N. Moayeri1, A. C. Krediet2, J. C. Welleweerd2, R. L. A. W. Bleys3 and G. J. Groen4*

1 Department of Neurosurgery, 2 Department of Anaesthesiology, and 3 Department of Anatomy, University Medical Centre Utrecht, Utrecht, The Netherlands
4 Pain Centre, Department of Anaesthesiology, University Medical Centre Groningen, University of Groningen, Mailstop EB 31, PO Box 30 001, 9700 RB Groningen, The Netherlands
* Corresponding author. E-mail: g.j.groen@umcg.nl

Editor’s key points

- Intraneural injection of local anaesthetic can cause nerve damage.
- A cadaver study of intraneural injection of 0.5 ml under ultrasound (US) guidance.
- Cryomicrotome section findings were compared with US image changes.
- Expansion of the nerve cross-sectional surface area gave reliable detection of intraneural injection.

Background. Intraneural injection of local anaesthetic agents carries a risk of neurological complications. Early detection of intraneural needle-tip position is very important in the initial phase of injection. Ultrasound (US) characteristics for real-time detection of intraneural injections have been described, but only for relatively large volumes (5–40 ml). This study assesses the reliability of various US criteria to detect early low volume (0.5 ml) intraneural injections. Intraneural deposition of an injected dye was confirmed by cryomicrotomy.

Methods. In nine unembalmed human cadavers, 0.5 ml methylene blue was injected intraneurally into the supraclavicular brachial plexus and subgluteal sciatic nerve on both sides. The sites of injection were subsequently removed en bloc. Consecutive cryomicrotomy cross-sections with a 50 μm interval were obtained to assess intraneural presence of the injectate. Two independent experts separately reviewed US video clips of the injections and scored each US criterion.

Results. Of the 36 injections, cryomicrotome cross-sections showed intraneural staining in 33 and extraneural staining in three. The best US criterion was expansion of the nerve cross-sectional surface area together with a change in echogenicity. It was observed in 35 injections, including two false positives. There was one true negative. Test precision was 94% [95% confidence interval (CI), 87–100%]. The mean increase in surface area was 8.7% (95% CI, 5.6–11.9).

Conclusions. Reliable detection of early low-volume intraneural injection using US is possible using expansion of the cross-sectional surface area of the nerve together with a change in echogenicity as markers.

Keywords: diagnostic imaging; nerve block; predictive value of tests; ultrasonography

Accepted for publication: 22 March 2012

Nerve damage is a rare but important complication of regional anaesthesia. One risk factor is thought to be intraneural injection of local anaesthetic agents. Although intraneural injections do not invariably cause neurological complications,1–7 most clinicians aim to inject outside rather than inside the nerve. This is not always possible as electrostimulation thresholds in the range of 0.3–0.5 mA have been shown to be unreliable in distinguishing between intra- and extraneural needle-tip position.2 8–10

Ultrasound (US) imaging is thought to decrease the risk of injection inside the nerve,11 but inadvertent intraneural injections have been reported.12–14 Real-time detection of intraneural injections is challenging due to limitations in both the equipment15 and the operator.16 In clinical studies, a number of ultrasonographic parameters have been proposed which could be of help to distinguish between intra- and extraneural injection. They include an increase in surface area, cross-sectional diameter,1 4 6 17 or both and visualization of the needle tip during indentation and penetration of the nerve wall.2 18 In pig brachial plexus/femoral nerves, intraneural injections were defined as nerve swelling19 and tissue expansion by hypoechoic fluid within a predominantly hyperechoic neural structure.20 However, they used relatively large volumes (4–5 ml). Local anaesthetics volumes such as these, and even smaller, may cause neurotoxic effects after intraneural injection.20–22 Thus, detection of intraneural needle-tip position is highly relevant in the initial phase, when only a small amount of local anaesthetic is injected.

In contrast to studies that use post-mortem histological assessment, the exact site of injection cannot be verified in clinical studies. As post-mortem manipulation of tissue might affect the spread of injected fluid, examination of
Early detection of intraneural injection

undisturbed anatomy is essential, and cryomicrotomy is the method of choice.23 24

We hypothesized that intraneural injection of as little as 0.5 ml could be reliably captured by US. Using a cadaver model, we applied three ultrasonographic criteria suggestive for intraneural injection: (i) visualization of the needle tip during penetration of the nerve or inside the nerve adjacent to hypoechoic spots; (ii) indentation of the nerve wall by the needle followed by puncture; and (iii) expansion of the cross-sectional surface area together with a change in echogenicity. Cryomicrotome cross-sections were used for confirmation. The ratio of correctly identified intraneural injections to all intraneural injections was used as the primary outcome.

Methods

Nine consecutive cadavers were selected within the donor programme of the Department of Anatomy of the University Medical Centre Utrecht, Utrecht, The Netherlands, after institutional review board approval from the same department. One additional cadaver was used for a pilot study. Exclusion criteria were the presence of brachial plexus or sciatic nerve pathology. All cadavers were examined within 12 h post-mortem.

For intraneural placement, the needle tip had to penetrate the outermost epineural layer of the nerve (intra-epineural injection). In the supraclavicular brachial plexus, the nerve fascicles are surrounded by epineural layers, whose configuration may differ depending upon the site of formation of the nerve trunks and cords. Close observation of these layers reveals that epineurium lies directly adjacent to the prevertebral and scalenic fasciae, with both layers being very thin (≤0.2 mm). Owing to their close relationship, we believe that breaching the outermost layer at this location will most likely puncture both layers, thus resulting in intra-epineural positioning of the needle tip.25 The sciatic nerve is internally divided into its tibial and common peroneal compartment by the Compton–Cruveilhier septum. Both divisions are enveloped by epineural connective tissue.26 The penetration of the epineurium of either tibial or common peroneal compartment is considered intraneural.

A pilot study was done to determine the potential diffusion capacity of the dye into the nerve after extraneural placement of the dye. Methylene blue has been previously used for accuracy and positioning studies of nerves.27 We chose a total of 0.5 ml of methylene blue 1% for detection of intraneural injection. In a fresh cadaver, a total of 0.5 ml was dripped directly on the tissue sheath of an exposed, unembalmed brachial plexus at the supraclavicular region just dorsal to the clavicle (site A) and 5 cm distally (site B). Sites A and B were only used for the pilot study. The injections during the formal study were performed in the supraclavicular fossa. Both locations were subsequently harvested. Tissue from site A was cross-sectioned with an interval of 0.5 mm and directly examined under the microscope to determine the extent of diffusion of the dye. Tissue from site B was immediately embalmed in a solution of 3.5% formaldehyde for 1 h and frozen to −30°C. After 24 h, histological and cryosections with an interval of 0.5 mm were obtained for microscopic examination. Microscopic examination of site A revealed dye infiltration in the epineurial and perineurial area. At site B, dye was only observed in the outer epineurial layer with no diffusion into the epineurial or perineurial area. Therefore, we chose the conservation technique of site B for all subsequent cadavers.

Intraneural injections

US scanning and injection of dye was performed by one investigator with experience in US-guided regional anaesthesia. All procedures were recorded as video clips for later analysis. In the supine position, both supraclavicular regions of each cadaver were scanned with an 18 MHz linear array transducer (LA435, Esaote, Maastricht, The Netherlands). The probe was positioned in the supraclavicular fossa according to the modified Plumb-Bob approach,28 in an oblique sagittal orientation and perpendicular to the axis of the brachial plexus. Under real-time US guidance, a 22 G, 50 mm, short bevel needle (Pajunk GmbH, Geisingen, Germany) was inserted in a posterolateral direction from the sternocleidomastoid–clavicle junction, parallel to the long axis of the transducer in the supraclavicular fossa. The brachial plexus appeared as distinct round- to oval-shaped hypoechoic nodules embedded in a hyperechoic area, and encircled by a hyperechoic line. The most superficially visible trunk of the brachial plexus was targeted. All injections were intended to be intraneural. A total of 0.5 ml of methylene blue 1% was injected during 5 s.

For the infragluteal region, an 8–12 MHz linear array transducer was used in both legs in the prone position. The transducer was placed caudal to the subgluteal fold, perpendicular to the axis of the sciatic nerve. In this region, the sciatic nerve appeared as a triangular to oval-shaped hypoechoic spots, and hyperechoic areas. A 22 G, 100 mm needle was used, and a total of 0.5 ml of methylene blue 1% were injected during 5 s. After the injection procedure, the cadaver was immediately embalmed and frozen as described above for pilot site B. In addition to the ultrasonographic assessment, pressure was also monitored during injection. A calibrated manometer (BSmart, Concert Medical LLC, Norwell, MA, USA) was attached between the syringe and the needle. The manometer provided a colour interval scale of the pressure throughout the injection (white, 1–15 psi; yellow, 15–20 psi; orange, >20 psi).

Cryomicrotomy and histology

To minimize the spread of the dye by manipulation of the nerves or surrounding tissues during dissection, the anatomy was left undisturbed. The complete area between the lateral side of the interscalene region and the midclavicular area was removed en bloc. For the infragluteal region, a similar procedure was done from 3 cm proximal to 3 cm distal to the injection site. The specimens were then frozen.
in carboxymethylcellulose gel at \(-30^\circ\text{C}\). Using a heavy-duty sledge cryomicrotome (PMV 450; LKB Instruments, Stockholm, Sweden), consecutive sagittal (supraclavicular region) or transversal (upper leg) sections (interval, 50 \(\mu\text{m}\)) of each specimen were obtained. The surface of each section was photographed (Nikon D1X; Nikon Corporation, Chiyoda-ku, Tokyo, Japan) at a resolution of 300 pixels in \(-1\). An independent investigator, blinded for the injections, assessed the images to confirm or reject the presence of methylene blue staining within the confines of the epineurium of the nerves (Fig. 1). Histological sections with an interval of 5 mm were stained using a modified Mallory-Cason procedure.\(^{29}\)

### Ultrasound

All US video clips were assessed separately by two independent experts, both with expertise in US-guided regional anaesthesia, who were blinded to the results of the cryomicrotomy sections. To assess the consistency of US, three individual criteria suggestive for intraneural injection were used (Fig. 2):

(i) visualization of the needle tip during penetration of the nerve or inside the nerve adjacent to the hypoechoic spots,

(ii) indentation of the nerve wall followed by puncture,

(iii) expansion of the cross-sectional surface area of the nerve together with a change in echogenicity.

The criteria could be rated as either ‘not visible’ or ‘visible’. We did not set a cut-off level for the increase in surface area. The relative increase in surface area was determined independently after the injection of dye.

### Statistical analysis

Data are presented as mean (SD) and proportions or percentages with 95% confidence intervals (95% CIs). The test precision (positive-predictive value) for each US criterion applied is defined as the ratio of true positives to all positives. Based on early findings of US to detect intraneural injection,\(^2\) we expected to find a ratio between 0.70 and 0.95. With 36 attempts of US-guided intraneural injection, it is possible...
to detect a value between 0.7 (0.57–0.83) and 0.95 (0.89–1.0), according to worst- and best-case scenario. For comparison of proportions and means, the dependent $\chi^2$ (McNemar’s test) and Student’s t-test were, respectively, used. $P$-values of <0.05 were considered significant. Statistical analysis was performed using SPSS Statistics version 17.0 (SPSS Inc., Chicago, IL, USA).

**Results**

A total of nine cadavers (eight female and one male) were included for final analysis. The mean age and BMI were 86.4 (6.8) yr (range 73–95 yr) and 21.6 (5.1) kg m$^{-2}$ (range 13.3–28.7 kg m$^{-2}$), respectively. A total of 36 attempts at intraneural injection were performed, using the supraclavicular brachial plexus and infragluteal sciatic nerve. The anatomic and histological cross-sections revealed intraneural presence of the injectate in 33 out of 36 cases (92%). There was no evidence of intraneural presence of dye in two attempts at the supraclavicular brachial plexus and one at the infragluteal sciatic nerve.

The test precision of the US criterion showed that the needle tip was visualized inside the nerve in 28 of 36 (78%) instances, of which 25 coincided with the cryomicrotomy assessment, a test precision of 89% (95% CI, 78–100) (Table 1). Indentation followed by puncture of the nerve wall was observed in 33 of 36 (92%) cases, of which 30 were correct, resulting in a test precision of 91% (95% CI, 81–100). Expansion of the nerve area together with change in echogenicity was visible in 35 of 36 (97%) samples, 33 of which were affirmed to be intraneural. In one attempt, no change in cross-sectional surface area and echogenicity was observed. In this case, cryomicrotomy confirmed the absence of injectate inside the nerve. The test precision of this ultrasonographic criterion was 94% (95% CI, 83–100%). In these confirmed intraneural injections, the cross-sectional surface area increased by 8.7% (95% CI, 5.6–11.9, $P<0.05$).
Table 1 Summary of findings in both the brachial plexus and the sciatic nerve (n=36 injections). +, present; –, absent; *test precision [%; (95% CI)]. Criterion 1: visualization of the needle tip during penetration of the nerve or inside the nerve adjacent to hypoechoic spots. Criterion 2: indentation of the nerve wall followed by puncture; Criterion 3: increase in the nerve cross-sectional area together with a change in echogenicity. Relationship between intraneural injection determined by US parameters and intraneural deposition of dye identified in cryomicrotomy cross-sections

<table>
<thead>
<tr>
<th>Intraneural presence of dye</th>
<th>US characteristics</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Criterion 1</td>
<td>Criterion 2</td>
</tr>
<tr>
<td>+</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>–</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><strong>89 (78–100)</strong></td>
<td><strong>91 (81–100)</strong></td>
<td><strong>94 (87–100)</strong></td>
</tr>
</tbody>
</table>

brachial plexus (8.0%; 95% CI, 3.4–12.6) and the sciatic nerve (9.4%; 95% CI, 4.6–14.2).

The calibrated manometer attached between the syringe and the needle showed that the pressure remained between 0 and 15 psi during all intraneural injections.

Discussion

This is the first study validating real-time intraneural injection criteria of US-guided blocks of supraclavicular brachial plexus and infragluteal sciatic nerve. This was achieved with a volume of only 0.5 ml. The ultrasonographic criterion ‘increase in cross-sectional surface area together with change in echogenicity’ identified intraneural injection with 94% precision. The cross-sectional surface area increased with 8–9% in both the supraclavicular brachial plexus and the subgluteal sciatic nerve.

An increase in cross-sectional surface area has previously been used as an indicator for intraneural injection. In some cases, swelling was accompanied by the appearance of a hypoechoic ring around the nerve (halo ring) and an increase in echo-lucent areas within the nerve.2 4 10 30 The latter observation has been incorporated into our third criterion as ‘change in echogenicity’.

An increase in nerve area of >15% has been proposed as a cut-off value to distinguish intraneural from extraneural injection in popliteal sciatic nerve block.4 6 This value represents an expert opinion, but experimental data are needed for different nerve locations and different volumes to establish a cut-off value that can be reliably detected by clinicians. In our study, we found that a lower percentage (9%) was sufficient for detection. Other reports on the amount of nerve swelling after intraneural injection show higher percentages, but they all used larger volumes: 57% increase (range: 14–200%) after 5 ml intraneural injection in pig brachial plexus10 and 158–706% increase after 10–20 ml intraneural injection in pig brachial plexus and femoral nerves.31 In humans, intraneural injection of 30–40 ml of local anaesthetic resulted in a nerve area increase of 45 (14%) in popliteal sciatic nerve block.5 Although volumes and tissue properties are different compared with our study, we have shown that earlier detection of a small amount of nerve swelling is possible and important, as it allows for needle repositioning and prevents injection of a larger amount of local anaesthetic. Although it has not been formally investigated, the risk of nerve damage is likely to be greater for high-volume injections. However, the type of local anaesthetic and its concentration are also important.

The terms ‘intraneural’ or ‘subepineurial’ for supraclavicular injection have been a source of debate.25 32 Although the configuration of epineural and other fascial layers around the brachial plexus is complex, we believe that a distinction can be made between intra- and extra-epineurial injections at the supraclavicular location. This has been the basis for the published work on intraneural vs extraneural stimulation thresholds in patients2 and in a study on the incidence of unintentional intraneural injection during US-guided supraclavicular block.3

The prevertebral and scalenic fasciae that encompass the entire plexus, specifically in the supraclavicular region, create a densely arranged nervous structure, which is internally separated by the epineuria of the individual cords. We argue that puncture of the outer layer of the brachial plexus and thus the epineural border and injection in the intra-epineural space would, as measured, increase the cross-sectional surface area of that specific part of the brachial plexus and, by definition, increase the entire cross-sectional surface area of the brachial plexus.

There are some important limitations to our study. Although the differences between cadaver and live subjects in ultrasonographic appearance of the brachial plexus and sciatic nerves are reported to be minimal,33 34 a cadaver model does not take into account the tissue oxygenation, blood circulation, and elasticity of the structures in vivo. This may alter the spread of injectate through the tissue. Although we used unembalmed cadavers within 12 h post-mortem, these differences may have played a role. The older age of the cadavers may also have affected nerve microanatomy.26

As soft tissues lose elasticity post-mortem, we assumed that injection pressures could be higher in cadavers than in living humans. However, all intraneural injections in this...
study had injection pressures < 15 psi. We can only speculate to whether the injection pressure would increase beyond this value in vivo when very low volumes are injected. Detection of a subtle increase in cross-sectional surface area of nerves (8–10%) may be more challenging in living subjects than in cadavers. Muscle contraction, pulsation, and respiration create motion in the US image that could hinder an accurate observation of nerve swelling. Detection of small changes in surface area (> 15%) has been found to be feasible in live subjects. The results of this study need to be tested for reproducibility in a clinical setting. Visualization of the nerve is dependent on the US equipment used, its settings, and the transducer, and use of different equipment could yield different findings. Finally, replication in an animal model may be advocated, provided that cryomicrotomy is used as a confirmatory test.

While the results of this study demonstrate that intraneural injection of a small volume can be detected with high precision, they do not provide evidence that the absence of nerve swelling corresponds to extraneural injection. Although clinically relevant, addressing this question in a statistically meaningful way would require a much larger sample size of at least double the current study. This was beyond the scope of our study.

In conclusion, we have shown in a cadaver model that intraneural injection can be detected by US by injecting a very small amount of injectate. Consequently, intraneural placement of the needle tip can be recognized at the initial phase of injection.

**Attribution**

This article should be attributed to Division of Vital Functions, Department of Anaesthesiology, and Department of Neurosurgery, both at the University Medical Centre Utrecht, Utrecht, the Netherlands, and to the Pain Centre, Department of Anaesthesiology, University Medical Centre Groningen, Groningen, The Netherlands.

**Acknowledgements**

The authors are indebted to Willem J.A. van Wolferen and Simon Plomp, both prosectors at the Department of Anatomy at the University Medical Centre Utrecht, The Netherlands, for their assistance during data collection. Also, we thank Linda M Peelen, statistician at the Department of Epidemiology, Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht, The Netherlands, for statistical advice.

**Declaration of interest**

None declared.

**Funding**

Support was provided from the Netherlands Organization for Scientific Research [Nederlandse Organisatie voor Wetenschappelijke onderzoek (NWO), The Hague, the Netherlands], grant 017.005.12.

**References**


15. Neale JM, Wedel DJ. Ultrasound guidance and peripheral nerve injury: is our vision as sharp as we think it is? Reg Anesth Pain Med 2010; 35: 335–7


25 Bigeleisen PE, Moayeri N, Groen GJ. Ultrasound-guided supraclavicular block may be intraneural. Anesthesiology 2010; 112: 251–2


32 Morfey D, Brull R. Ultrasound-guided supraclavicular block: what is intraneural? Anesthesiology 2010; 112: 250–1; author reply 251–2
