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Published in:
Regional Anesthesia and Pain Medicine

DOI:
10.1136/rapm-2022-104282

Publication date:
2023

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Document Version
Peer reviewed version

Link to publication in Discovery Research Portal

Citation for published version (APA):

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The accuracy of injection pressure measurement at peripheral nerves using high resolution 40MHz ultrasound in an anesthetized porcine model

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This article has been accepted for publication in Regional Anesthesia & Pain Medicine (2023) following peer review, and the Version of Record can be accessed online at https://dx.doi.org/10.1136/rapm-2022-104282. This manuscript version is made available under the CC-BY-NC 4.0 license.
Conflicts of Interest:

Nil

Funding:

Nil

Running Head: Injection pressure measurement at peripheral nerves

Word count: 3028

Keywords: Ultrasonography; Pressure; Injury; Fascicle: Mechanisms

Clinical trial number and registry URL: N/A

Contributors: GML: Project development, data collection and management, data analysis and manuscript writing. AC: Data collection; AS: Data collection and Data analysis. PW: Data collection; FW: Data collection. Manuscript editing; MAR: Data analysis, manuscript writing and editing. All authors have read and approved the manuscript.

Funding sources: Funded by the NIAA BJA/RCOA PhD studentship 2014/R2/05 and Sinapse, Scottish Imaging Network.

Disclosure and conflicts of interest: No competing interests.

Patient consent for publication: Not required.

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ABSTRACT

Fluid injection pressure measurement is promoted as a marker of needle tip position that discriminates between tissue layers. However, clinical ultrasound has insufficient resolution to identify the exact position of the needle tip. Our primary objective was to use 40MHz ultrasound in anesthetized pigs in order to precisely locate the tip of the needle, and measure opening injection pressure in muscle, at close epineurium and in subepineurium.

Methods: We surgically exposed the axillae of four anesthetized pigs. Two operators placed a 40MHz ultrasound transducer over the pectoral muscle and imaged axillary, median and radial nerves. Injections (0.5 ml) were randomized to in-plane and out-of-plane needle trajectories and flow rates of 1, 6 and 12 ml.min⁻¹

Results: We identified 541 fascicles in 23 nerves. The ratio of fascicle area to nerve area remained constant at ~ 0.30 for all nerves. Axillary nerves were smaller than median and radial nerves, difference in diameter (95%CI) 1.61 (0.87 to 2.36) mm, P < 0.001 and 1.59 (0.82 to 2.36) mm, P = 0.001 respectively. Axillary nerves had less fascicles per nerve than median nerves, difference 7.63 (2.43 to 12.83) and radial nerves, difference 9.02 (3.64 to 14.40). We visualised the circumneurium and injection within the subcircumneural compartment. Intraneural injection increased nerve area (SD) from 5.7 (2.2) mm² to 13.7 (5.5) mm², difference 8.0 (5.4 - 10.6) mm², P < 0.001. Mean injection pressure was greater in subepineurium compared to muscle, geometric ratio 2.29 (1.30 to 4.10), P < 0.001; and greater on epineurium compared to muscle, geometric ratio 1.73 (1.03 to 3.00), P = 0.01. Twenty-two out of 23 injections in muscle, 14 out of 23 injections at epineurium, and 11 out of 22 injections in subepineurium were < 138 kPa (20psi).

Conclusion: Needle tip position was not discernible using pressure monitoring. The circumneurium and subcircumneural injection compartment were observed, but not intrafascicular injection.

KEY WORDS: Ultrasonography, Nerve, Needle, Intraneural injection, Nerve trauma, Pressure
- **What is already known on this topic**
  
  Intrafascicular needle placement is not possible with regional anesthesia needles. Fluid injection pressure monitoring differentiates between nerve contact and perineural tissue.

- **What this study adds**
  
  All intraneural injections occurred between and not within fascicles.
  
  Fluid injection pressure is not a marker of needle tip position.

- **How this study might affect research, practice or policy**
  
  Fluid injection pressure monitoring offers no additional benefit over and above clinical ultrasound.
  
  Micro-ultrasound offers real-time visibility of circumneurium and subcircumneural injection.
INTRODUCTION

High fluid injection pressure > 138 kPa (20 psi) in fascicles has been associated with axonal damage in anesthetized dogs\(^1-3\), and assumed to be an important clinical indicator of nerve damage during regional anesthesia\(^4-9\). As such, fluid injection pressure has been investigated as a marker of needle tip position in order to make regional anesthesia safer. In animal\(^2; 3\) and human cadaver\(^7; 10\) models, peak injection pressure was consistently higher after intraneural injection compared to extraneural (perineural) injection. In patients, fluid injection pressure accurately identified contact with the collagen fibres that comprise the epineurium of the ventral nerve roots of the brachial plexus and femoral nerve\(^11; 12\). Given this evidence, threshold pressures of 103 kPa (15 psi) and 138 kPa (20 psi) are used in commercial devices (BSmart, BBraun, Melsungen, Germany and SAFIRA®, Medovate, Cambridge, UK). However, such systems are inherently inaccurate as fascial penetration also generates high pressures.

Our recent evidence questions current beliefs about the nature of nerve damage, and the application and interpretation of fluid injection pressure measurement. First, histology studies characterised the microscopic anatomy of nerves\(^13; 14\); delineated spread using a novel heparinised blood marker\(^15\) within adipocyte containing neural compartments\(^16\); and questioned the value of extrapolating results obtained from different animal models\(^17\). Second, Thiel cadaver studies identified the wide, log-normal distribution of pressure and force data at tissue interfaces\(^18\), and dependence of injection pressure on flow rate\(^19\). Third, application of 40 MHz micro-ultrasound of the axillary brachial plexus in anaesthetized pigs showed highly detailed anatomy, and precise location of needle tips relative to the target nerve\(^20\).

Fundamentally, we question the role of pressure monitoring as a clinical tool. No study on anaesthetized large animals or humans has measured fluid injection pressure in perineural tissues, on epineurium and within nerves using high resolution micro-ultrasound that identifies the exact position of the needle tip. We hypothesized, from our previous work, that pressures had a wide range of values at all tissue interfaces, were less accurate than previously claimed\(^11; 12\), were dependent on flow rate, and arose from a single source – injection between and not within fascicles.
Therefore, our primary objective was to compare fluid injection pressures during micro-ultrasound-guided injection in muscle, on epineurium and within subepineurium in the anaesthetized pig model using the same 0.5ml injected volumes and infusion rates as in our soft embalmed cadaver model. In addition, we wished to identify, using ultra-high 40 MHz ultrasound resolution, fluid spread within the muscle, within different fat compartments demarcated by the circumneurium, within the nerve (subepineurium), or between or within fascicles.
MATERIAL AND METHODS

The study was carried out in the animal research laboratories of Sunnybrook Health Science Network, Toronto, Canada. Ethical approval was obtained from the Animal Care Committee of Sunnybrook Research Institute, 2017. We complied to the ARRIVE guidelines 2.0: author checklist\textsuperscript{21}. Four male "Yorkshire" pigs weighing between 20 kg and 50 kg, were supplied by Caughell farms, Fingal, Ontario. Investigators were trained on animal handling. The study was funded by the NIAA BJA/RCOA PhD studentship 2014/R2/05 and travel costs funded by Sinapse, the Scottish Imaging Network.

After overnight fasting, pigs were anesthetized by a trained laboratory technician using intramuscular ketamine 15 mg.kg\textsuperscript{-1} and atropine 0.05 mg.kg\textsuperscript{-1}. Ringer’s Lactate solution was infused at a rate of 10 ml.kg\textsuperscript{-1}.h\textsuperscript{-1} through a 22g cannula inserted in an ear vein. Anesthesia was maintained using oxygen 100% and isoflurane 2 - 3%. The trachea was intubated with a size 6 to size 7 endotracheal tube, and lungs ventilated at a tidal volume of 8 ml.kg\textsuperscript{-1} and respiratory rate between 12 and 15 bpm in order to maintain normocapnia. ECG, SpO\textsubscript{2} and non-invasive blood pressure were monitored continuously. Temperature was maintained using an electrically heated pad and warmed blankets.

We bluntly dissected the left and right axillae of each pig and exposed the brachial plexus and pectoral muscle. We chose not to dissect the sciatic nerve as it lies very deep in the pig leg, or the cervical nerve roots because neck skin is very thick in pigs.

Once the brachial plexus was exposed, we placed a 40 MHz transducer (Vevo 2100, FUJIFILM Visualsonics, Toronto, Canada) over the axillary brachial plexus using the tail of the pectoral muscle as an imaging bridge. At the end of the experiment the pig was euthanized by the technician using 144 mg.kg\textsuperscript{-1} of Pentobarbital Sodium (Euthanyl, Bimeda-MTC Animal Health Inc., Cambridge, ON).

Pressure measurement

A polysulphone fluid pressure sensor (PendoTech, New Jersey) with a measurement range between 48 kPa (7 psi) and 517 kPa (75 psi) was connected in-line between the side tubing of a 22g, 50 mm needle (Stimuplex A, B.Braun, Melsungen AG) and a 30 ml syringe of saline (9 mg.ml\textsuperscript{-1}) held within a programmable electro-mechanical infusion pump (PHD Ultra, Harvard Apparatus, Holliston, MA).
The 22g Stimuplex A needle has a 30° bevel, 0.71 mm outer diameter and 0.13 mm wall thickness. Fluid pressure was recorded continuously at a frequency of 0.5 Hz using the same digital biological manometer (Pressure-MAT, PendoTech, New Jersey) used in clinical trials. The infusion pump was programmed to deliver 0.5 ml because this volume is easily recognisable using in-plane and out-of-plane approaches using micro-ultrasound. The 1, 6 and 12 ml.min⁻¹ injection rates replicated the rates used in previous animal and clinical studies, and were below the 15 ml.min⁻¹ threshold infusion rate identified by Patil et al. Infusion rates > 15 ml.min⁻¹ are associated with non-laminar flow and a high rate of false positive pressure measurements. Opening injection pressure was defined as the peak pressure generated following injection.

**Needle insertion and injection procedure**

The imaging, needle insertion and injection procedures were performed by two operators, one a regional anesthesiologist, and the other a PhD engineering student, trained previously to conduct ultrasound guided nerve injection on Thiel cadavers and pigs. We identified the radial, median and axillary nerves according to the schemata provided by Steinfeld et al. The 22g nerve block needle was inserted randomly in-plane or out-of-plane and placed at 3 locations:

1. 1 to 2 mm from the target nerve within the muscle bridge
2. Onto the collagen surface of epineurium (direct contact with epineurium)
3. Within subepineurium (intraneural)

Injection flow rates were randomized to 1, 6 and 12 ml.min⁻¹ using computer software (GraphPad Prism, San Diego, CA). Our secondary objectives were to measure: (i) nerve and fascicle area dimensions before and after intraneural injection; and (ii) the accuracy of opening injection pressure overall, and at each infusion rate.

**Image analysis**

A single, trained independent investigator outlined and measured nerve and fascicle areas, diameters and circularity using Corel Draw (Corel Corporation, Ottawa, Canada) and ImageJ with Fiji (v 2.0.0-rc-69/1.52p). Measurements were taken before needle insertion and at maximum nerve expansion.
following injection. The ratio of total fascicle area to nerve area was calculated for each nerve at each site. Feret’s diameter was defined as the longest distance between any two points along the boundary.

We defined nerve circularity (Circ) as:

$$Circ = \frac{4 \cdot \pi \cdot Area}{Perimeter^2}$$

Nerve sizes were also measured after subepineural injection. Subepineural injection was categorized in two ways according to the region of nerve expansion (“ peripheral” or “central”), and whether epineurium was ruptured or not.

Video recordings were exported as DICOM files. Pressure measurements were matched to injection, needle position observed on ultrasound and independently evaluated by three anesthesiologists. The maximum pressure measured after injection in muscle, on epineurium and within nerves was noted.

**Statistical analysis**

The distributions of data were assessed using normal probability plots and the D’Agostino - Pearson test. Nerve dimensions are presented as mean (SD). Pressure measurements had a log-normal distribution and are presented as geometric mean (95% CI). Data were analyzed using generalized linear mixed models. Significance was defined as $P<0.05$ (two-sided). Markers of needle tip accuracy were the ROC area under the curve and the Youden Index ($J$) (sensitivity + specificity -1). The cut-off point was calculated from the Youden index. Analyses were performed using Number Cruncher Statistical Systems (NCSS) 2020, (Kaysville, Utah), GraphPad Prism 9, (San Diego CA).
RESULTS

Nerve and fascicle characteristics

We identified 541 fascicles (Table 1) in 23 nerves. We failed to measure the dimensions of one radial nerve. Axillary nerves were smaller than median and radial nerves, difference in diameter 1.61 (0.87 to 2.36) mm, \( P < 0.001 \) and 1.59 (0.82 to 2.36) mm, \( P = 0.001 \) respectively. Axillary nerves had less fascicles per nerve than median nerves, difference 7.63 (2.43 to 12.83) and radial nerves, difference 9.02 (3.64 to 14.40). The ratio of fascicle area to nerve area remained constant at \( \sim 0.30 \) for all nerves. The fascicle diameters of the three nerves are available in Table 1.

**Table 1.** Nerve and fascicle characteristics. Median and radial nerves had a greater diameter than axillary nerves. Radial nerves had more fascicles than axillary nerves. Fascicles within median and radial nerves were larger than fascicles within axillary nerves. The ratio of total fascicle area to nerve area was \( \sim 0.30 \) for all nerves. Results presented as mean (SD) and difference between means (95%CI).

<table>
<thead>
<tr>
<th></th>
<th>Axillary nerve</th>
<th>Median nerve</th>
<th>Radial nerve</th>
<th>Comparison</th>
<th>Geometric mean (95%CI) difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nerves (n)</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nerve area</td>
<td>3.83 (0.42)</td>
<td>7.23 (2.31)</td>
<td>7.62 (1.56)</td>
<td>( A \text{ vs } M ) [ A \text{ vs } R ]</td>
<td>3.41 (1.78 to 3.04) [ 3.79 (2.10 to 5.48) ]</td>
<td>0.469</td>
</tr>
<tr>
<td>(mm²)</td>
<td></td>
<td></td>
<td></td>
<td>( M \text{ vs } R )</td>
<td>0.38 (-1.30 to 2.07)</td>
<td>0.199</td>
</tr>
<tr>
<td>Nerve diameter</td>
<td>3.43 (0.48)</td>
<td>5.04 (1.00)</td>
<td>5.02 (0.64)</td>
<td>( A \text{ vs } M ) [ A \text{ vs } R ]</td>
<td>1.61 (0.87 to 2.36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(mm)</td>
<td></td>
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<td></td>
<td></td>
<td>0.001</td>
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<td></td>
<td></td>
<td></td>
<td>M vs R</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1.59 (0.82 to 2.36)</td>
<td>0.951</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.02 (-0.08 to 0.08)</td>
<td></td>
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<tr>
<td>Nerve circularity</td>
<td>0.67 (0.08)</td>
<td>0.61 (0.14)</td>
<td>0.63 (0.09)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>A vs M</td>
<td></td>
<td>0.07 (-0.04 to 0.18)</td>
<td>0.537</td>
<td></td>
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<tr>
<td></td>
<td>A vs R</td>
<td></td>
<td>0.18</td>
<td>0.728</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>M vs R</td>
<td></td>
<td>0.04 (-0.07 to 0.16)</td>
<td>0.728</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.03 (-0.09 to 0.14)</td>
<td></td>
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</tr>
<tr>
<td>Fascicle count (n)</td>
<td>145</td>
<td>206</td>
<td>190</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fascicles per nerve (n)</td>
<td>18.1 (3.6)</td>
<td>25.8 (5.7)</td>
<td>27.1 (6.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A vs M</td>
<td></td>
<td>7.63 (2.43 to 12.83)</td>
<td>0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A vs R</td>
<td></td>
<td>12.83</td>
<td>0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M vs R</td>
<td></td>
<td>9.02 (3.64 to 14.40)</td>
<td>0.609</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.39 (-3.99 to 6.77)</td>
<td></td>
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<tr>
<td>Fascicle area (mm²)</td>
<td>0.06 (0.03)</td>
<td>0.08 (0.04)</td>
<td>0.083 (0.05)</td>
<td></td>
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<tr>
<td></td>
<td>A vs M</td>
<td></td>
<td>0.02 (0.01 to 0.03)</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A vs R</td>
<td></td>
<td>0.03</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M vs R</td>
<td></td>
<td>0.02 (0.01 to 0.03)</td>
<td>0.643</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.00 (-0.01 to 0.01)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fascicle diameter (mm)</td>
<td>0.36 (0.10)</td>
<td>0.41 (0.18)</td>
<td>0.411 (0.14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A vs M</td>
<td></td>
<td>0.05 (0.03 to 0.08)</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A vs R</td>
<td></td>
<td>0.08</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M vs R</td>
<td></td>
<td>0.05</td>
<td>0.851</td>
<td></td>
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</tr>
</tbody>
</table>
### Fascicle circularity

<table>
<thead>
<tr>
<th></th>
<th>Fascicle circularity</th>
<th>A vs M</th>
<th>A vs R</th>
<th>M vs R</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.829 (0.07)</td>
<td>0.02</td>
<td>0.04</td>
<td>0.03</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>0.805 (0.08)</td>
<td>0.04</td>
<td>0.05</td>
<td>0.01</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>0.800 (0.08)</td>
<td></td>
<td></td>
<td></td>
<td>0.540</td>
</tr>
</tbody>
</table>

### Fascicle total area (mm²)

<table>
<thead>
<tr>
<th></th>
<th>Fascicle total area (mm²)</th>
<th>A vs M</th>
<th>A vs R</th>
<th>M vs R</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.18 (0.15)</td>
<td>0.90</td>
<td>1.28</td>
<td>1.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>2.09 (0.48)</td>
<td></td>
<td>1.46</td>
<td>0.68</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>2.25 (0.42)</td>
<td></td>
<td>0.56</td>
<td>-0.22</td>
<td>0.396</td>
</tr>
<tr>
<td></td>
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</tbody>
</table>

### Fascicle to nerve ratio

<table>
<thead>
<tr>
<th></th>
<th>Fascicle to nerve ratio</th>
<th>A vs M</th>
<th>A vs R</th>
<th>M vs R</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.31 (0.02)</td>
<td>0.01</td>
<td>0.04</td>
<td>0.01</td>
<td>0.800</td>
</tr>
<tr>
<td></td>
<td>0.30 (0.03)</td>
<td></td>
<td>0.04</td>
<td>-0.02</td>
<td>0.800</td>
</tr>
<tr>
<td></td>
<td>0.30 (±0.04)</td>
<td></td>
<td>0.04</td>
<td>0.04</td>
<td>0.851</td>
</tr>
</tbody>
</table>

### Injection

Intraneural injection was achieved in 22 out of 23 nerves. Nerve area (SD) increased from 5.7 (2.2) mm² to 13.7 (5.5) mm², difference (95%) 8.0 (5.4 - 10.6) mm², P < 0.001 (Fig. 1). Nerve diameter (SD) increased from 4.4 (1.1) mm² to 5.4 (1.3) mm², difference (95%) 0.9 (0.3 - 1.6) mm², P = 0.008. Nerve
cross-sectional area (SD) increased (%) by 187 (48%), 252 (78%) and 283 (59%) in the radial, median and axillary nerves respectively. Circularity (SD) increased from 0.62 (0.11) mm$^2$ to 0.78 (0.09) mm$^2$, difference (95%) 0.16 (0.08 - 0.25) mm$^2$, $P = 0.001$. Epineural rupture was determined by the location of the needle tip within the nerve: 4 out of 18 (82.4%) central injections, and 1 out of 4 peripheral injections, resulted in nerve rupture, RR 1.2 (0.2 to 7.3), $P = 0.99$.

Ultrasound examples of intramuscular, epineural and intraneural injection and epineural rupture are shown in Fig 1.

**Pressure**

The distribution of pressure data at perineural, epineural and subepineural sites was log-normal (Fig 2). Geometric mean fluid injection pressure (95% CI:) was 64.7 (45.6 to 93.8) kPa, 99.5 (73.7 to 134.3) kPa, 133.0 (97.5 to 183.1) kPa for intramuscular, epineural and subepineural injection respectively (Fig. 2). Twenty-two out of 23 injections in muscle, 14 out of 23 injections at epineurium, and 11 out of 22 injections in subepineurium were < 138 kPa (20psi).

Injection pressure was greater in subepineurium compared to muscle, geometric ratio 2.29 (1.30 to 4.10), $P < 0.001$; and greater on epineurium compared to muscle, geometric ratio 1.73 (1.03 to 3.00), $P = 0.01$. An example of ultrasound images paired with fluid injection pressure is shown in Fig 3. Images (i) to (iii) clearly show continuous layer(s) suggestive of circumneurium that lie between the epimysium and epineurium.

Fluid injection pressure did not differ between flow rates of 12 ml.min$^{-1}$ and 1 ml.min$^{-1}$, geometric ratio 1.39 (0.77 – 2.56), $P = 0.61$ (Fig 2). The maximum difference was between pig 1 and pig 4, geometric ratio 1.82 (1.00 to 3.29), $P = 0.19$. In addition, there was no difference between operators, geometric ratio 0.94 (0.66 – 1.35) $P = 0.12$; between in-plane or out-of-plane needle trajectories, geometric ratio 1.09 (0.76 – 1.32), $P = 0.24$; sides, geometric ratio 0.64 (0.45 to 1.12) $P = 0.07$; or between nerves, median vs axillary geometric ratio 1.35 (0.79 – 2.29) $P = 1.00$, median vs radial, geometric ratio 1.34 (0.44 - 1.26), $P = 0.72$

**Accuracy**
Accuracy results of needle tip position are given in Table 2.

### Table 2. Accuracy of fluid injection pressure at epineurium and subepineurium overall and at three flow rates 1 ml.min⁻¹, 6 ml.min⁻¹ and 12 ml.min⁻¹.

<table>
<thead>
<tr>
<th>Flow Rate (ml.min⁻¹)</th>
<th>Epineurium</th>
<th>Subepineurium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>AUC</td>
<td>0.55</td>
<td>0.75</td>
</tr>
<tr>
<td>ROC</td>
<td>(0.11 to 0.99)</td>
<td>(0.54 to 0.96)</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>40 (7 to 77)</td>
<td>71 (47 to 87)</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>100</td>
<td>88</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>(18 to 100)</td>
<td>(53 to 99)</td>
</tr>
<tr>
<td>Youden index</td>
<td>70</td>
<td>80</td>
</tr>
<tr>
<td>Log cut-off point (kPa)</td>
<td>4.35</td>
<td>4.07</td>
</tr>
<tr>
<td>Cut-off point (kPa)</td>
<td>77.5</td>
<td>58.6</td>
</tr>
<tr>
<td>Cut-off point (psi)</td>
<td>11.6</td>
<td>8.8</td>
</tr>
</tbody>
</table>
DISCUSSION

We showed that fluid injection pressure is a poor biomarker of needle tip position: a wide range of overlapping measurements were recorded when the needle tip was lodged within muscle, epineurium or subepineurium. Using ultrasound, we observed the circumneural layers and subcircumneural injection. We did not observe an increase in size of any fascicle following intraneural injection.

We conducted this study because there is still a preconception amongst many experts\textsuperscript{4-9}, despite evidence to the contrary\textsuperscript{16}, that high pressure intrafascicular injection contributes to nerve damage.

The principal strength of our study was that our imaging system enabled us to visualize the precise position of the needle tip relative to anatomical tissue at resolutions of approximately 100µm, and match tip position with pressure changes, and, in doing so, allowed us unravel the mechanisms of both nerve block and nerve injury.

For clarity in the following discussion, we define: injection outside the nerve as sub-circumneural injection; injection on first contact with nerve as epineurial (albeit that minimal amounts of injectate may also pass intraneurally depending on the relative position of the needle orifice); and intraneural injection as subepineural injection outwith the fascicles.

We confirm previous studies that regional anesthesia block needles are not able to penetrate fascicles\textsuperscript{16; 20}. Scanning electron microscopy has verified that the endoneurium is a rigid structure resistant to the entry of liquids and not a soft, fragile tissue - a concept only recently acknowledged\textsuperscript{13}.

Mean injection pressures on epineural contact and during subepineural injection were similar to those obtained in previous experiments on anesthetized pigs\textsuperscript{7}, the soft embalmed cadaver\textsuperscript{19; 28} and during needle nerve contact in patients\textsuperscript{11; 12}. However, the range of values varied considerably at all sites. The wide range of pressure results may be explained by: consideration of resistance to fluid injection through a needle orifice that only partially covers fascicular tissue; to the physics of injection at all
tissue layers; and the means of fluid delivery.

First, fluid injected at high pressure liquid is directed towards compliant intraneural fat which offers much less resistance than fascicular tissue\textsuperscript{29}. In this scenario, injectate does not enter the fascicle but instead exits towards the intraneural fat, and provides an element of safety\textsuperscript{16}. For example, a needle bevel angle of 15° will have a larger elliptical orifice area than a 30° needle even if both needles have the same external diameter, and thus reduces the vulnerability of the nerve to intrafascicular injection. The figure in the Appendix helps explain this concept. Indeed, in nerves that do not have fat between the fascicles, and only have interlaced collagen fibers, it is impossible not only to make an intrafascicular injection but also an extrafascicular injection. The resistance of both the endoneurium within the fascicles and the collagen fibres that provide a second protective layer to each of the fascicles, prevents the entry of any solution into extrafascicular tissue\textsuperscript{14}.

The same principles apply when considering extraneural injection. The sub-circumneurium fat compartment offers low resistance whereas injection close to the collagen-rich external surface of epineurium meets greater resistance. Thus, local anesthetic will flow in the path of least resistance creating the classical “donut” pattern of spread. Similarly, encapsulation of the needle orifice within intramuscular fibrotic tissue, and away from adipose tissue, may explain the sole high-pressure response to intramuscular injection in one cadaver.

Second, the wide range of opening pressure measurements at epineurium and in subepineurium, reflect the exponential relationship between the axial force applied by a needle tip (stress) and the displacement of tissue (strain) encountered at all tissue interfaces such as fascia and within contained compartments such as nerves. This means that minimal changes in needle tip position are associated with considerable changes in fluid injection pressure.

Third, injection was given by hand and not an electromechanical infusion pump, and contributed to the high variability of pressure measurement.
Thus, it is not surprising unsurprising, given these fundamental principles, that needle tip position was indiscernible between tissue types < 138 kPa (20 psi) and that peak pressures reached 572 kPa (83 psi). If translated to clinical practice, such a range of opening injection pressures would not offer any help to the anesthesiologist over and above clinical observation using ultrasound.

Our high resolution images also give credence to a recent hypothesis regarding the efficacy of regional anesthesia that proposes-intimates that experts obtain better anesthesia because they inject both within the subcircumneural compartment and unintentionally within the subepineurium. By inference, more tentative novices inject further from the nerve within muscle, and fail to provide sufficient anesthesia because local anesthetic has further to diffuse. Fig 3 (images (i) to (iii)) and the corresponding video in the Appendix show fascial planes between the collagenous border of epineurium and the epimysium with injectate occupying both spaces. We would further hypothesize that injection within the subcircumneural compartment but close to epineurium is associated with slightly higher initial resistance to injection compared to injection 1 to 2 mm distant from the nerve, but is more likely to provide better, longer anaesthesia because more local anesthetic enters the nerve. Fick’s law of diffusion suggests that the closer to the nerve, the greater the concentration gradient and the fewer the fascial barriers, then the greater the amount of local anaesthetic entering the nerve.

The weaknesses of our study were the use of an animal model and failure to measure pressure at the needle tip. Nevertheless, it should be noted that Server et al. [11] demonstrated that pig and sheep models represent the closest simulated conditions to humans for in vivo study. Direct measurement of pressure at the needle tip, rather than in-line measurement, is possible using Fabry-Perot interferometry but needles are not yet available commercially. An air-filled, glass bound cavity is bonded to the tip of an optical fibre secured to the side-wall of a regional anesthesia block needle. Optical fibres have numerous advantages: they are light, flexible, dielectric (a non-conductor of DC current) and provide a continuous graphical output. However, the accuracy of such sensors to differentiate between anatomical interfaces also needs to be evaluated using realistic models and with known sensor position.
In future studies, it will be necessary to define which types of needles, depending on their external
diameter and orifice size, are considerably larger than fascicles and can be safely used at each nerve
site.

In conclusion, identification of the precise location of the needle tip was not possible using injection
pressure monitoring. Intrafascicular injection was not observed. Consideration of our microultrasound
observations allow us to hypothesize that excessive pressures are not associated with subperineural
injection (intrafascicular injection). and that placement of the needle tip and orifice and injection of
local anaesthetic within the subcircumneural compartment (in close contact with epineurium) block has
the potential to offer a more effective anesthetic block, with less latency and longer duration. Our
findings have implications for other clinical interventions. outside outwith One example is
administration of intraneural drugs for the treatment of different neurological pathologies in which in
situ administration is essential to reach the target. However, more studies will be needed to confirm our
affirmations.
REFERENCES


http://dx.doi.org/10.1136/rapm-2018-100087

Acknowledgments: The authors wish to sincerely thank the staff of the Sunnybrook Research Institute Comparative Research Centre, Dr C Demore and A Chandra.
TABLES

Table 1. Nerve and fascicle characteristics. Median and radial nerves had a greater Feret’s diameter than axillary nerves. Radial nerves had more fascicles than axillary nerves. Fascicles within median and radial nerves were larger than fascicles within axillary nerves. The ratio of total fascicle area to nerve area was ~0.30 for all nerves. Results presented as mean (SD) and difference between means (95%CI)

Table 2. Accuracy of fluid injection pressure at epineurium and subepineurium using three flow rates 1 ml.min$^{-1}$, 6 ml.min$^{-1}$ and 12 ml.min$^{-1}$. Data show best accuracy at epineurium using 12 ml.min$^{-1}$

FIGURES

Figure 1. Examples of microultrasound imaging of nerves before and after injection. Image (a) shows individual changes in nerve size after injection. Image (b) shows nerves scanned before injection and overlying the pectoral muscle bridge. The arrow in image (c) indicates injection within the muscle layer adjacent to the median nerve. The proportional increase in area did not differ between nerves, image (d). Image (e) shows a 22g regional anesthesia block needle inserted in-plane with distal aperture visualized and expansion of the axillary nerve. The arrow in image (f) indicates epineural rupture of the radial nerve. M - Median nerve; Ax - Axillary nerve; R - Radial nerve; F - Fluid.

Figure 2. Muscle, epineural and subepineural fluid injection pressures. Difference in log injection pressure (image a) between muscle and subepineural injection pressures (P = 0.042). Image (b) shows log-normal distribution of data at all interfaces. Image (c) shows injection pressures at each interface categorized according to flow (1, 6 and 12 ml.min$^{-1}$). No difference in pressure according to flow rate.

Figure 3. Ultrasound images paired with fluid injection pressure. Images (a) to (d) captured at four time points (i) to (iv). (i) preinjection; (ii) intramuscular injection, and subepineural injection; (iii) subepineural and subcircumneural injection. The asterisks in (ii) and (iii) indicate the circumneurium
of the nerve; (iv) intraneural injection outwith the fascicles. The corresponding video is available in the Appendix. Image (f) is the log transformed pressure profile of image (e).

Appendix

Video corresponding to Fig 3.

Human ulnar nerve and nerve regional anesthesia block needles. A: Difference in needle orifice at same external diameter (22g, 0.70 mm diameter) and 15° or 30° bevel angle.

B: Human ulnar nerve. Needle tip placed at intrafascicularly (label 1) or at epineurium (label 2). The third option in subepineurium is showed as label 3 (peripheral adipose tissue).

This image was built superimposing images using Adobe Photoshop (Adobe, San Jose, California, USA). The images from Dr Reina’s archives obtained in his lab, was captured at same magnification had been stained with haematoxylin-eosin. Magnification: original acquisition at x20. The images show the alternative place where a needle tip can be introduced, but the conclusion obtained from looking at the image is only valid for ulnar nerve and not extrapolated to other nerves because the proportion between fascicles sizes, distribution of them, and thickness of extrafascicular adipose tissue will be different.
The accuracy of injection pressure measurement at peripheral nerves using high resolution 40MHz ultrasound in an anesthetized porcine model

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Conflicts of Interest:
Nil

Funding:
Nil

Running Head: Injection pressure measurement at peripheral nerves

Word count: 3028

Keywords: Ultrasonography; Pressure; Injury; Fascicle: Mechanisms

Clinical trial number and registry URL: N/A

Contributors: GML: Project development, data collection and management, data analysis and manuscript writing. AC: Data collection; AS: Data collection and Data analysis. PW: Data collection; FW: Data collection. Manuscript editing; MAR: Data analysis, manuscript writing and editing. All authors have read and approved the manuscript.

Funding sources: Funded by the NIAA BJA/RCOA PhD studentship 2014/R2/05 and Sinapse, Scottish Imaging Network.

Disclosure and conflicts of interest: No competing interests.

Patient consent for publication: Not required.

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ABSTRACT

Fluid injection pressure measurement is promoted as a marker of needle tip position that discriminates between tissue layers. However, clinical ultrasound has insufficient resolution to identify the exact position of the needle tip. Our primary objective was to use 40MHz ultrasound in anesthetized pigs in order to precisely locate the tip of the needle, and measure opening injection pressure in muscle, at close epineurium and in subepineurium.

Methods: We surgically exposed the axillae of four anesthetized pigs. Two operators placed a 40MHz ultrasound transducer over the pectoral muscle and imaged axillary, median and radial nerves. Injections (0.5 ml) were randomized to in-plane and out-of-plane needle trajectories and flow rates of 1, 6 and 12 ml.min\(^{-1}\).

Results: We identified 541 fascicles in 23 nerves. The ratio of fascicle area to nerve area remained constant at ~ 0.30 for all nerves. Axillary nerves were smaller than median and radial nerves, difference in diameter (95%CI) 1.61 (0.87 to 2.36) mm, \(P < 0.001\) and 1.59 (0.82 to 2.36) mm, \(P = 0.001\) respectively. Axillary nerves had less fascicles per nerve than median nerves, difference 7.63 (2.43 to 12.83) and radial nerves, difference 9.02 (3.64 to 14.40). We visualised the circumneurium and injection within the subcircumneural compartment. Intraneural injection increased nerve area (SD) from 5.7 (2.2) mm\(^2\) to 13.7 (5.5) mm\(^2\), difference 8.0 (5.4 - 10.6) mm\(^2\), \(P < 0.001\). Mean injection pressure was greater in subepineurium compared to muscle, geometric ratio 2.29 (1.30 to 4.10), \(P < 0.001\); and greater on epineurium compared to muscle, geometric ratio 1.73 (1.03 to 3.00), \(P = 0.01\). Twenty-two out of 23 injections in muscle, 14 out of 23 injections at epineurium, and 11 out of 22 injections in subepineurium were < 138 kPa (20psi).

Conclusion: Needle tip position was not discernible using pressure monitoring. The circumneurium and subcircumneural injection compartment were observed, but not intrafascicular injection.

KEY WORDS: Ultrasonography, Nerve, Needle, Intraneural injection, Nerve trauma, Pressure
• What is already known on this topic
  Intrafascicular needle placement is not possible with regional anesthesia needles.
  Fluid injection pressure monitoring differentiates between nerve contact and perineural tissue

• What this study adds
  All intraneural injections occurred between and not within fascicles.
  Fluid injection pressure is not a marker of needle tip position

• How this study might affect research, practice or policy
  Fluid injection pressure monitoring offers no additional benefit over and above clinical ultrasound.
  Micro-ultrasound offers real-time visibility of circumneurium and subcircumneural injection
INTRODUCTION

High fluid injection pressure > 138 kPa (20 psi) in fascicles has been associated with axonal damage in anesthetized dogs 1-3, and assumed to be an important clinical indicator of nerve damage during regional anesthesia4-9. As such, fluid injection pressure has been investigated as a marker of needle tip position in order to make regional anesthesia safer. In animal 2; 3 and human cadaver 7; 10 models, peak injection pressure was consistently higher after intraneural injection compared to extraneural (perineural) injection. In patients, fluid injection pressure accurately identified contact with the collagen fibres that comprise the epineurium of the ventral nerve roots of the brachial plexus and femoral nerve11; 12. Given this evidence, threshold pressures of 103 kPa (15 psi) and 138kPa (20 psi) are used in commercial devices (BSmart, BBraun, Melsungen, Germany and SAFIRA®, Medovate, Cambridge, UK). However, such systems are inherently inaccurate as fascial penetration also generates high pressures.

Our recent evidence questions current beliefs about the nature of nerve damage, and the application and interpretation of fluid injection pressure measurement. First, histology studies characterised the microscopic anatomy of nerves13; 14; delineated spread using a novel heparinised blood marker15 within adipocyte containing neural compartments16; and questioned the value of extrapolating results obtained from different animal models17. Second, Thiel cadaver studies identified the wide, log-normal distribution of pressure and force data at tissue interfaces18; and dependence of injection pressure on flow rate19. Third, application of 40 MHz micro-ultrasound of the axillary brachial plexus in anaesthetized pigs showed highly detailed anatomy, and precise location of needle tips relative to the target nerve20.

Fundamentally, we question the role of pressure monitoring as a clinical tool. No study on anaesthetized large animals or humans has measured fluid injection pressure in perineural tissues, on epineurium and within nerves using high resolution micro-ultrasound that identifies the exact position of the needle tip. We hypothesized, from our previous work, that pressures had a wide range of values at all tissue interfaces, were less accurate than previously claimed11; 12, were dependent on flow rate, and arose from a single source – injection between and not within fascicles.
Therefore, our primary objective was to compare fluid injection pressures during micro-ultrasound-guided injection in muscle, on epineurium and within subepineurium in the anaesthetized pig model using the same 0.5ml injected volumes and infusion rates as in our soft embalmed cadaver model\textsuperscript{19}. In addition, we wished to identify, using ultra-high 40 MHz ultrasound resolution, fluid spread within the muscle, within different fat compartments demarcated by the circumneurium, within the nerve (subepineurium), or between or within fascicles.
MATERIAL AND METHODS

The study was carried out in the animal research laboratories of Sunnybrook Health Science Network, Toronto, Canada. Ethical approval was obtained from the Animal Care Committee of Sunnybrook Research Institute, 2017. We complied to the ARRIVE guidelines 2.0: author checklist\(^{21}\). Four male “Yorkshire” pigs weighing between 20 kg and 50 kg, were supplied by Caughell farms, Fingal, Ontario. Investigators were trained on animal handling. The study was funded by the NIAA BJA/RCOA PhD studentship 2014/R2/05 and travel costs funded by Sinapse, the Scottish Imaging Network.

After overnight fasting, pigs were anesthetized by a trained laboratory technician using intramuscular ketamine 15 mg.kg\(^{-1}\) and atropine 0.05 mg.kg\(^{-1}\). Ringer’s Lactate solution was infused at a rate of 10 ml.kg\(^{-1}\).h\(^{-1}\) through a 22g cannula inserted in an ear vein. Anesthesia was maintained using oxygen 100% and isoflurane 2 - 3%. The trachea was intubated with a size 6 to size 7 endotracheal tube, and lungs ventilated at a tidal volume of 8 ml.kg\(^{-1}\) and respiratory rate between 12 and 15 bpm in order to maintain normocapnia. ECG, SpO\(_2\) and non-invasive blood pressure were monitored continuously. Temperature was maintained using an electrically heated pad and warmed blankets.

We bluntly dissected the left and right axillae of each pig and exposed the brachial plexus and pectoral muscle. We chose not to dissect the sciatic nerve as it lies very deep in the pig leg, or the cervical nerve roots because neck skin is very thick in pigs.

Once the brachial plexus was exposed, we placed a 40 MHz transducer (Vevo 2100, FUJIFILM Visualsonics, Toronto, Canada) over the axillary brachial plexus using the tail of the pectoral muscle as an imaging bridge. At the end of the experiment the pig was euthanized by the technician using 144 mg.kg\(^{-1}\) of Pentobarbital Sodium (Euthanyl, Bimedia-MTC Animal Health Inc., Cambridge, ON).

Pressure measurement

A polysulphone fluid pressure sensor (PendoTech, New Jersey) with a measurement range between 48 kPa (7 psi) and 517 kPa (75 psi) was connected in-line between the side tubing of a 22g, 50 mm needle (Stimuplex A, B.Braun, Melsungen AG) and a 30 ml syringe of saline (9 mg.ml\(^{-1}\)) held within a programmable electro-mechanical infusion pump (PHD Ultra, Harvard Apparatus, Holliston, MA).
The 22g Stimuplex A needle has a 30° bevel, 0.71 mm outer diameter and 0.13 mm wall thickness. Fluid pressure was recorded continuously at a frequency of 0.5 Hz using the same digital biological manometer (Pressure-MAT, PendoTech, New Jersey) used in clinical trials\textsuperscript{11, 12}. The infusion pump was programmed to deliver 0.5 ml\textsuperscript{22} because this volume is easily recognisable using in-plane and out-of-plane approaches using micro-ultrasound\textsuperscript{20}. The 1, 6 and 12 ml.min\textsuperscript{-1} injection rates replicated the rates used in previous animal\textsuperscript{7, 10, 23} and clinical\textsuperscript{11, 12} studies, and were below the 15 ml.min\textsuperscript{-1} threshold infusion rate identified by Patil et al\textsuperscript{24}. Infusion rates > 15 ml.min\textsuperscript{-1} are associated with non-laminar flow and a high rate of false positive pressure measurements\textsuperscript{24}. Opening injection pressure was defined as the peak pressure generated following injection.

**Needle insertion and injection procedure**

The imaging, needle insertion and injection procedures were performed by two operators, one a regional anesthesiologist, and the other a PhD engineering student, trained previously to conduct ultrasound guided nerve injection on Thiel cadavers\textsuperscript{18, 25} and pigs\textsuperscript{20}. We identified the radial, median and axillary nerves according to the schemata provided by Steinfeld et al\textsuperscript{26}.

The 22g nerve block needle was inserted randomly in-plane or out-of-plane and placed at 3 locations:

1. 1 to 2 mm from the target nerve within the muscle bridge
2. Onto the collagen surface of epineurium (direct contact with epineurium)
3. Within subepineurium (intraneural)

Injection flow rates were randomized to 1, 6 and 12 ml.min\textsuperscript{-1} using computer software (GraphPad Prism, San Diego, CA). Our secondary objectives were to measure: (i) nerve and fascicle area dimensions before and after intraneural injection; and (ii) the accuracy of opening injection pressure overall, and at each infusion rate.

**Image analysis**

A single, trained independent investigator outlined and measured nerve and fascicle areas, diameters and circularity using Corel Draw (Corel Corporation, Ottawa, Canada) and ImageJ with Fiji (v 2.0.0-rc-69/1.52p)\textsuperscript{27}. Measurements were taken before needle insertion and at maximum nerve expansion.
following injection. The ratio of total fascicle area to nerve area was calculated for each nerve at each site. Feret’s diameter was defined as the longest distance between any two points along the boundary.

We defined nerve circularity (Circ) as:

\[ \text{Circ} = \frac{4 \cdot \pi \cdot \text{Area}}{\text{Perimeter}^2} \]

Nerve sizes were also measured after subepineural injection. Subepineural injection was categorized in two ways according to the region of nerve expansion (“peripheral” or “central”), and whether epineurium was ruptured or not.

Video recordings were exported as DICOM files. Pressure measurements were matched to injection, needle position observed on ultrasound and independently evaluated by three anesthesiologists. The maximum pressure measured after injection in muscle, on epineurium and within nerves was noted.

**Statistical analysis**

The distributions of data were assessed using normal probability plots and the D’Agostino - Pearson test. Nerve dimensions are presented as mean (SD). Pressure measurements had a log-normal distribution and are presented as geometric mean (95% CI). Data were analyzed using generalized linear mixed models. Significance was defined as \( P < 0.05 \) (two-sided). Markers of needle tip accuracy were the ROC area under the curve and the Youden Index \( (J) \) (sensitivity + specificity -1). The cut-off point was calculated from the Youden index. Analyses were performed using Number Cruncher Statistical Systems (NCSS) 2020, (Kaysville, Utah), GraphPad Prism 9, (San Diego CA).
RESULTS

Nerve and fascicle characteristics

We identified 541 fascicles (Table 1) in 23 nerves. We failed to measure the dimensions of one radial nerve. Axillary nerves were smaller than median and radial nerves, difference in diameter 1.61 (0.87 to 2.36) mm, P < 0.001 and 1.59 (0.82 to 2.36) mm, P = 0.001 respectively. Axillary nerves had less fascicles per nerve than median nerves, difference 7.63 (2.43 to 12.83) and radial nerves, difference 9.02 (3.64 to 14.40). The ratio of fascicle area to nerve area remained constant at ~ 0.30 for all nerves. The fascicle diameters of the three nerves are available in Table 1.

Table 1. Nerve and fascicle characteristics. Median and radial nerves had a greater diameter than axillary nerves. Radial nerves had more fascicles than axillary nerves. Fascicles within median and radial nerves were larger than fascicles within axillary nerves. The ratio of total fascicle area to nerve area was ~ 0.30 for all nerves. Results presented as mean (SD) and difference between means (95%CI)

<table>
<thead>
<tr>
<th></th>
<th>Axillary nerve</th>
<th>Median nerve</th>
<th>Radial nerve</th>
<th>Comparison</th>
<th>Geometric mean (95%CI) difference</th>
<th>P-value</th>
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<td>Nerves (n)</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td></td>
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<tr>
<td>Nerve area (mm²)</td>
<td>3.83 (0.42)</td>
<td>7.23 (2.31)</td>
<td>7.62 (1.56)</td>
<td>A vs M</td>
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<td></td>
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<td>A vs R</td>
<td>3.79 (2.10 to 5.48)</td>
<td>0.199</td>
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<td>M vs R</td>
<td>0.38 (-1.30 to 2.07)</td>
<td>0.639</td>
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<tr>
<td>Nerve diameter (mm)</td>
<td>3.43 (0.48)</td>
<td>5.04 (1.00)</td>
<td>5.02 (0.64)</td>
<td>A vs M</td>
<td>1.61 (0.87 to 2.36)</td>
<td>&lt;0.001</td>
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<td></td>
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<td></td>
<td></td>
<td>A vs R</td>
<td>1.59 (0.82 to 2.36)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>M vs R</td>
<td>0.02 (-0.08 to 0.08)</td>
<td>0.951</td>
</tr>
<tr>
<td>Nerve circularity</td>
<td>0.67 (0.08)</td>
<td>0.61 (0.14)</td>
<td>0.63 (0.09)</td>
<td>A vs M</td>
<td>0.07 (-0.04 to 0.18)</td>
<td>0.537</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A vs R</td>
<td>0.04 (-0.07 to 0.16)</td>
<td>0.728</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M vs R</td>
<td>0.03 (-0.09 to 0.14)</td>
<td>0.728</td>
</tr>
<tr>
<td>Fascicle count (n)</td>
<td>145</td>
<td>206</td>
<td>190</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fascicles per nerve (n)</td>
<td>18.1 (3.6)</td>
<td>25.8 (5.7)</td>
<td>27.1 (6.0)</td>
<td>A vs M</td>
<td>7.63 (2.43 to 12.83)</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A vs R</td>
<td>9.02 (3.64 to 14.40)</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M vs R</td>
<td>1.39 (-3.99 to 6.77)</td>
<td>0.609</td>
</tr>
<tr>
<td>Fascicle area (mm²)</td>
<td>0.06 (0.03)</td>
<td>0.08 (0.04)</td>
<td>0.083 (0.05)</td>
<td>A vs M</td>
<td>0.02 (0.01 to 0.03)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A vs R</td>
<td>0.02 (0.01 to 0.03)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M vs R</td>
<td>0.00 (-0.01 to 0.01)</td>
<td>0.643</td>
</tr>
</tbody>
</table>
### Injection

Intraneural injection was achieved in 22 out of 23 nerves. Nerve area (SD) increased from 5.7 (2.2) mm² to 13.7 (5.5) mm², difference (95%) 8.0 (5.4 - 10.6) mm², P < 0.001 (Fig. 1). Nerve diameter (SD) increased from 4.4 (1.1) mm² to 5.4 (1.3) mm², difference (95%) 0.9 (0.3 - 1.6) mm², P = 0.008. Nerve cross-sectional area (SD) increased (%) by 187 (48%), 252 (78%) and 283 (59%) in the radial, median and axillary nerves respectively. Circularity (SD) increased from 0.62 (0.11) mm² to 0.78 (0.09) mm², difference (95%) 0.16 (0.08 - 0.25) mm², P = 0.001. Epineural rupture was determined by the location of the needle tip within the nerve: 4 out of 18 (82.4%) central injections, and 1 out of 4 peripheral injections, resulted in nerve rupture, RR 1.2 (0.2 to 7.3), P = 0.99.

Ultrasound examples of intramuscular, epineural and intraneural injection and epineural rupture are shown in Fig 1.

### Pressure

The distribution of pressure data at perineural, epineural and subepineural sites was log-normal (Fig 2). Geometric mean fluid injection pressure (95% CI:) was 64.7 (45.6 to 93.8) kPa, 99.5 (73.7 to 134.3) kPa, 133.0 (97.5 to 183.1) kPa for intramuscular, epineural and subepineural injection respectively (Fig. 2). Twenty-two out of 23 injections in muscle, 14 out of 23 injections at epineurium, and 11 out of 22 injections in subepineurium were < 138 kPa (20psi).
Injection pressure was greater in subepineurium compared to muscle, geometric ratio 2.29 (1.30 to 4.10), P < 0.001; and greater on epineurium compared to muscle, geometric ratio 1.73 (1.03 to 3.00), P = 0.01. An example of ultrasound images paired with fluid injection pressure is shown in Fig 3. Images (i) to (iii) clearly show continuous layer(s) suggestive of circumneurium that lie between the epimysium and epineurium.

Fluid injection pressure did not differ between flow rates of 12 ml.min\(^{-1}\) and 1 ml.min\(^{-1}\), geometric ratio 1.39 (0.77 – 2.56), P = 0.61 (Fig 2). The maximum difference was between pig 1 and pig 4, geometric ratio 1.82 (1.00 to 3.29), P = 0.19. In addition, there was no difference between operators, geometric ratio 0.94 (0.66 – 1.35) P = 0.12; between in-plane or out-of-plane needle trajectories, geometric ratio 1.09 (0.76 – 1.32), P = 0.24; sides, geometric ratio 0.64 (0.45 to 1.12) P = 0.07; or between nerves, median vs axillary geometric ratio 1.35 (0.79 – 2.29) P = 1.00, median vs radial, geometric ratio 1.34 (0.44 - 1.26), P = 0.72.

**Accuracy**

Accuracy results of needle tip position are given in Table 2.
Table 2. Accuracy of fluid injection pressure at epineurium and subepineurium overall and at three flow rates 1 ml.min\(^{-1}\), 6 ml.min\(^{-1}\) and 12 ml.min\(^{-1}\).

<table>
<thead>
<tr>
<th></th>
<th>Epineurium</th>
<th>Subepineurium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 ml.min(^{-1})</td>
<td>6 ml.min(^{-1})</td>
</tr>
<tr>
<td>AUC ROC</td>
<td>0.55</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>(0.11 to 0.99)</td>
<td>(0.54 to 0.96)</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>40 (7 to 77)</td>
<td>71 (47 to 87)</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>100 (18 to 100)</td>
<td>88 (53 to 99)</td>
</tr>
<tr>
<td>Youden index</td>
<td>70 (7 to 77)</td>
<td>80 (47 to 87)</td>
</tr>
<tr>
<td>Log cut-off point (kPa)</td>
<td>4.35</td>
<td>4.07</td>
</tr>
<tr>
<td>Cut-off point (kPa)</td>
<td>77.5</td>
<td>58.6</td>
</tr>
<tr>
<td>Cut-off point (psi)</td>
<td>11.6</td>
<td>8.8</td>
</tr>
</tbody>
</table>
DISCUSSION

We showed that fluid injection pressure is a poor biomarker of needle tip position: a wide range of overlapping measurements were recorded when the needle tip was lodged within muscle, epineurium or subepineurium. Using ultrasound, we observed the circumneural layers and subcircumneural injection. We did not observe an increase in size of any fascicle following intraneural injection.

We conducted this study because there is still a preconception amongst many experts\textsuperscript{4-9}, despite evidence to the contrary\textsuperscript{16}, that high pressure intrafascicular injection contributes to nerve damage.

The principal strength of our study was that our imaging system enabled us to visualize the precise position of the needle tip relative to anatomical tissue at resolutions of approximately 100µm, and match tip position with pressure changes. and, in doing so, allowed us unravel the mechanisms of both nerve block and nerve injury.

For clarity in the following discussion, we define: injection outside the nerve as sub-circumneural injection; injection on first contact with nerve as epineurial (albeit that minimal amounts of injectate may also pass intraneurally depending on the relative position of the needle orifice); and intraneural injection as subepineural injection outwith the fascicles.

We confirm previous studies that regional anesthesia block needles are not able to penetrate fascicles\textsuperscript{16};\textsuperscript{20}. Scanning electron microscopy has verified that the endoneurium is a rigid structure resistant to the entry of liquids and not a soft, fragile tissue - a concept only recently acknowledged\textsuperscript{13}.

Mean injection pressures on epineural contact and during subepineural injection were similar to those obtained in previous experiments on anesthetized pigs\textsuperscript{7}, the soft embalmed cadaver\textsuperscript{19,28} and during needle nerve contact in patients\textsuperscript{11,12}. However, the range of values varied considerably at all sites. The wide range of pressure results may be explained by: consideration of resistance to fluid injection through a needle orifice that only partially covers fascicular tissue; to the physics of injection at all
tissue layers; and the means of fluid delivery.

First, fluid injected at high pressure liquid is directed towards compliant intraneural fat which offers much less resistance than fascicular tissue\(^\text{29}\). In this scenario, injectate does not enter the fascicle but instead exits towards the intraneural fat, and provides an element of safety\(^\text{16}\). For example, a needle bevel angle of 15° will have a larger elliptical orifice area than a 30° needle even if both needles have the same external diameter, and thus reduces the vulnerability of the nerve to intrafascicular injection. The figure in the Appendix helps explain this concept. Indeed, in nerves that do not have fat between the fascicles, and only have interlaced collagen fibers, it is impossible not only to make an intrafascicular injection but also an extraneural injection. The resistance of both the endoneurium within the fascicles and the collagen fibres that provide a second protective layer to each of the fascicles, prevents the entry of any solution into extraneural tissue\(^\text{14}\).

The same principles apply when considering extraneural injection. The sub-circumneurium fat compartment offers low resistance whereas injection close to the collagen-rich external surface of epineurium meets greater resistance. Thus, local anesthetic will flow in the path of least resistance creating the classical “donut” pattern of spread. Similarly, encapsulation of the needle orifice within intramuscular fibrotic tissue, and away from adipose tissue, may explain the sole high-pressure response to intramuscular injection in one cadaver.

Second, the wide range of opening pressure measurements at epineurium and in subepineurium, reflect the exponential relationship between the axial force applied by a needle tip (stress) and the displacement of tissue (strain) encountered at all tissue interfaces such as fascia and within contained compartments such as nerves. This means that minimal changes in needle tip position are associated with considerable changes in fluid injection pressure.

Third, injection was given by hand and not an electromechanical infusion pump, and contributed to the high variability of pressure measurement.
Thus, it is not surprising unsurprising, given these fundamental principles, that needle tip position was
indiscernible between tissue types $< 138$ kPa (20 psi) and that peak pressures reached $572$ kPa (83 psi).
If translated to clinical practice, such a range of opening injection pressures would not offer any help
to the anesthesiologist over and above clinical observation using ultrasound.

Our high resolution images also give credence to a recent hypothesis regarding the efficacy of
regional anesthesia that proposes-intimates that experts obtain better anesthesia because they inject
both within the subcircumneural compartment and unintentionally within the subepineurium. By
inference, more tentative novices inject further from the nerve within muscle, and fail to provide
sufficient anesthesia because local anesthetic has further to diffuse. Fig 3 (images (i) to (iii)) and the
corresponding video in the Appendix show fascial planes between the collagenous border of
epineurium and the epimysium with injectate occupying both spaces. We would further hypothesize
that injection within the subcircumneural compartment but close to epineurium is associated with
slightly higher initial resistance to injection compared to injection 1 to 2 mm distant from the nerve,
but is more likely to provide better, longer anaesthesia because more local anesthetic enters the nerve.
Fick’s law of diffusion suggests that the closer to the nerve, the greater the concentration gradient and
the fewer the fascial barriers, then the greater the amount of local anaesthetic entering the nerve.

The weaknesses of our study were the use of an animal model and failure to measure pressure at the
needle tip. Nevertheless, it should be noted that Server et al. [11] demonstrated that pig and sheep
models represent the closest simulated conditions to humans for in vivo study. Direct measurement of
pressure at the needle tip, rather than in-line measurement, is possible using Fabry-Perot
interferometry but needles are not yet available commercially. An air-filled, glass bound cavity is
bonded to the tip of an optical fibre secured to the side-wall of a regional anesthesia blockade needle.
Optical fibres have numerous advantages: they are light, flexible, dielectric (a non-conductor of DC
current) and provide a continuous graphical output. However, the accuracy of such sensors to
differentiate between anatomical interfaces also needs to be evaluated using realistic models and with
known sensor position.
In future studies, it will be necessary to define which types of needles, depending on their external diameter and orifice size, are considerably larger than fascicles and can be safely used at each nerve site.

In conclusion, identification of the precise location of the needle tip was not possible using injection pressure monitoring. Intrafascicular injection was not observed. Consideration of our microultrasound observations allow us to hypothesize that excessive pressures are not associated with subperineural injection (intrafascicular injection), and that placement of the needle tip and orifice and injection of local anaesthetic within the subcircumneural compartment (in close contact with epineurium) block has the potential to offer a more effective anesthetic block, with less latency and longer duration. Our findings have implications for other clinical interventions. One example is administration of intraneural drugs for the treatment of different neurological pathologies in which in situ administration is essential to reach the target. However, more studies will be needed to confirm our affirmations.
REFERENCES


http://dx.doi.org/10.1136/rapm-2018-100087

Acknowledgments: The authors wish to sincerely thank the staff of the Sunnybrook Research Institute Comparative Research Centre, Dr C Demore and A Chandra.
TABLES

Table 1. Nerve and fascicle characteristics. Median and radial nerves had a greater Feret’s diameter than axillary nerves. Radial nerves had more fascicles than axillary nerves. Fascicles within median and radial nerves were larger than fascicles within axillary nerves. The ratio of total fascicle area to nerve area was ~ 0.30 for all nerves. Results presented as mean (SD) and difference between means (95%CI).

Table 2. Accuracy of fluid injection pressure at epineurium and subepineurium using three flow rates 1 ml.min\(^{-1}\), 6 ml.min\(^{-1}\) and 12 ml.min\(^{-1}\). Data show best accuracy at epineurium using 12 ml.min\(^{-1}\).

FIGURES

Figure 1. Examples of microultrasound imaging of nerves before and after injection. Image (a) shows individual changes in nerve size after injection. Image (b) shows nerves scanned before injection and overlying the pectoral muscle bridge. The arrow in image (c) indicates injection within the muscle layer adjacent to the median nerve. The proportional increase in area did not differ between nerves, image (d). Image (e) shows a 22g regional anesthesia block needle inserted in-plane with distal aperture visualized and expansion of the axillary nerve. The arrow in image (f) indicates epineural rupture of the radial nerve. M - Median nerve; Ax - Axillary nerve; R - Radial nerve; F - Fluid.

Figure 2. Muscle, epineural and subepineural fluid injection pressures. Difference in log injection pressure (image a) between muscle and subepineural injection pressures (P = 0.042). Image (b) shows log-normal distribution of data at all interfaces. Image (c) shows injection pressures at each interface categorized according to flow (1, 6 and 12 ml.min\(^{-1}\)). No difference in pressure according to flow rate.

Figure 3. Ultrasound images paired with fluid injection pressure. Images (a) to (d) captured at four time points (i) to (iv). (i) preinjection; (ii) intramuscular injection, and subepineural injection; (iii) subepineural and subcircumneural injection. The asterisks in (ii) and (iii) indicate the circumneurium.
of the nerve; (iv) intraneural injection outwith the fascicles. The corresponding video is available in the Appendix. Image (f) is the log transformed pressure profile of image (e).

Appendix

Video corresponding to Fig 3.

Human ulnar nerve and nerve regional anesthesia block needles. A: Difference in needle orifice at same external diameter (22g, 0.70 mm diameter) and 15° or 30° bevel angle.

B: Human ulnar nerve. Needle tip placed at intrafascicularly (label 1) or at epineurium (label 2). The third option in subepineurium is showed as label 3 (peripheral adipose tissue).

This image was built superimposing images using Adobe Photoshop (Adobe, San Jose, California, USA). The images from Dr Reina’s archives obtained in his lab, was captured at same magnification had been stained with haematoxylin-eosin. Magnification: original acquisition at x20. The images show the alternative place where a needle tip can be introduced, but the conclusion obtained from looking at the image is only valid for ulnar nerve and not extrapolated to other nerves because the proportion between fascicles sizes, distribution of them, and thickness of extrafascicular adipose tissue will be different.
Pre-injection
Post-injection

Nerve area (mm²)

<0.0001

Axillary Median Radial

Percent change in area (%)

0.4689
0.1993
0.3638

(a)

(b)

(c)

(d)

(e)

(f)

5 mm

5 mm

5 mm