Incidence of sub-perineural injection using a targeted intracluster supraclavicular ultrasound-guided approach in cadavers

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Abstract

Background: Multi-injection targeted intracluster injection ultrasound-guided supraclavicular brachial plexus block has been advocated to provide a faster onset of anaesthesia compared with a double injection technique. By placing the needle within clusters of hypoechoic structures, corresponding to neural tissue, this technique may increase needle trauma and the incidence of nerve injury. This study assessed the rate of sub-perineural needle placement with a single intracluster brachial plexus injection in the supraclavicular fossa of human cadavers.

Methods: A single ultrasound-guided intracluster brachial plexus injection was performed bilaterally at the supraclavicular fossa on 21 lightly embalmed clinical grade cadavers. Using an in-plane technique, an echogenic needle was positioned to target the middle or lower trunk 'cluster', where 0.2 ml black India ink was injected. An effort was taken to avoid the hypoechoic structures with the needle tip. Tissue samples were assessed histologically by two experienced reviewers.

Results: All 42 injections were sonographically assessed to be within the 'main cluster'. Ink was extra-epineural in 13/41 (32%), sub-epineural but outside perineurium in 18/41 (44%), and sub-perineural in 10/41 sections (24%; 95% confidence interval, 13–41%). The histology from one injection was uninterpretable. Of the 10 sub-perineural deposits, the ink was intrafascicular in nine sections.

Conclusions: We observed a high rate of sub-perineural injection with a single intracluster injection. Thus the targeted intracluster injection supraclavicular block cannot be recommended until further evidence is available regarding the safety of this technique.

Keywords: brachial plexus; cadaver; histology; nerve injury; regional anaesthesia; supraclavicular block; ultrasonography; ultrasound-guided nerve block
First described in the early 20th century, the supraclavicular brachial plexus block did not gain popularity until the introduction of high-quality ultrasonography in the past 20 yrs. The ability of ultrasound imaging to show the lung and vessels has reduced the risk of pneumothorax and intravascular injection while allowing precise placement of the needle tip to provide reliable anaesthesia with a high degree of patient satisfaction. However, the introduction of ultrasound as a nerve localisation technique has not been shown to reduce the incidence of postoperative neurological symptoms. The incidence of neurological symptoms after supraclavicular blocks can be as high as 14% at 1 week.

The optimal needleng technique for the supraclavicular block is still under investigation. Various strategies of where to inject the local anaesthetic and how many injections to perform around the brachial plexus have been described. Recently the ‘targeted intracluster injection’ technique for supraclavicular brachial plexus block has been described to provide comparable success rates with other ultrasound approaches with the advantage of a shorter block onset time. This approach involves placing the needle in the middle of ‘nerve clusters’ or hypoechoic structures, representing neural tissue, at the level of the brachial plexus trunks and divisions. Multiple needle passes are required to target all the clusters for the technique, relying only on the use of a small volume of injected saline (<1 ml) to ensure the needle tip is correctly positioned inside the intended targets (main or satellite clusters).

The targeted intracluster injection technique contrasts to other ultrasound approaches, which attempt to avoid needle nerve contact while still producing efficient and effective anaesthesia of the plexus. The risk of neurological complications associated with the targeted intracluster injection technique has not been established. In particular, no correlation of intracluster injection with histology in cadavers has been performed to show the incidence of needle trauma when the tip is placed in the middle of tightly bundled hypoechoic structures within the trunks or divisions of the brachial plexus. The objective of this study was to assess the rate of sub-perineural needle placement with a single brachial plexus injection in the supraclavicular fossa using an intracluster technique.

**Methods**

After research ethics board approval (REB number 1020775, Nova Scotia Health Authority, Halifax, Canada) was obtained, bilateral supraclavicular intracluster injections were performed on 21 lightly embalmed clinical grade cadavers. Preparation of the cadavers as described results in supple tissues, high-quality ultrasound images and excellent histology. A single regional anaesthesia fellow (S.R.) supervised by the same experienced regional anaesthesiologist (J.S.) performed all injections.

**Block procedure**

Using a Sonosite M-turbo ultrasound machine (Fujifilm Sonosite Inc., Bothell, WA, USA), a linear array transducer (7–13 MHz), and a 22G, 50 mm echogenic needle (Pajunk SonoPlex NanoLine, Geisingen, Baden-Württemberg, Germany), an in-plane lateral to medial technique was performed. The transducer was positioned at the level of the C5 nerve root in the interscalene groove. As the transducer moved caudally towards the supraclavicular fossa, the C5 and C6 nerve roots were observed to coalesce forming the upper trunk, and the plexus was followed distally into the supraclavicular fossa. Our objective was to position the needle within the middle or lower main ‘cluster’ deep to the superior trunk as a target for a single injection, as we felt this to be clinically relevant and representative of a typical injection within the supraclavicular fossa. Although the desired needle position was within the ‘cluster’, an effort was taken to avoid unnecessary movements and hypoechoic structures with the needle tip. Daler Rowney FW Black (India) Acrylic Ink, 0.2 ml, was injected using a Harvard Apparatus Pump (Holliston, MA, USA) at 0.4 ml min⁻¹. Ultrasound stills and cine clips were recorded. Two experienced regional anaesthesiologists not present for the injections assessed the videos to determine if successful intracluster injection was performed and if expansion of a hypoechoic structure was seen during injection.

**Tissue fixation and histology**

Tissue blocks were removed by RS, immediately placed in 1 L containers with 10% buffered neutral formaldehyde (Fischer Scientific, Whitby, ON, Canada), and agitated (Max Q 2000-Thermo Scientific, Iowa USA, Model #SHKE2000, Serial # 141009037517) to maximise fixative penetration and hardening. The 42 specimens were then trimmed and bisected through the anticipated needle injection point to create mirrored tissue planes of the site. The surface containing the needle injection point was placed face downwards in labelled cassettes. Bisection of the tissue samples and meticulous attention to the placement of the tissues in the cassettes ensured the microtome site would be as close as possible to the site of deepest ink deposition.

Blocks were coded with random numbers to blind examiners of its source. Tissues were post-fixed for 4–5 weeks. After fixation, tissues were washed with two changes of 70% ethanol, dehydrated, cleared of dehydrating solutions, and paraffin wax infiltrated overnight using an automated tissue processor (Leica ASP300; Leica Biosystems, Concord, ON, Canada). Tissues were embedded into wax blocks (Leica EG1150H/C Tissue Embedding Centre; Leica Biosystems) for sectioning, with 12 μm sections cut using a microtome (Leica RM2255; Leica Biosystems) mounted on glass slides (VWR Microslides, Colorfrost Gold, Fischer Scientific, Pittsburgh, PA, USA), allowed to dry in an oven overnight at 45°C, and deparaffinised by xylene and graded ethanol. Slides were stained with Harris Hematoxylin (Surgipath; Leica Biosystems, Richmond, IL, USA) and eosin, using a manual capillary system
Stained slides were dehydrated and mounted with a non-aqueous mounting media (Cytoseal XYL; Richard-Allan Scientific, Kalamazoo, MI, USA) and cover-slipped. Slides were prepared by a blinded technician (PC), coded with a random number to blind the examiners (JS and KK) to the source of the tissue, and examined using a stereomicroscope (Olympus SZ40; Olympus Optical Co., Tokyo, Japan). Deposition of ink was classified according to the deepest layer in which it was found as: ink present on the slide, ink in muscle only, extra-epineural, sub-epineural but outside perineurium, and sub-perineural. Distortion of fascicles by ink was assessed for the sub-perineural deposits (intrafascicular).

Sample size estimation
In the absence of data regarding the incidence of sub-epineural injection after the ‘targeted intracluster injection’ supraclavicular block, we used data from the study by Szerb and colleagues, who estimated the incidence of sub-epineural injection after intraplexus approach for inter-scalene block to be 11.5%. Sample size was calculated using the precision approach for the confidence interval. With a precision estimate of 0.1, sample size was calculated to be a total of 37.5 cadavers. Accounting for 10% inconclusive histology, total sample size of 42 cadavers was used.

Statistical analysis
Data analysis was performed using SPSS 24 (IBM SPSS Statistics for Windows, version 24.0; IBM Corp., Armonk, NY, USA). The incidence of extra-epineural, sub-epineural, sub-perineural, and intrafascicular ink deposits is expressed as a proportion (%) with 95% confidence interval (CI). Inter-rater agreement between the operator and the expert reviewers of the intracluster technique for confirming intracluster was assessed using percent agreement (i.e. the percentage of times both raters had the same rating) and AC1 (i.e. an inter-rater agreement statistic that controls for chance agreement, and is preferred over kappa when events are rare). AC1 can be interpreted in a similar fashion to kappa (0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; 0.81–1.0, almost perfect agreement). Association between operator’s and reviewers’ observations of intrafascicular expansion and histology findings were analysed using Fischer exact test.

Results
Bilateral intracluster injection was performed at the supraclavicular level of the brachial plexus block in 21 lightly embalmed clinical grade cadavers. Histology of one of the 42 tissue specimens obtained was uninterpretable because of the inadvertent oblique sectioning angle. The operator assessed all 42 injections to conform to the intracluster technique. Reviewer 1 assessed 36/42 to be intracluster, four not intracluster, and was undecided for two injections. Reviewer 2 assessed 39/42 to be intracluster, and three not to be intracluster. Inter-rater agreement between operator and Reviewer 1, when the undecided responses were treated as missing data, was 90% (AC1=0.89). When these undecided responses for Reviewer 1 were treated as not intracluster, the inter-rater agreement for operator and Reviewer 1 was 86% (AC1=0.84). Reviewer 2 had no undecided responses, and had 98% agreement (AC1=0.98) with the operator regarding performance of intracluster injection.

The incidence of extra-epineural ink deposits (Fig 1) was 13/41 (32%), sub-epineural (but extra-perineural) (Fig 2) ink deposits was 18/41 (44%), and sub-perineural ink deposits was 10/41 (24%; 95% CI, 13–41%). The ink distorted fascicles in nine of the 10 sub-perineural deposits indicating intrafascicular injection (Fig 3).

The operator had a sensation of a tactile pop while entering the cluster in 40/42 injections (95.2%). Expansion of hypoechoic structures during injection was seen by the operator in 15/42 injections (36%) and by offline reviewers 3/42 (7%) and

<table>
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<th>Histology sub-perineural</th>
<th>P-value</th>
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<tr>
<td>Operator: No</td>
<td>22</td>
<td>4</td>
<td>0.130</td>
</tr>
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<td>29</td>
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<td>2</td>
<td>1</td>
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</tr>
<tr>
<td>Reviewer 2: No</td>
<td>18</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Reviewer 2: Yes</td>
<td>13</td>
<td>3</td>
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</tbody>
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Fig 1. Haematoxylin and eosin stained slide demonstrating extra-epineural ink.
There was no significant association between hypoechoic structure expansion observed by the operator on ultrasound and sub-perineural deposits (Fisher’s exact test; $P = 0.130$). No association was found between sub-perineural deposits and hypoechoic structure expansion observed by offline reviewers, respectively ($P = 1.000$ and $P = 0.712$) (Table 1).

**Discussion**

The intracluster technique resulted in a 24% sub-perineural injection rate with just a single needle pass. No association was found between hypoechoic structure expansion observed during injection and sub-perineural ink deposits. Although there has been some debate regarding the safety of intraneural injection, the current evidence suggests that an intraneural injection is harmful and should be avoided. The reason for harm is thought to be multifactorial including direct injury by the needle, injectate, and secondary inflammation. Historically, a needle position under the epineurium has been called ‘intraneural’. However, this definition of intraneural can be further subdivided into sub-epineural and sub-perineural. Although an injection deep to the epineurium generally leads to reversible anaesthesia, sub-perineural injections are associated with long-term nerve injury. Therefore, all efforts should be made to avoid sub-perineural placement of needle and injection of local anaesthetic.

The studies that have compared the targeted intracluster injection technique to conventional approaches show that the technique provides a faster median onset time (8–10 min), without a difference in the success rate. However, these studies consistently found that this approach required a higher number of needle passes. Given a 24% sub-perineural rate, each additional pass into the ‘clusters’ of hypoechoic structures would result in a cumulative increase in the chance of sub-perineural needle tip placement and injection. Furthermore, this technique results in an increased incidence of paraesthesias compared with axillary or infraclavicular approaches, and new neurological symptoms are more common in patients with procedure-induced paraesthesia.

The targeted intracluster injection technique studies used hydro-dissection with 1 ml saline to determine needle tip position and observed the internal elements of the clusters to ‘roll away’ when contacted by the needle tip. Low volume injectate (0.5 ml) does not allow discrimination of needle tip position as either intraneural (sub-epineural or intra-fascicular) or extra-epineural. Even small volumes of crystallloid hydro-dissection can cause inflammation and axonal degeneration when deposited intraneurally. As a result, hydro-dissection may not increase the safety of the targeted intracluster injection technique, and paraesthesias noted during the this block may represent subclinical neurological injury.

Histologic observation of the brachial plexus shows that the initially mono- and oligo-fascicular roots become poly-fascicular with an increase in the relative amount of connective tissue to neural tissue. This has led to the belief that risk of inadvertent intrafascicular needle placement within the brachial plexus may be lower in the supraclavicular fossa compared with the interscalene groove with use of a short-bevel needle. Our findings are in stark contrast to previous findings that needles tend to traverse nerves without disturbing the fascicles, or that a short-bevel blunt needle is less likely to pierce the perineurium. The degree of intrafascicular disruption seen in our study is consistent with previous work that shows that once the perineurium is breached a short-bevel needle causes significant trauma compared with a long-bevel needle.

In a recent study comparing supraclavicular targeted intracluster injection to infraclavicular brachial plexus block,
the authors stated that ‘our cumulative clinical experience so far (>300 ultrasound-guided targeted intracluster injection supraclavicular blocks) has not revealed any case of a motor deficit at 1 week or sensory symptoms (numbness) exceeding 1 month’.\textsuperscript{12} This should not be reassuring, as it has previously been suggested that 70 000 subjects per group might be required to prove through a randomised trial that the risk of a severe, permanent neurological complication (4/10 000) can be halved.\textsuperscript{32} The logistics required to perform this randomised controlled trial are likely insurmountable. As a result, it is possible that histological evidence may be the best available surrogate for needle-related nerve trauma during peripheral nerve block.

There was considerable variation in the perception of injections resulting in hypoechoic structure expansion between operators and reviewers. There was no significant association between hypoechoic structure expansion during the block and sub-perineural ink found with histology, meaning that neither the operator nor the offline sonography reviewers were reliably able to determine which injection leads to sub-perineural ink deposition. This may be explained by the low accuracy of ultrasound to detect small volume injectate, the interpretive variability in the assessment of the ultrasound image, the lack of lateral resolution of the high-frequency linear ultrasound transducer to detect small fascicles (1 mm at 10 MHz), and the imprecision with which surrounding connective tissue can be distinguished from embedded neural tissue.\textsuperscript{23,26,32,33}

A limitation of our study is that a cadaver histological study cannot prove that sub-perineural injections would have led to an increase in neurological complications compared with injections outside the perineurium or epineurium. In addition, cadaver preparation could affect the integrity of the perineurium, although we have previously verified that it is impervious to the ink used,\textsuperscript{15} which is similar to the results of other researchers.\textsuperscript{34} Our method might underestimate the frequency of sub-epineurial ink if the deepest deposition of ink was not within the microme sample. This risk was minimised by bisecting each tissue sample. We did not use hydrodissection, as saline or dextrose would have diluted the ink leading to diffuse tissue staining. We were unable to measure the injection pressure because of the small injectate volume (0.2 ml) and ink viscosity. Avoiding elevated injection pressure might have reduced the rate of intrafascicular injection.\textsuperscript{35} Similarly, avoidance of an evoked motor response with a current below 0.2 mA within a cluster might allow the needle to be repositioned extrafascicularly, but no previous studies with the targeted intracluster injection technique have used this modality, which we are unable to test in our model.

In conclusion, we observed a 24% sub-perineural injection rate with a single intracluster injection and no significant association between sonographic hypoechoic structure expansion and sub-perineural ink deposits in a study of lightly embalmed human cadavers. Until further evidence is available regarding the safety of this technique, use of the targeted intracluster injection supraclavicular block cannot be recommended for an only modest improvement in onset time.

Authors’ contributions
Conception and design of the study: JJS, KK, VU.
Drafting of the protocol: JJS.
Cadaver injections: SR, JJ.

Gross dissections: RS.
Production of the slides: PC.
Histology analysis: JJS, KK.
Analysis of results: JJS, KK, VU.
Drafting of the manuscript: JJS, KK, VU.
Revising manuscript and approval for publication: all authors.

Declaration of interests
VU has been a principal investigator for a Recro Pharma (Devault, PA, USA) funded clinical trial on i.v. meloxicam. However, this did not directly or indirectly affect the conduct or reporting of this study. The other authors have no disclosures.

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