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Histological Evaluation of Fascicular Damage Following Repeated Sciatic Nerve Transfixion: An Anatomical Study

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ABSTRACT

Background

Ultrasound guidance has improved the accuracy and safety of peripheral nerve blocks, yet intraneural and even intrafascicular needle placement remains possible. The true incidence of fascicular injury after direct nerve transfixion is uncertain due to limited histological data. This study aimed to determine the frequency of fascicular damage after needle transfixion of the sciatic nerve using a human anatomical model.

Methods

A sciatic nerve segment was harvested from a fresh-frozen male cadaver without neurological disease. One hundred full-thickness transfixions were performed using a 22-gauge sharp needle, simulating accidental intraneural penetration. Each transfixion was replicated using a standardized technique. The nerve was fixed in formalin, sectioned every 5 millimeters, embedded in paraffin, and stained with hematoxylin and eosin for histological evaluation. The presence of fascicular injury or displacement was assessed microscopically. Descriptive statistics were used to summarize findings, and proportions with 95 percent confidence intervals were calculated using the Wald method.

Results

All 100 needle trajectories were successfully identified histologically. Clear fascicular damage was observed in 30 of 100 transfixions, corresponding to an incidence of 30 percent (95 percent confidence interval, 21 to 39 percent). In four additional cases, the needle passed in close proximity to fascicles, causing their displacement without visible structural injury, yielding an incidence of 4 percent (95 percent confidence interval, 0.2 to 7.8 percent). In the remaining cases, the needle path did not involve any fascicles.

Conclusions

One third of simulated sciatic nerve transfixions performed with a sharp needle resulted in direct fascicular injury, while a smaller proportion produced fascicular displacement without structural disruption. These findings suggest that fascicular penetration is more common than previously believed when sharp needles are used and highlight the potential for mechanical nerve injury during regional anesthesia procedures. The use of sharp needles for peripheral nerve blocks should be avoided, and further research should explore how needle design and tip geometry influence the likelihood of fascicular injury.

Key-words: Sciatic nerve; Fascicular injury; Needle transfixion; Regional anesthesia; Intraneural injection; Nerve damage; Histology

INTRODUCTION

The introduction of ultrasound into regional anesthesia practice has significantly improved both our understanding of nerve anatomy and our ability to locate neural structures and track their course accurately. However, despite the use of ultrasound guidance, nerve transfixion and intraneural injection remains possible [1]. In fact, this occurrence has been suggested to be more common than previously believed [2] and has been estimated to occur in 16% of cases with expert practitioners and up to 35% with trainees performing peripheral nerve blocks [3,4] but does not invariably result in nerve injury [2]. Although a subclinical reduction in the amplitude of nerve potentials is common after peripheral nerve blocks [5], clinically significant post-block neurological dysfunction remains a rare complication [6]. In some cases, mild transient symptoms have been reported in up to 7% of patients after peripheral nerve blocks; however, permanent neurological deficits occur in less than 0.1% of cases [7].

Recent studies have challenged the hypothesis that injectate-induced mechanical damage occurs, particularly when injections are performed under low-pressure conditions [8], while it is unlikely that a needle is directly transfixed to a fascicle during its trajectory [9]. Despite these findings, the extremely small sample sizes in existing studies investigating mechanical transfixion mean that uncertainty persists, even in the face of limited experimental evidence using standardized procedures. In this setting, the contribution of experimental clinical anatomy can help fill the knowledge gap [10]. The purpose of this research is to determine the incidence of fascicle damage after needle transfixion in an anatomical model of the isolated sciatic nerve.

METHODS

All the procedures performed in this study involved human bodies from the Veneto Region Reference Center for the preservation and use of gifted bodies (Deliberation of the Regional Council of the Veneto Region No. 245, Mar 8th, 2019; No. 389897), in accordance with the national laws, and the ethical standards of the regional/national research committees, as well as with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was provided to join the body donation program. According to the Body Donation Program of the Institute of Anatomy of the University of Padova, a sciatic nerve was harvested from a fresh frozen male body of 84 years of age hosted at our Reference Center for the Preservation and Use of Donated Bodies [11,12]. As a certified standard, we use a routine protocol according to which we receive the bodies 48 hours after death and on the same day they are frozen at -20°C with a remote temperature control system, until they are used with programmed thawing. The donor had no diseases that could interfere with the study. The length of the harvested sciatic nerve was 11 cm.

On the bench, the accidental transfixion of the sciatic nerve was simulated during the anesthesiological procedure. For this purpose, 100 transfixions were repeated and performed using a 22 G sharp needle for the syringe (BD Microlance, Becton Dickinson, Oslo, Norway) and a polypropylene monofilament 3/0 suture (Prolene, Eticon, New Jersey, United States), using the TruePass® procedure [13].

Briefly, the TruePass® procedure first involves full-thickness side-to-side sciatic nerve transfixion by the 22G needle, and subsequently a coaxial wire was inserted into the lumen of the needle and then held in place while the needle was removed. These steps were repeated identically 100 times. Once the whole procedure was completed, we preserved the sciatic nerve in 4% m/v formalin solution at room temperature. After 5 days of fixation, the nerve was cut at 5mm intervals, processed for paraffin embedding, and cut every 5 µm up to make out of all 100 wires. All sections were stained for hematoxylin and eosin (H&E) staining according to the routine protocol. Briefly, After formalin fixation, the sciatic nerve was cut every 5 mm, processed according to the routine protocol, and paraffin was embedded. From each sample, 5 µm thick sections were cut and stained with

Hematoxylin-Eosin (W01030708, BioOptica Milano, Italy) for histological characterization. The sections were dipped in xylene for deparaffination, immersed in a decreasing scale of alcohol solutions for hydration, and treated with the staining dyes of the kit. Subsequently, dehydration was performed through immersion on an increasing scale of alcohol solutions, clarification in xylene, and mounting with Eukitt medium (09-00250, BioOptica Milano, Italy) on all nerve sections. Hematoxylin-Eosin staining was performed to observe the tissue morphology and architecture of each and every thick section of the sciatic nerve.

To measure the surface area corresponding to the removed tissue, and the total area of a given fascicle, the RAW uncompressed images were analyzed using Image-J software, version 1.8.0 [14].

Fascicular damage evaluation was performed through ImageJ software (ImageJ 1.54f, Rasband W. and contributors, National Institutes of Health, USA) analyzing each histological section, previously acquired at 5x magnification in bright field under a Leica D4500B microscope (Leica Microsystems, Wetzlar, Germany) connected to a digital camera (Leica DC 200, Leica Microsystems, Wetzlar, Germany). Specifically, percentage damaged area of the fascicles was calculated by subtracting the area sum of the two halves of each remnant fascicle from the whole fascicle estimated area. Where the residual contour of the fascicle had been distorted by the passage of the needle, manual interpolation was performed between the detectable morphological profiles. Areas were measured by selecting fascicles perimeter through area selection and measurement tools (Figure 3).

Statistical analysis

Descriptive statistics were used to summarize the incidence of fascicular injury and near miss events after sciatic nerve transfixion. Proportions were calculated and reported as number and percentage, and the corresponding 95% confidence intervals (CI) were estimated using the Wald method for large samples. All analyses were performed using R software (version 3.3.4, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The nerve sample had an anterior-posterior diameter of 10 mm (range 7-11 mm) and a medial-lateral diameter of 15 mm (range 12-18 mm).

The procedure was performed as described in the Methods section without deviation, obtaining a total of 100 histological slides, and the path of the needle was successfully identified on all slides.

In general, in 30 out of 100 transfixions, clear lesions to the sciatic nerve fascicles were observed (Figure 1). Simultaneous damage to multiple fascicles by a single transfixion has been observed 4 times, in which at most 2 fascicles were affected at the same time.

In four additional cases, the needle passed in close proximity to the fascicles, causing their displacement in a distinct manner without resulting in structural damage (Figure 2). In the remaining cases, the needle path did not involve any fascicles. There were no discernible differences between intrafascicular and interfascicular damage patterns, and in this last case no vascular injuries were identified (Figure 1 and 2).

Based on these findings, the estimated incidence of fascicular damage after direct needle transfixion was 30% (95% CI 21 to 39%), while the incidence of needle contact without fascicular injury was 4% (95% CI: 0.2 to 7.8%).

In case of fascicular damage caused by direct needle transfixion, the proportion of the surface area of the removed tissue compared to the total area of the damaged fascicle section corresponded to the percentage of tissue removed (19.3% ; range 4.0-37). Taking into account the worst-case scenario, in which the removed tissue portion coincides with the needle path that overlaps the whole diameter of the fascicle, it has been calculated that the percentage varies from 24.7% to 34.1% (n = 2).

DISCUSSION

The possibility of fascicle penetration due to needle insertion is a matter of debate in regional anesthesia. It has been postulated that fascicle penetration should be not possible [15] due to the endonerium being a rigid structure that exhibits high resistance to needle insertion [16].

The main finding of our study is that the sciatic nerve iatrogenic lesion due to mechanical interaction with the needle during its insertion occurs at a higher rate (approximately 30%) than previously reported (less than 1%). The discrepancy between the high prevalence observed experimentally on the bench in this study and the infrequent sequelae related to events reported in clinical practice is only apparently contradictory. It should be kept in mind that in the real world it is a matter of a composite probability of an event, that is, it refers to the proportion of subjects who experienced clinically detectable sequelae, among those who underwent the procedure performed with the specific intent of making an intraneural injection or in which this interaction is unwanted. An interesting dynamic of the mechanical needle-fascicle interaction was highlighted in the case of no nerve injury, while, on the other hand, some consequences of neural injury can be clinically undetectable, temporary, or functionally compensated, and are so difficult to be detected and evaluated [17,18].

The damage that may be caused by direct mechanical injury is an issue deserving further investigation. The clinical manifestation of direct mechanical damage to the nerve fascicle caused by needle transfixion of the sciatic nerve is still understudied due to the lack of histopathological correspondence with the clinical manifestation ascertained in livings and the lack of clinical correspondence with the morphological alteration detectable in cadavers. However, we believe that this clinical manifestation has basically an extremely variable expression, due to the many variables involved. In fact, it would be necessary to know the number of fascicles damaged by the passage of the needle, and the magnitude of lesion of the fascicle (trajectory coinciding with the diameter or with a marginal trend). The demonstration that the proportion of tissue removed by the needle passage is small compared to the total area of the damaged fascicle (around 20%) supports this hypothesis. Moreover, the functional "weight" of a single fascicle, the territorial destination of that fascicle and the overall number of fascicles destined for the same anatomical area, the recovery capacity of the damaged tissue, the chance that other fascicles can over time replace and compensate for the functional deficit, the detectability of the deficit in the case of subjects with pre-existing nervous deficits, with reduced mobility or even bedridden, the degree of cognitive impairment in olders potentially impacting on the patient's perception of a functional deficit and its reporting for further investigations.

Moreover, the true prevalence of the event is likely unknown, as its publication could be under-reported. A study with a similar design to ours reported 4 fascicular injuries out of 8 simulated sciatic nerve transfixions by a sharp needle, involving a total of 134 fascicles located near the needle trajectory, resulting in an estimated nerve damage rate of 3%. While this percentage is lower than the incidence observed in our study, it is important to highlight a key methodological difference: the denominator in their calculation included all fascicles in the vicinity of the needle path, regardless of whether the needle made contact. In our view, this approach may not be appropriate, as fascicles that are only near the needle but not directly contacted are unlikely to sustain mechanical damage. Including such fascicles in the denominator probably underestimates the true incidence of nerve injury after direct needle transfixion [19].

Another study using a median and ulnar nerve model reported a very low rate of fascicular injury (<1%, 3 out of 400 transfixions). This discrepancy may be explained by anatomical differences: the median and ulnar nerves contain a higher number of fascicles, but these are smaller in diameter, particularly in the forearm (0.47 ± 0.18 mm and 0.38 ± 0.18 mm, respectively) [20], whereas fascicles in the sciatic nerve can be considerably larger (up to 1.2 ± 0.7 mm) [21].

It is important to note that the tip of the needle could probably play a role in mechanical damage, as sharp needles have been associated with mechanical damage both in experimental [16,19] and clinical settings [20]. Our work also shows that fascicles are not inevitably cut by the needle tip, but can instead undergo a process of displacement, an effect previously noted and described in response to needle insertion [20,21]. However, there were no discernible differences between intrafascicular and interfascicular damage patterns that could explain this different behaviour, as also observed by other authors [20,21].

Our study has several limitations that merit discussion. First, the use of a sciatic nerve model from a deceased donor can only demonstrate mechanical damage to the nerve, but not the potential biological responses to injury, such as inflammation or cytotoxicity. However, this was the most appropriate model for our objective, as animal models would not adequately reflect human nerve structure [15]. Second, the use of sharp needles does not fully align with common clinical practice, where non-cutting needles are often preferred for regional anesthesia. However, our primary objective was to demonstrate that fascicular damage can indeed occur due to mechanical forces. Future studies should investigate the incidence of fascicular damage with different needle designs and gauges. Third, the use of a standardised technique has allowed us to estimate for the first time the stochastic probability of iatrogenic injury to nerve fascicles in the case of transfixion while performing an anesthetic block of the sciatic nerve.

Conclusions

One third of sciatic nerve transfixions performed with a sharp needle resulted in fascicular damage. For this reason, the use of sharp needles should be always avoided and intraneural injection should be prevented. Further studies should investigate alternative needle designs to quantify the incidence of fascicular damage across different outer diameters and tip configurations.

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Human Ethics and Consent to Participate declarations: All the procedures performed in this study involved human bodies from the Veneto Region Reference Center for the preservation and use of gifted bodies (Deliberation of the Regional Council of the Veneto Region No. 245, Mar 8th, 2019; No. 389897), in accordance with the national laws, and the ethical standards of the regional/national research committees, as well as with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was provided to join the body donation program.

Conflicts of interest: Alessandro De Cassai and Burhan Dost serve as Associate Editors for the *Journal of Anesthesia, Analgesia and Critical Care*. These roles had no influence on the editorial or peer review process. The other authors declare no conflicts of interest.

Fundings: None

Data Availability: All the data used to prepare this manuscript are presented in the manuscript

Author Contributions

RBB and ADC conceived the study and drafted the first version of the manuscript. MC and VM performed the histological analyses and contributed to data interpretation. AP supervised the work and provided critical revisions. RDC and VM contributed anatomical and methodological expertise and assisted in data validation. BD and ST contributed to the study design and provided clinical interpretation within the context of regional anesthesia. AB contributed to manuscript editing and refinement of the discussion. VM and MC assisted in figure preparation and data presentation. All authors reviewed and approved the final version of the manuscript.

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FIGURE LEGEND

Figure 1. Sciatic nerve with fascicular injury following needle transfixion

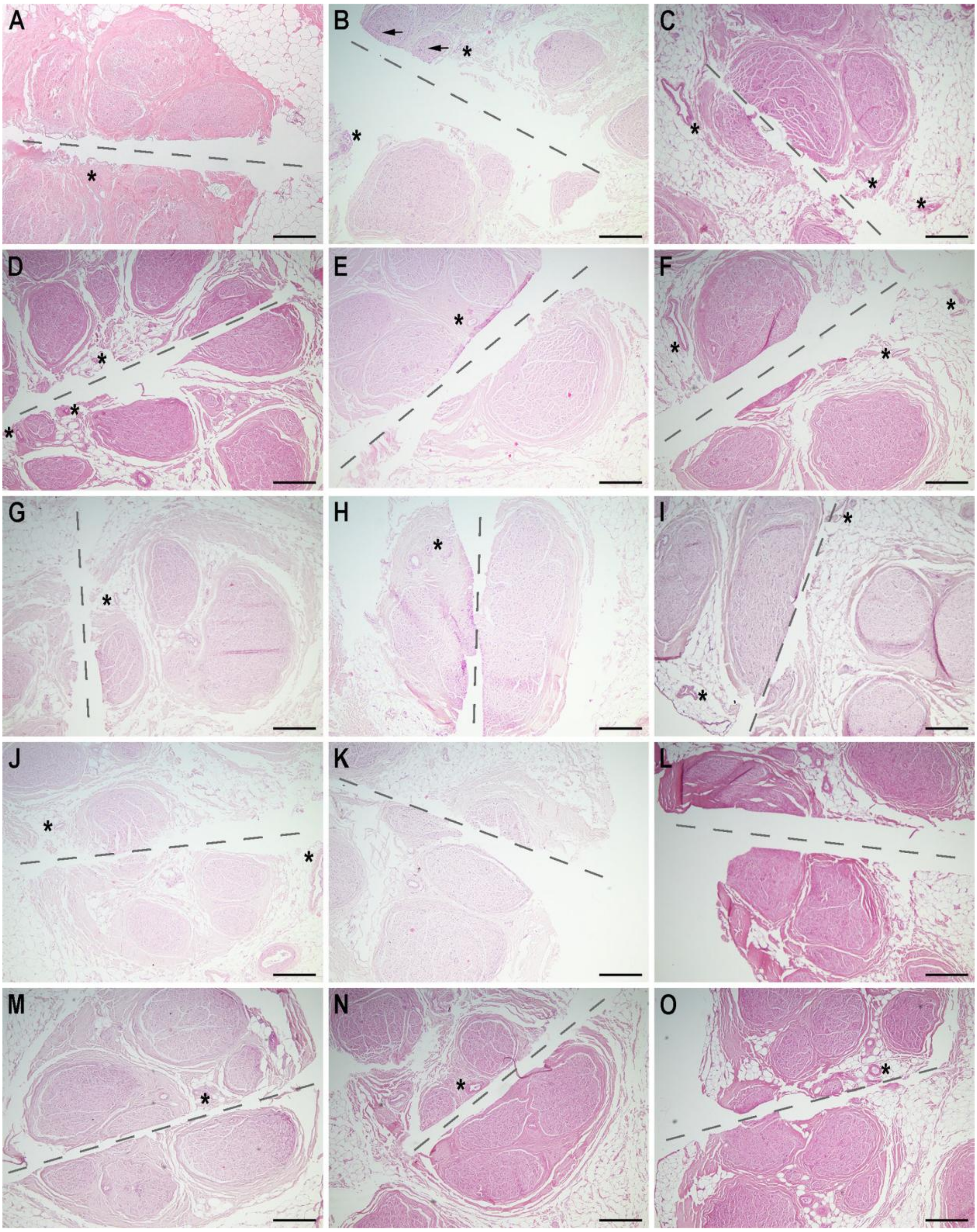
Histological analyses of damaged sciatic nerve sections stained with haematoxylin and eosin (A-O). The needle trajectories throughout the sciatic nerve and perineurium/endoneurium damage due to the mechanical insertion of the needle are shown. All the trajectories of the needle are shown in grey dotted lines. Blood vessels are indicated by asterisks. Arrows (B) indicate the fascicles damaged simultaneously by the trajectory of a single needle transfixion. Scale bars: 150 µm.

Figure 2. Sciatic nerve without fascicular injury following needle transfixion

Morphological analyses of non-damaged sciatic nerve sections stained with hematoxylin and eosin (A-D). Cases in which no nerve fascicles were damaged by needle transfixion are shown. Needle-paths (grey dotted lines) throughout the sciatic nerve did not affect the perineurium/endoneurium, but only adipose tissue. Blood vessels are indicated by asterisks. Scale bars: 150 µm.

Figure 3. Morphological assessment of damaged sciatic nerve fascicles

The needle-path throughout the sciatic nerve is shown by a grey dotted line. The outline of the area corresponding to the portions of fascicle remaining after the transfixion by needle has passed is indicated with a solid black line. The outline of the area corresponding to the overall surface of the original fascicle is indicated with a blue dotted line, with the necessary manual interpolation due to the distortion caused by the transfixion. Scale bars: 150 µm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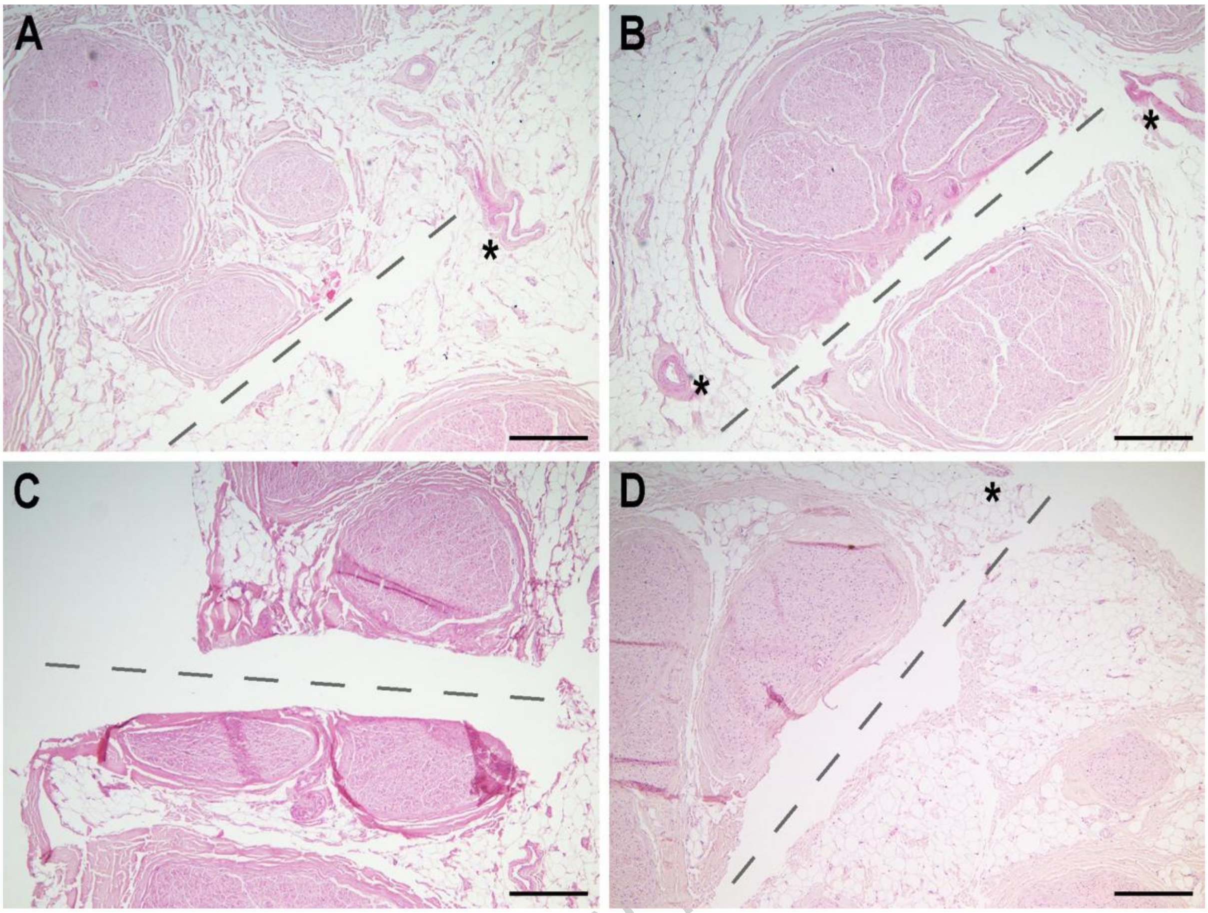
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