and therapy response. We added the following sentence and the according references to the discussion part:
   “Moreover, CSA alterations in longitudinal disease course of patients with CIDP were described to correlate to clinical disease course and treatment response.” (page 8)

- Fisse, A.L. et al. Clinical, Sonographic, and Electrophysiologic Longitudinal Features of Chronic Inflammatory Demyelinating Polyneuropathy. Journal of Neuroimaging. 9, 402 (2018).

- Fionda, L. et al. Changes of clinical, neurophysiological and nerve ultrasound characteristics in CIDP over time: a 3-year follow-up. Journal of Neurology. 1–9 (2021).
- Härtig, F. et al. Nerve Ultrasound Predicts Treatment Response in Chronic Inflammatory Demyelinating Polyradiculoneuropathy-a Prospective Follow-Up. Neurotherapeutics. 15 (2), 439–451 (2018).
- Décard, B.F., Pham, M., Grimm, A. Ultrasound and MRI of nerves for monitoring disease activity and treatment effects in chronic dysimmune neuropathies - Current concepts and future directions. Clinical neurophysiology. 129 (1), 155–167 (2018).

Reviewer #4:
Manuscript Summary:
The manuscript describes several aspects of nerve ultrasound in diagnosis of dysimmue neuropathies, based on a protocol from single German center.

Major Concerns:
The manuscript appears to be mainly driven by authors own work, and unpublished data that should also receive validation in independent cohorts.

> We agree with the reviewer, that the focus of this manuscript is to describe the method of nerve ultrasound performed in our department and the aim of this work is to show the nerve ultrasound examination itself. Of course, we can only show how we actually perform it, but our examination protocol corresponds to many previously published protocols and includes nerve sites that have been repeatedly described internationally.
>
> Also, this manuscript does not aim to review the suitability of nerve ultrasound for diagnosis of certain diseases, nor to review different nerve ultrasound scores for evaluation of polyneuropathies. For this we have to refer to other work.

Several methodological items warrant extensive revision and further clarification.

> We revised methods, protocol and discussion extensively.

Finally, limitations of nerve ultrasound and relevant imaging mimics warrant a more balanced discussion.

> We added a subheading to the discussion part “Limitations of the technique” to be more specific. Also, the limitations were described more clearly: “There are anatomical limitations for nerve ultrasound i.e., examination of cervical nerve roots can be difficult to impossible in patients with obesity and short neck. Also, imaging of proximal nerve roots of the lower extremity nerves or the lumbosacral plexus is not possible due to limited penetration depth of the ultrasonic rays.” (page 8).
> As this is a technical and methodological paper and not a paper about interpretation of nerve ultrasound alterations or different diseases, we focused on technical and methodological limitations, not on imaging mimics.

Minor Concerns:
Several items in introduction and technical description are lacking or need rewording.
Title: I would change title to 'nerve ultrasound protocol to detect dysimmune neuropathies: a video instruction and review on its practical implementation'

> As suggested, we changed the title to “Nerve ultrasound protocol to detect dysimmune neuropathies: a video instruction”. As this manuscript does not aim to be a review on nerve ultrasound, we did not implement this part.

Abstract:
The authors suggest that nerve ultrasound can reliably distinguish between hereditary demyelinating and dysimmune neuropathies, but overlap is far from uncommon.

> We agree with the reviewer, there are patients with both hereditary and dysimmune neuropathies, therefore changed the formulation to “CIDP may be distinguished from hereditary and other polyneuropathies by measuring extent and pattern of nerve swellings (CSA increase).” (page 1)

Refrain from speculation on (validated) histologic correlation of sonomorphologic abnormalities.

> We omitted the corresponding sentence from the abstract as suggested.

Introduction:
This section lacks a balanced introduction of relevant concepts and appropriate use of diagnostic tests

in polyneuropathy. I would urge the authors to consider the following:
- Diagnosis of polyneuropathy is primarily clinical, based on adequate identification of relevant signs and symptoms.
- The main adjunctive tests in polyneuropathy include laboratory testing to identify possible causal mechanisms, and electrodiagnosis to confirm loss of sensory and motor axons. However, contribution of electrodiagnosis itself is usually small in diagnostic strategies on polyneuropathy [1, 2]. Only in a subset there is a true added value, particularly in cases where genetic groups (axonal vs demyelinating) and the rarer dysimmune neuropathies are considered. Genetic testing is needed in the former. Expanded electrodiagnostic (EDX) protocols are often deployed in patients suspected with potentially treatable neuropathies [3-6]. Other supportive tests are primarily used to obtain more certainty on diagnosis when EDX fails to provide required evidence (e.g. in suspected CIDP), and include imaging (MRI or ultrasound), cerebrospinal fluid testing and in selected cases nerve biopsy [7, 8].
- There are several caveats to test characteristics of EDX in patients with suspected CIDP, as sensitivity and specificity are far from perfect. Several other neuropathy causes (e.g. demyelinating hereditary neuropathies, IgM neuropathy, POEMS syndrome, amyloid neuropathy, GBS, etc.) may lead to similar abnormalities on EDX, formally fulfilling electrodiagnostic consensus criteria for CIDP.
- The terminology 'axonal' and 'demyelinating' are clinical concepts with several limitations. The fact that diverse set of disease processes can lead to similar electrodiagnostic abnormalities such as severe conduction slowing, also imply that the latter is not simply 'demyelination'. Previous histologic investigations have similarly shown us that overlap of abnormalities between these to clinical categories is common.

> We agree with what the reviewer wrote on diagnostics in polyneuropathies. However, we want to remind, that this manuscript is not a review on diagnostics of polyneuropathies but aims to describe methodology of nerve ultrasound supported by video instructions. Therefore, we did not implement a comprehensive recommendation on all aspects of diagnostics.

- The recently revised EAN/PNS criteria on CIDP have already embraced the use of nerve ultrasound and pointed out several caveats such as lower specificity and caution on interpretation in light of relevant imaging mimics [7].

> The revised EFNS / PNS criteria were published in the meantime. We have therefore revised the manuscript in this regard and cited the new criteria.
> “As this was proven in several studies, the 2021 revised EFNS/PNS criteria, implemented the use of nerve ultrasound as additional tool in diagnosis of CIDP5.” (page 1/2)

- Prognostic potential of nerve ultrasound in CIDP and other dysimmune neuropathies remains debatable, with mixed results from previous studies [9-12]. Given the diagnostic perspective of nerve ultrasound as main aim of this manuscript, I would ask the authors to omit this.

> We removed the statement that nerve ultrasound can be used for disease monitoring on page 2, as it is not the main aim to discuss this in the manuscript.

- The authors should use more nuanced wording on nerve ultrasound features. Nerve size appears to be most robust parameter, consistently reported in wide range of different neuropathies [13]. At present, other sonographic parameters that have been proposed have received less attention in published literature. Evaluation of fascicle size has been used previously by several groups [14-17], but larger validation studies are still lacking and the added value over nerve size alone is likely limited.

> As most data exist on measuring nerve size by CSA, we focused on this in the manuscript. Regarding echogenicity and fascicle size, we agree with the reviewer, that there are not enough data to report on this method here. Still, we believe that referring to these other sonographic parameters than CSA is still necessary. Concluded we omitted the part from the introduction but left it in the results and discussion part.

Speculation on histologic correlates should be removed, as this was never formally investigated (few had indirect comparison with distal sensory leg nerve segment vs mixed and arm nerves), and also warrants validation when appropriate testing confirms this.

We removed details on histologic correlates of CSA increase in the introduction. Nevertheless, we find it important to have at least an idea of what the correlate of morphology is, so we left a sentence in the discussion with the limitation that it still needs to be examined:
“Histologic correlate of increased CSA is assumed to be acute inflammation and repeated de- and remyelination, however this remains to be investigated”. (page 6)

- A recent neuromuscular expert panel reviewed the available protocols, ranging from practical to more elaborate protocols with more complex scoring systems [18].

We do not aim to compare the published scoring systems. Therefore, we did not extensively discuss this point. However, we added the suggested literature to the manuscript.

- The present technical section is rather superficial, either expand to appropriate details (e.g. include basic physics on nerve ultrasound, describe basic rules of ultrasound (instrumentation considerations, probe and device handling, scanning rules including image optimization, measurements (including why perpendicular and within hyperechoic rim), appropriate annotation and reporting) or simply reduce it to single sentence and refer to more dedicated papers [19, 20].

We believe that our technical section is helpful as an overview of technique in neuromuscular ultrasound. However, we referred to more detailed articles as addition: “More details on technical ultrasound can be found elsewhere.” (page 3).
In this manuscript, we do not aim to include technical basic physics and rules of ultrasound.

Protocol:
Omit selection of patient, probe and preset setting in this section, and also omit 'bilateral' and 'save' items.

There are quite specific author guidelines, which as for this detailed description, therefore we did not change these parts.

I would urge the authors to use video and images rather than text here, given the highly visual operation of the ultrasound devices this would appeal potential readers more than plain text.

This work includes creation of video files with help of the journal on the methods after completion of review and acceptance of the manuscript.

The ultrasound protocol proposed by the authors appear to be mainly driven by German data sets, from the same few centers.

Please see answer to you first comment.

Representative results:
- The authors appear to push their own scoring system, that has not been published or validated yet, whereas some of the other scoring systems have been validated (including original Bochum scoring system that achieved considerably lower diagnostic performance than original publications) [21, 22].

We explained the scoring system developed Bochum as example and as this is part of our evaluation of nerve ultrasound in our center. As this manuscript does not aim to review different scoring systems, this section was kept short and we did not discuss other scoring systems in detail but referred to other sources.

- The reference values from their own meta-analysis also includes important bias (e.g. mix data from Asian and Western populations, young and older age categories (including children up to 10 14 years), different probe frequencies) all leading to smaller nerve sizes [23-25]. The fact that normal nerve sizes are quite different from disease specific cut-offs further compound the complexity here for potential readers.

Our meta-analysis to which we refer in the article has not included CSA data of children < 18 years and also different origin and age were taken into account, which can be read in the original publication.

However, we agree with the reviewer, that establishing cut-offs for CSA is complex, which is why we do not suggest simply using the cited cut off values, but confirmation and adjustment for each ultrasound laboratory on its own.

- What are the criteria for significantly enlarged nerves? How does this compare to previous studies on disease specific cut-offs?

To give an example of cut-off values for normal respective enlarged nerves, we refer to reference values in table 1, which need to be adapted by each center. Disease specific cut-offs for inflammatory neuropathies are not yet validated enough to recommend clinical use.

- Several scoring systems have been proposed (short and elaborate protocols on nerve size only at specific sites, distribution of abnormal nerve size along length of nerves, distribution of abnormal nerve and fascicle sizes, echogenicity, all using different cut-offs) that warrant more balanced description here [13, 26].

We do not aim to discuss and review different scoring systems in this manuscript. Therefore, we refer to other work for this topic.

- What is the difference between modified ultrasound pattern sumscore (UPSS) and modified Bochem ultrasound score? The similarity is more striking than previous versions. Again, just dropping sensitivity/specificity data here from unpublished work cannot be justified.

Differences from aBUS and UPSS are included nerve sites (aBUS: 6 sites, UPSS: 12 sites), and scoring (aBUS: 0 or 1 point per site, UPSS 0-2 points per site), as well as evaluated patient groups (aBUS was validated for patients with possible CIDP according to EFNS/PNS criteria and with secondary axonal damage). Again, discussing these differences is beyond the aim of this article, therefore we did not further elucidate this.

Discussion:
This section is mainly driven by unpublished results by own work of authors, implying considerable bias.

Discussion section was re-structured according to the requirements of the journal and does not include discussion of any unpublished results.

In contrast to statement of the authors, reproducibility of nerve ultrasound has been studied, and demonstrated confidence of consistent measurements between raters and different (machine) setups [27-35]. Formal consensus on required training and criteria for certain levels of expertise have been published recently [19, 36].

We agree with the reviewer in this point and this is also what we state in our manuscript. If used by a trained examiner and with adequate protocols, nerve ultrasound is a reliable method. Therefore we do not see a contradiction here.

Again, refrain from speculation on ultrasound correlates with (distant) histologic findings.

Please see comment above on the same issue.

The recently revised EAN/PNS guideline has already implemented nerve ultrasound along with previous option of plexus MRI [7].

Please see comment above on the same issue.

Finally, I would urge the author to provide balanced discussion here on imaging mimics that should be considered when performing nerve ultrasound and caution warranted on interpretation of heterogenous results.

Please see comment above on the same issue.

References

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