Ultrasound-guided approach to nerves (direct vs. tangential) and the incidence of intraneural injection: a cadaveric study

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Summary
This study evaluated the incidence of nerve puncture and intraneural injection based on the needle approach to the nerve (direct vs. tangential). Two expert operators in regional anaesthesia performed in-plane ultrasound-guided nerve blocks (n = 158) at different levels of the brachial plexus in cadavers, aiming either directly for the nerve (n = 77) or tangentially inferior to the nerve (n = 81). After reaching the outer limit of the nerve, the needle was intentionally advanced approximately 1 mm in both approaches, and 0.2–0.5 ml of saline was injected. Each operator classified (in real time) the needle tip and injectate as intraneural or not. Video clips showing the final position of the needle and the injection were evaluated in the same manner by seven independent expert observers who were blinded to the aims of this study. In addition, 20 injections were performed with ink for histological evaluation. Intraneural injections of saline were observed by the operator in 58% (45/77) of cases using the direct approach and 12% (10/81) of cases using the tangential approach (p < 0.001). The independent observers agreed with the operator in a substantial number of cases (Cohen’s kappa index 0.65). Histological studies showed intraneural spread in 83% (5/6) of cases using the direct approach and in 14% (2/14) of cases using the tangential approach (p = 0.007). No intrafascicular injections were observed. There was good agreement between the operators’ assessment and subsequent histological evaluation (Cohen’s kappa = 0.89). Simulation of an unintentional/accidental advancement of the needle ‘beyond the edge’ of the nerve suggests significantly increased risk of epineural perforation and intraneural injection when a direct approach to the nerve is used, compared with a tangential approach.

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Introduction
The potential risk for neurological insult resulting from peripheral nerve block is of major concern for every anaesthetist [1]. While there is controversy regarding the aetiology of these injuries, nerve puncture and/or intraneural injection are associated with neurological complications, and prevention of puncture and injection into nerves is likely to be a good strategy for avoiding long-term complications.

Many anaesthetists use monitoring systems such as low-current peripheral nerve stimulation and/or injection pressure monitors to aid the identification of intraneural puncture and injection. Neither of these techniques has been proven to be highly specific, and using algorithms to minimise the risk of nerve damage has also been suggested [2, 3].

Despite real-time visualisation of the needle as it approaches neural structures, the use of ultrasound guidance in clinical practice cannot prevent needle to nerve contact or epineurium perforation, even when performed by skilled individuals [4–6]. Inspection of the ASA Closed Claims Database suggests that nerve damage is much more prevalent among patients who receive upper limb blocks [7].

There are very few publications that describe how needle approaches may influence the incidence of epineural penetration. The incidence is strongly dependent on human factors, including: the expertise of the block performer; visualisation of the needle tip and outer limits of the nerve; force and speed of needle advancement; nerve depth; surrounding tissues; and needle angle. Also, some technical issues may play a role, such as the type and orientation of the needle bevel, the echogenicity of the needles and the resolution of ultrasound machines. After considering all possible human and technical factors that might make a procedure suboptimal, we believe that the angle of needle approach may influence the incidence of epineural penetration.

Common sense and practical experience suggest that if a needle is directed at a peripheral nerve margin (below or above the nerve), rather than targeting the centre of the nerve, it may affect the incidence of intraneural puncture and injection. In clinical practice, we have observed that, when using a tangential approach to the nerve, needle–nerve contact causes rotation of the nerve that prevents puncture of the epineurium (Fig. 1). We therefore aimed to evaluate the incidence of intraneural puncture and injection (as a primary outcome) in various nerves of the upper limb of an experimental cadaveric model. We simulated unintentional/accidental advancement of the needle ‘beyond the edge’ of the nerve using two different approaches, namely a direct and tangential approach to the same nerve. As a secondary outcome, we measured interrater agreement between the expert operators and independent expert observers.

Figure 1 Likely outcomes for the tangential and direct approaches when the needle is advanced approximately 1 mm after the needle tip has reached the presumed outer limits of the nerve, based on ultrasound (US) views. (a) Schematic representation. (b) Corresponding US images of the ulnar nerve at the arm.
Methods
This study was performed at the Universities of Barcelona and Antwerp between May 2014 and April 2015. The research protocol (involving cadaver specimens) was approved by the internal committee of the Anatomy and Embryology Unit of the School of Medicine at the University of Barcelona, Spain. Five fresh human cadavers tested for biological risk (Human immunodeficiency virus, Hepatitis B virus and Hepatitis C virus at 24–72 h postmortem) were used to perform multiple ultrasound-guided blocks (Linear 18-MHz US transducer, Focus 800; BK-medical, Copenhagen, Denmark). Exclusion criteria included: the presence of upper limb nerve pathology; anatomical macroscopic pathology; or ultrasonographic pathology. Following randomisation, a short-bevelled echogenic needle (Sonoplex 50 mm needle; Pajunk, Geisingen, Germany) was advanced in-plane towards different nerves and at different levels of the brachial plexus (Table 1).

After a pre-procedure ultrasound scan to determine puncture site, the skin was marked. Two approaches were performed at each nerve/level, separated by 2–3 cm. In one approach, the needle was aimed directly at the middle of the nerve. The second approach was tangential, i.e. the needle was directed immediately under the nerve with the bevel upward. The intention was to put the needle tip close to the nerve and then advance the needle by approximately 1 mm until the nerve clearly moved (Fig. 1). Two experts in ultrasound-guided regional anaesthesia (UGRA) completed all procedures (authors LS and XB). Relying on both visual and tactile inputs while advancing the needle, it was possible to ascertain whether the needle-tip position was intraneural. With the tip of the needle in place and visible on the monitor screen, a small volume of saline (0.2–0.5 ml) was injected. The two operators recorded whether the saline had been injected intra- or extraneurally, based on the presence or absence of neuronal swelling (expansion of the cross-sectional surface area of the nerve associated with a change in echogenicity), or by the observation of spread between fascicles (hypo-echoic structures inside the nerve).

During each procedure, a 10-s video clip was recorded to show the final position of the needle and the injection of saline. All video clips were then assessed by seven independent experts in UGRA. These observers were blinded to the clinical project and study objectives and were instructed to answer two simple questions, each with three possible responses:

- Do you think that the needle tip is intraneural? (Yes/No/Not possible to evaluate)
- Do you think that intraneural injection occurred? (Yes/No/Not possible to evaluate)

An additional cadaver was used for the histological study. Approaches were performed at the same levels of the brachial plexus as described for the ‘saline’ group (Table 1). Applying the same methods,

| Table 1 | Operator evaluations of incidence of intraneural needle positioning and intraneural solution spread at the different levels of the brachial plexus, based on the needle approach to the nerve. Values are number (proportion). |
|---------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|
|         | Direct approach |                                                                                                                                  | Tangential approach |
|         | Needle position | Spread (%)                                                                        | Needle position | Spread (%) |
| Root    |                  |                                                                                                                                  |                  |
| C5      | 5/6 (83%)        | 2/6 (33%)                                                                        | 5/12 (42%)      | 2/12 (18%) |
| C6      | 11/11 (100%)     | 3/11 (27%)                                                                        | 0/10 (0%)       | 0/10 (0%)  |
| Median; nerve |                  |                                                                                                                                  |                  |
| Axillary | 8/10 (80%)       | 6/10 (60%)                                                                        | 4/10 (40%)      | 3/10 (30%) |
| Elbow   | 10/10 (100%)     | 9/10 (90%)                                                                        | 2/10 (20%)      | 2/10 (20%) |
| Forearm | 9/10 (90%)       | 7/10 (70%)                                                                        | 0/10 (0%)       | 0/10 (0%)  |
| Radial; nerve |                  |                                                                                                                                  |                  |
| Axillary | 8/10 (80%)       | 7/10 (70%)                                                                        | 1/9 (11%)       | 0/9 (0%)   |
| Ulnar; nerve |                  |                                                                                                                                  |                  |
| Axillary | 8/10 (80%)       | 5/10 (50%)                                                                        | 2/10 (20%)      | 2/10 (20%) |
| Forearm | 9/10 (90%)       | 6/10 (60%)                                                                        | 2/10 (20%)      | 1/10 (10%) |
| Total   | 68/77 (88%)      | 45/77 (58%)                                                                        | 16/81 (19.7%)   | 10/81 (12.5%) |

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0.1–0.2 ml of ink (instead of saline) was injected. After ink injection, an anatomist dissected the nerve. The dissected nerves were then fixed in Carnoy’s solution, processed and embedded in paraffin blocks according to standard protocols. Sections of 5 μm thickness were prepared and stained for haematoxylin and eosin. Two pathologists (who were blinded to the study purposes) microscopically examined the samples to determine whether ink had entered the epineurium. In addition, they looked for signs of fascicular damage i.e. perineurium infiltration [8].

According to our clinical experience and hypothesis, when using the direct approach 90% of needle passes resulted in the needle tip being introduced into the nerve, compared with less than 40% when using the tangential approach. Thirteen samples in each group were sufficient to validate the hypothesis with a β error of 20% and an α error of 5%. Chi-square was used to test if the incidence of intraneural needle-tip position or intraneural injection was independent from needle approach. A total of 158 needle passes were performed, including 77 direct and 81 tangential. In the histological part of the study, 6 direct and 14 tangential approaches were used. Due to the small sample size, Fisher’s exact test was used in this part of the study. A p value of 0.05 was considered significant.

Fleiss’ kappa was used to determine concordance between the seven independent observers [9]. A consensus between the seven observers was assigned by majority vote. When no consensus was reached (this was the case in nine samples because some of the seven observers answered ‘Not possible to evaluate’), those samples were excluded from further analyses in which the consensus vote was used. Cohen’s kappa value was used to evaluate the concordance of the consensus with respect to the two observer’s decisions during the procedure. Landis–Koch classification was performed to evaluate inter-observer agreement [10, 11]. Statistical analysis was performed using R (R 3.3.1; The R Foundation for Statistical Computing, Vienna, Austria).

Results
In the 158 procedures (direct vs. tangential, expert evaluation study), operators observed intraneural needle-tip positioning in 88% (68/77) of the cases when using a direct approach to the nerve, and in 19% (16/81) of the cases when using a tangential approach (p < 0.001). They also observed local anaesthetic injections inside the nerve in 58% (45/77) of the cases using the direct approach and in 12% (10/81) of the cases using the tangential approach (p < 0.001).

The incidence of intraneural needle positioning and intraneural solution spread at the different levels of the brachial plexus (based on the needle approach to the nerve) is shown in Table 1. Independent and blinded expert anaesthesiters agreed with these results (Fig. 2).

Measuring multirater agreement between the blinded experts for qualitative (categorical) items, Fleiss’s kappa index was 0.51 for intraneural needle position and 0.58 for the intraneural spread of injectate. According to the Landis–Koch classification, our results suggest moderate agreement between observers (Kappa index between 0.41 and 0.60) [9, 11].

Based on the assumption that intraneural needle position and injection occurred when at least four of the seven experts agreed, the relative risk indicates that the needle position is 3.5-fold more likely to be intraneural, and the incidence of injections is likely to be fourfold higher when the needle is aimed directly at the nerve (Table 2).

To measure the inter-rater agreement between the two operators and the blinded observers for qualitative (categorical) items (secondary outcome), we used Cohen’s kappa index. The Kappa values were 0.56 for intraneural needle placement and 0.65 for the intraneural spread of injectate, which indicated moderate and substantial agreement (Kappa index between 0.61 and 0.80, respectively) according to the Landis–Koch classification.

Histological analysis of 20 samples showed that intraneural injection occurred in 83% (5/6) of cases when using the direct approach, and in 14% (2/14) of cases when using the tangential approach (p = 0.007). There was no intrafascicular infiltration of the ink in any case, and the fascicles were not damaged (Fig. 3).

The relative risk of intraneural injection when using a straight direct needle approach was 5.8-fold higher than that when using a tangential approach (Table 2).

As mentioned in the Methods, we used Cohen’s kappa index to measure inter-rater agreement between the operators and the histological assessment for the qualitative (categorical) item (intraneural spread of
injectate). This produced a mean index of 0.89 for the evaluations. In accordance with the Landis–Koch classification, our results suggest almost perfect agreement (Kappa index between 0.8 and 1.0) [11].

Discussion

Our study has demonstrated that direct approach of the needle towards the nerve during UGRA in upper-limb blocks results in a higher incidence of intraneural injections than when using a tangential approach to the same nerve. The risk of nerve damage in regional anaesthesia, although infrequent, is a concern when performing peripheral nerve blocks [12, 13]. Permanent nerve damage is a complication that should be avoided at all costs. Even though not every intraneural injection will lead to nerve damage, this fact cannot be easily appreciated with the tools available today [14, 15]. The challenge faced by clinicians is how to diagnose epineural intrusion by the needle tip and intraneural injection when using ultrasound [16, 17].

The advantage of using cadavers in practise ultrasound-guided needle technique is well established [18, 19]. They make it possible to simulate and evaluate the consequences of inappropriate ultrasound-guided needle techniques, and may help to improve safety.

Since the introduction of UGRA there have been many published articles that described unintentional neuronal penetration, intentional intraneural injection, and the anatomical and histological features of these insulted nerves [4, 20]. It has also been suggested that the addition of other techniques, such as adding low-current peripheral nerve stimulation, injection pressure monitoring and hydro-localisation, should decrease the incidence of intraneural needle placement and injection [21]. Even the presence of paraesthesia is not a reliable sign, as this usually results in the withdrawal of the needle tip before injection in the clinical setting. The use of short-bevelled regional anaesthesia needles has been demonstrated to decrease the incidence of intrafascicular needle-tip placement and should, therefore, be associated with a lower incidence of permanent nerve damage [22, 23].

The neural structure is of utmost importance, and it varies greatly depending on position along the upper limb. There are obvious changes in the epineurium and connective tissues and in the number and size of the fascicles present [24]. This difference in neuronal composition was evident and important when using peripheral nerve stimulators before the widespread availability of ultrasound [25]. Differences in surrounding tissues, such as fascia, fat and muscle, can influence the approach used to perform peripheral nerve block and may help to detect epineural penetration. Moreover, the term 'intraneural injection' seems less controversial in the context of complex neural structures such as the sciatic nerve, or when using

Figure 2 Box plot of the results of the video evaluation by the independent observers. The needle location, i.e. intraneural vs. extraneural (a), and the intraneural or extraneural spread of the injectate were evaluated (b). Observers were blinded to the type of needle approach (□, direct, ◦, tangential).
particular techniques, such as the supraclavicular approach to the brachial plexus [15, 26, 27].

The primary benefit of ultrasound is that it enables the limits of the nerve to be determined and the needle-tip position to be monitored. Visualised movement of surrounding tissues and the nerve during needle insertion aids correct needle-tip placement. However, if not appreciated, this tissue movement may result in increased intraneural needle-tip placement. Techniques such as hydro-dissection and hydro-localisation can also be used to confirm needle-tip position, and should be used in conjunction with the previously mentioned techniques to decrease the incidence of intraneural needle-tip placement. Perforation of the epineurium is not always easy to observe. The detection of intraneural needle placement may be influenced by additional factors, such as resolution of the ultrasound machine, characteristics of the nerve and experience of the anaesthetist. However, the most important factor to consider when evaluating intraneural positioning of the needle is subepineural injection of the first fraction of the anaesthetic solution, characterised by increased injection pressure relative to extraneural injection and nerve swelling [28].

Despite an extensive search of published articles, our group did not find any studies that evaluated the risk of puncture and intraneural injection based on the approach taken to the nerve, nor have we been able to find evidence of the risk associated with needle approach described in the methods section of our article. Our study establishes that the approach can be important when estimating the incidence of intraneural injection. We believe that the introduction of a systematic tangential approach could significantly reduce the risk of nerve penetration and intraneural injection. Although perforation of the epineurium and subsequent injection does not always result in neurological damage [14], we should remain vigilant and use all tools at our disposal to avoid intraneural injection [29, 30]. Since we are performing the tangential approach routinely in clinical practice, we have no concerns about the effectiveness of the block [31].

**Table 2** Comparison of risk of intraneural puncture with needle approach, analysis according to operator, expert observer and histological evaluations.

<table>
<thead>
<tr>
<th>Incidence of intraneural puncture</th>
<th>Direct</th>
<th>Tangential</th>
<th>p value</th>
<th>Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operator</td>
<td>58% (45/77)</td>
<td>12% (10/81)</td>
<td>&lt; 0.001</td>
<td>4.7 (2.5–8.7)</td>
</tr>
<tr>
<td>Observer</td>
<td>58% (42/72)</td>
<td>14% (11/77)</td>
<td>&lt; 0.001</td>
<td>4.0 (2.2–7.2)</td>
</tr>
<tr>
<td>Histological</td>
<td>83% (5/6)</td>
<td>14% (2/14)</td>
<td>0.007</td>
<td>5.8 (1.5–22.1)</td>
</tr>
</tbody>
</table>

**Figure 3** Microscopic images (40x) of the median nerve at the elbow after the injection of 0.1–0.2 ml of Indian ink. (a) After a direct approach, Indian ink is present in the epineurium, but does not enter the perineurium or endoneurium of the nerve fascicles. (b) After a tangential approach, the Indian ink remains outside the nerve and does not enter the epineurium.
This study has some limitations. The most important limitation is the methods used. It might be considered ‘not conforming with actual practice’ and ‘unconventional’ because we advanced the needle after reaching the edge of the nerve. Needle approaches, direct or tangential to the nerve, were performed by seeking contact and advancing the needle slowly and progressively, simulating unintentional advancement of the needle that may occur under various suboptimal conditions. Such a simulation is akin to the performance of car crash tests by car manufacturers, which are valid even though drivers are supposed to stop for an obstacle. The difficulty of avoiding personal bias by favouring the new ‘tangential’ approach is another important limitation. This study considers the different human factors between operators, determined mostly by differences in experience and expertise. There are individual variations in visualisation of the needle and nerve as well as the force and speed of advancement of the needle. However, for both approaches, the operators used the same nerves, the same ultrasound machine, and had a rigorous intent ‘not to perforate’, thus enabling a valid comparison. Although perforation of the epineurium was not intended, it was a potential risk (Fig. 1). The speed of needle advancement was slow enough to minimise the risk of this factor in nerve perforation, and the same procedure was performed in both approaches.

The way in which the needle was advanced in this study would not be usual while performing a peripheral nerve block in the clinical setting, or by experts in UGRA, but could happen unintentionally. Anaesthetists who have limited experience may not visualise the needle tip correctly, may use too much force and may accidentally penetrate the epineurium. Moreover, in clinical practice the resolution of ultrasound machines is generally not sufficient to define the epineurium [32]. Thus, for beginners and experts, the risk of unintentionally penetrating the epineurium might be higher when using a direct approach. Another limitation is the use of cadavers, in which the consistency of the tissue and the muscle tone is altered, particularly tension between anatomical structures. Similarly, there was an obvious lack of blood flow in the vasculature, which would have had an impact on the tactile and visual aspects of the techniques used. Finally, the cadaver had a non-physiological tissue-dependent temperature and working environment (approximately 18 °C in an anatomy laboratory). We cannot determine whether these alterations had any influence on the absolute results. These alterations might have played a role in the increased incidence of epineural penetrations in both approaches. However, the bodies used were fresh, not previously frozen, and the elapsed time since death largely avoided the presence of rigor mortis. It was not possible to use a nerve stimulator, and our measurements with the pressure monitor were not conclusive due to the absence of physiological conditions.

The authors (LS and XB) who conducted the experiment had complete information on the technical process; however, the independent observers did not have all the information that is normally available to a performer of a block. Specifically, visualisation of the needle approach, the movements of the different tissues, and the tactile sensation when passing through those different layers are important in determining whether the outer limit of the nerve has been reached. The independent observers had only 10 s of video material. They could only see the final position of the needle tip and the start of the injection to help them evaluate whether a needle was in place and whether there was intraneural injection. The lack of visual and tactile information, as well as the actual resolution of ultrasound machines, makes it difficult to identify the outer limit of a nerve, as was shown by the differences in scores between operators and independent observers.

Other factors could have influenced the scores assigned by the independent observers, such as the influence of the hyperechogenicity of the needle on the surrounding nerve structures, injection near a hypoechoic structure, back-flow of saline (even when the needle is definitely intraneural) and an injection on both sides of the epineurium.

Despite these limitations, our study shows important differences between the incidence of intraneural injection in the direct and tangential nerve approaches. We suggest that even though this study was performed on cadavers and is subject to the limitations described above, future articles describing the evaluations of the incidence of intraneural
injection should include information on the type of needle approach. This study was conducted on human specimens and should be generalisable to clinical settings. Although further investigation in living animal specimens would be desirable, the results of this simulation of unintentional needle advancement appear sufficient to conclude that the tangential approach, i.e. aiming above or below the nerve, may help to avoid nerve penetration and should be included in algorithms for performing peripheral nerve block in order to prevent puncture and intra-neural injection. Moreover, we have not experienced any reduced block efficacy when performing the tangential approach in our daily practice (Fig. 4, modification from X. Sala-Blanch) [30].

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Competing interests
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