Nerve injury by needle nerve perforation in regional anaesthesia: does size matter?

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Background. In regional anaesthesia, there is a risk of direct nerve injury. The purpose of this study was to determine whether the diameter of the applied needle is associated with the magnitude of nerve injury after needle nerve perforation.

Methods. In five anaesthetized pigs, the brachial plexus were exposed bilaterally. Up to eight nerves underwent needle nerve perforation using a 24 G pencil-point cannula (small diameter) or a 19 G pencil-point needle (large diameter). After 48 h, the nerves were resected during anaesthesia. The specimens were processed for visual examination and the detection of inflammatory cells, myelin damage and intraneural haematoma. The grade of nerve injury was scored ranging from 0 (no injury) to 4 (severe injury).

Results. Forty-eight nerves were examined. The applied injury score was significantly lower in the small-diameter group [median (inter-quartile range) 2.0 (2.0–2.0)] compared with the large-diameter group [3.5 (3.0–4.0) P<0.01]. Myelin damage and intraneural haematoma occurred predominantly in the large-diameter group. Signs of post-traumatic regional inflammation were comparable among both groups.

Conclusions. The severity of nerve injury after needle nerve perforation was related to the diameter of the applied cannula. However, no such difference exists for regional inflammation. Functional consequences of these findings need to be determined. Currently, small-diameter cannulae may be advisable for peripheral nerve blocks to minimize the risk of nerve injury in the case of nerve perforation.

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Ultrasound-guided regional anaesthesia suggests that direct needle-nerve contact or even intraneural needle placement may not necessarily result in adverse outcome.1–4 However, reports about functional or structural consequences of needle-nerve contact or nerve perforation are limited.5 6 Interestingly, whether smaller or larger needles result in different trauma after nerve perforation has so far been neglected. Nevertheless, for clinical use, different needle sizes for the same purposes are commercially available.

One reason for the obvious lack of data may be the challenging methodology with respect to nerve injury assessment in both human and animal studies. In humans, only clinical or functional assessment of putative nerve injury is conceivable, whereas in animals, histological analysis is feasible. Nevertheless, histological examination of nerve tissue with conventionally applied techniques, such as dissection and haematoxylin–eosin staining followed by light microscopy, is easily compromised by artifacts after the post-processing of specimens.7–10 Although direct nerve trauma may be visible, indirect signs such as neuroinflammation11–14 have been cited as evidence for significant nerve trauma. Bias introduced by processing
methods seems unlikely. However, regional signs of inflammation after traumatic nerve injury may occur in vivo not earlier than 24 h after peripheral nerve trauma. Besides neuroinflammation, occurrence of intraneural haematoma may well serve as an indirect sign of nerve trauma. Apart from secondary measures of nerve injury, histomorphological assessment of myelin integrity serves as a valid diagnostic sign.

The aim of this study was to challenge the hypothesis that needle nerve perforation with small-diameter cannulae causes less nerve injury compared with cannulae with larger diameters. Primary defined endpoints of the study were the presence and magnitude of post-traumatic regional inflammation, occurrence of intraneural haematoma, and signs of myelin damage.

**Methods**

**Animals**

The experimental procedures were approved by local authorities (Ref: 50/2007, Regional Board, Giessen, Germany) and the study was performed in compliance with the Helsinki convention for the use and care of animals. In this study, five female pigs (Deutsche Landrasse) weighing 38–49 kg (mean 42.3 kg) were used.

**Anaesthesia**

On the day of experimentation, general anaesthesia (propofol: 0.2 mg kg\(^{-1}\) min\(^{-1}\), i.v.; sufentanil: 0.5 μg kg\(^{-1}\) h\(^{-1}\), i.v.) was administered after premedication as described recently. No neuromuscular blocking agents were administered. After tracheostomy, the pigs’ lungs were ventilated with pressure-controlled ventilation (Siemens Servo 300; Maquet Critical Care, Darmstadt, Germany) with 30% oxygen and nitrogen. Haemodynamics and respiration were monitored by ECG (Servomed Monitor; Hellige, Freiburg i.B., Germany), capnometry (DM 8020, Dräger AG, Luebeck, Germany), and pulse oximetry (Biox 3740; Ohmeda, Louisville, CO, USA). The pigs were kept anaesthetized for 48 h. Adequate anaesthesia was ascertained by adapting the dosage of propofol (maximum 0.3 mg kg\(^{-1}\) min\(^{-1}\)) and sufentanil (maximum 1.5 μg kg\(^{-1}\) h\(^{-1}\)) corresponding to signs of stress or awareness such as spontaneous breathing, stress-related tachycardia, or shivering. Fluid maintenance was with Ringer’s lactate solution 3–5 ml kg\(^{-1}\) h\(^{-1}\). The body temperature was assessed and kept constant using warming blankets (Bair Hugger Model 540; Arizant Healthcare Inc., Eden Prairie, MN, USA).

**Surgical procedures and needle placement**

The anaesthetized animals were placed in the supine position with both forelimbs slightly abducted. Using an aseptic technique, the axillary region was opened carefully by blunt dissection on both sides. The surgical approach was minimized due to prevention of bleeding by dissection of muscular tissue and larger vessels. After cautious dissection of the vascular nerve sheath, the brachial plexus was exposed. Contact by surgical instruments to nerves was avoided. Nerve connective tissue within the vascular nerve sheath was not removed. Thereafter, the investigator had a direct view of each plexus nerve. Before needle placement, sutures serving as visual references had been inserted. In addition, anatomic landmarks and the localization of sutures were documented photographically.

On each side, the nerves of the brachial plexus were identified and defined (Fig. 1). The musculocutaneous, the median, the radial, and the axillary nerves were identified for needle nerve perforation, thus in each pig, eight nerve perforations were scheduled. Twenty-four (UniPlex NanoLine®, Sprotte® 24 G; Pajunk, Geisingen, Germany) and 19 G (PlexoLong NanoLine®, Sprotte® 19 G; Pajunk) atraumatic pencil-point needles were selected for needle nerve perforation. The needle placements were scheduled from cranial to cannula on both sides, beginning with the right-hand side. The selection of cannula diameter and side of plexus was random. Nevertheless, either small (Group 24 G, n=4) or large-bore (Group 19 G, n=4) cannula was used per side. The needle tips were placed perpendicular to the target nerves. The needles were then slowly pushed forward until the needles perforated the nerves. The cannulae were left in this position for 40 s. Then, the needles were carefully retracted. After completion of the intervention, the tissue around the brachial plexus was carefully closed and sutured. Cefuroxim...
80 mg kg$^{-1}$ day$^{-1}$ i.v. was administered to all animals and anaesthesia was maintained.

After 48 h, the wound was reopened under general anaesthesia and the nerves of the brachial plexus were dissected. The photographs and visual references (sutures) guided the removal of treated nerve tissue (Fig. 1). The right caudal pectoral nerve of each pig was designated a control (non-treatment brachial plexus), that is, these nerves were not exposed to any treatment. After histological analysis, we were able to determine whether the surgical procedure for the axillary approach had any influence on the accumulation of inflammatory cells corresponding to a nerve trauma. Additionally, the gluteal region was opened and the left sciatic nerve was resected. The sciatic nerve represented a nerve tissue that was neither exposed to any potential surgical trauma (non-treatment sciatic nerve), nor any needle treatment. Thereby possibly confounding variables with respect to neuroinflammation such as systemic inflammation after needle placement, surgery and anaesthesia, antimicrobial therapy, or any other intervention could be identified.

All animals were killed at the end of the study period by an i.v. injection of potassium chloride (4 mmol kg$^{-1}$).

**Histology**

Each specimen (1–1.5 cm in length) was fixed by immersion in formalin for 48 h. After fixation, all tissue blocks were extensively washed in 70% 2-propanol and processed for paraffin embedding. Series of tissue slices (7 μm) were taken throughout the specimen length.

Nerve specimens of ~1 cm were cut and every third slice was haematoxylin–eosin stained. The initial histological analysis by light microscopy focused on the detection of the puncture site which was usually characterized by circumscribed accumulation of inflammatory cells or haematoma plus infiltration with inflammatory cells adjacent to the particular nerve. Within the puncture area, the pathologist searched for the most distinctive area of inflammatory response or the combination of inflammation and haematoma to locate the puncture site. Subsequently, at least four adjacent slices in both directions were alternately stained for either macrophages or myelin. Myelin was stained applying the technique by Kluver–Barrera$^{16,17}$ to differentiate vital and avital myelin tissue. CD68-labelling$^{18}$ was applied for the identification of macrophages and monocytes representing characteristic target cells with regard to neuroinflammation after nerve injury (Fig. 2)$^{11}$ For the purpose of this study, we developed a specific ‘injury score’ (Table 1), adopting aspects from Hirata and colleagues.$^{19}$ The score facilitates the appraisal of the grade of inflammatory response (haematoxylin–eosin), the occurrence of haematoma, and the presence of myelin damage. The score value was separately assessed by three trained outcome assessors who were unaware of the treatment groups. Discordant evaluation exceeding one score grade were discussed. Disagreements within one score grade were resolved determining the arithmetic mean.

The relative number of CD68-positive monocytic cells (macrophages, monocytes) to leucocytes was assessed by counting five representative visual fields including intra- and extravascular areas ($\times200$ magnification).

**Immunohistochemistry**

Endogenous peroxidise was blocked in deparaffinized sections using methanol (Fluka, Steinheim, Germany) and hydrogen peroxide 30% (Merck, Darmstadt, Germany). Trypsinization for antigen demasking was carried out using 0.1% trypsin solution (Sigma-Aldrich, St Louis, MO, USA). After rinsing with distilled water and phosphate buffered saline (PBS; Roth, Karlsruhe, Germany), an incubation step with 10% horse serum followed (Vector, Burlingame, CA, USA). Since there is no specific antibody available for macrophage detection in pigs, we used a modified method for mice. Hence, the incubation with a monoclonal primary mouse antibody (Clone P6-M1, Dako, Glostrup, Denmark) against human CD68-antigen diluted 1:100 in ChemMate Antibody-Diluent (Dako) was carried out for 60 min at...
After rinsing with wash buffer (WashBuffer, Dako), the incubation of sections with a 1:100 diluted, biotinylated secondary antibody [Anti-Mouse IgG (H&L), Vector] was performed for 30 min at room temperature in a humid chamber. After rinsing with wash buffer (WashBuffer, Dako), samples were incubated with avidin–biotin complex (Vectastain ABC-Kit Standard, Vector) for 30 min. Rinsing with PBS was followed by incubation with 1% 3,3′-di-aminobenzidine tetrachloride (Sigma-Aldrich), 0.05 M Tris–HCl (pH 7.4), and 0.075% H₂O₂. After final

<table>
<thead>
<tr>
<th>Score value</th>
<th>Definition</th>
<th>Staining methods</th>
<th>Legends</th>
<th>Notations</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No signs of neural injury or inflammation</td>
<td>Haematoxylin–eosin</td>
<td>N, nerve tissue; A, artifact. Magnification: a, ×100; b, ×100; c, ×200</td>
<td>No inflammatory cells according to both staining methods. Vital myelin (Kluver–Barrera) appears deep blue; nerve tissue appears compact</td>
</tr>
<tr>
<td>1</td>
<td>Areas with slight accumulation of inflammatory cells</td>
<td>Haematoxylin–eosin</td>
<td>I, inflammatory cells; A, artifact; N, nerve tissue. Magnification: d, ×100; e, ×100; f, ×200</td>
<td>Few inflammatory cells are located around vessels and fascicles of nerve tissue. No signs of myelin damage</td>
</tr>
<tr>
<td>2</td>
<td>Areas with distinctive signs of inflammation</td>
<td>Haematoxylin–eosin</td>
<td>N, nerve tissue; I, inflammatory cells; M, myelin. Magnification: g, ×100; h, ×100; i, ×400</td>
<td>Abundant perivascular, perineural, or both infiltration of inflammatory cells adjacent to nerve tissue. No signs of myelin damage</td>
</tr>
<tr>
<td>3</td>
<td>Areas with distinctive signs of inflammation plus haematoma</td>
<td>Haematoxylin–eosin</td>
<td>H, haematoma; N, nerve tissue; I, inflammatory cells. Magnification: j, ×100; k, ×100</td>
<td>Haematoma located next to an abundant invasion of inflammatory cells adjacent to nerve tissue. No signs of myelin damage</td>
</tr>
<tr>
<td>4</td>
<td>Areas with distinctive signs of inflammation plus myelin damage</td>
<td>Haematoxylin–eosin</td>
<td>I, inflammatory cells; N, nerve tissue; M, myelin; H, haematoma. *Injured area. Magnification: l, ×100; m, ×100; n, ×400</td>
<td>Signs of myelin damage with inconsistently and lower stained myelin (Kluver–Barrera). Myelin appears stretched and swollen (Fig. l compared with Fig. n)</td>
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rinsing with distilled water, haematoxylin staining (Merck) and dehydration in alcohol were performed.

Statistical analysis
The primary outcome measure was nerve damage after needle nerve perforation with a small-diameter needle (Group 24 G) or a large-diameter needle (Group 19 G) according to the grading of the ‘injury score’. The sample size was chosen to provide a 90% power to detect a score value difference of 1.0 between the non-treatment group of the brachial plexus, the small-diameter group (Group 24 G), and the large-diameter group (Group 19 G) using Tukey–Kramer’s all-pairs test (pairwise comparisons). A type-I error of 5% and a standard deviation (sd) of 0.5 in each group were assumed. Since most differences were expected between the non-treatment group of the brachial plexus and the perforation groups (24 G, 19 G), an unequal design with regard to the sample size and the allocation of the non-treatment group and the needle placement groups was executed. A specimen allocation of 1:3:3 was scheduled (non-treatment group: brachial plexus:Group 24 G:Group 19 G). The non-treatment sciatic nerve, that is, the control for systemic effects and confounders irrespective of any planned experimental intervention, has not been considered for the sample size calculation.

Using the PASS 2002 statistical package (Numbers Cruncher Statistical Systems, Kaysville, UT, USA), it was calculated that a total number of at least 35 specimens were required. We planned five pigs to allow at least four intended needle tip placements per needle placement group and animal.

Data are presented as median [inter-quartile range (IQR)]. Differences among the groups regarding score value were determined by the Tukey–Kramer test. A P-value of ≤0.05 was selected as the criterion of significance. Descriptive statistics have been applied with respect to the relative value of monocytic cells to leucocytes (mean and sd). However, a score value >1 was required for the assessment of monocytic cells.

Statistical analysis was performed using SPSS software for Windows (Release 15.0, SPSS, Chicago, IL, USA).

Results

Animals
None of the animals showed signs of local or systemic infection. Neither fever (>38°C) nor cardiopulmonary complications occurred throughout the experiments.

Needle placement and immediate macroscopic evaluation
After retraction of 19 G cannulae (large diameter), the perforation holes were easy to locate and the nerves developed a small but visible haematoma adjacent to the perforation. In contrast, after the retraction of the 24 G needle (small diameter), diligence was necessary to identify the site of puncture.

Resected nerve specimens
Two nerves of the large-diameter group (19 G) developed a distinctive haematoma during nerve resection (i.e. an iatrogenic lesion independent of the index intervention). These nerves were excluded from microscopic analysis. After 48 h, none of the perforation holes by small-diameter cannulae was identifiable macroscopically in contrast to those after 19 G treatment (large diameter).

Assessment of nerve injury score
Artifacts, that is, fascicle destruction or axonal damage in the absence of inflammatory cells or avital myelin, were found in the treatment and in the control groups (Fig. 3). The presence of haematoma or myelin damage was associated with the accumulation of inflammatory cells (Table 1). Haematoma and myelin damage (Fig. 4, Table 2) were predominantly found in the large size diameter group compared with the small-diameter group. In cases with a score value >1, that is, signs of inflammatory response, we assessed the relative number of monocytic cells (i.e. the percentage of macrophages and monocytes to leucocytes). Nerves with signs of neuroinflammation contained considerably larger numbers of monocytic cells compared with the control (Table 2).

Nerve injury
Corresponding to the primary outcome in the treatment groups, a significant difference was found (Fig. 4, Table 2). The median score value for nerve injury was significantly higher [3.5 (3.0–4.0)] after needle nerve perforation using large-diameter cannulae compared with a small-diameter needle [2.0 (2.0–2.0)].

Discussion
The present study demonstrates a direct dependency of needle diameter and nerve trauma after intentional nerve perforation in an experimental setting. At first glance, this may seem trivial; however, in clinical practice, nerve trauma related to regional anaesthesia is of relevance. Therefore, any method potentially limiting deleterious side-effects, for example, use of smaller diameter cannulae, may prove beneficial.

Interestingly, cannulae sizes and related injury have not been investigated before. Several authors observed the
nerve integrity after nerve perforation with pencil-point (22 G), long and short bevelled cannulae. They found out that nerve perforation with pencil-point needles results in a minor nerve damage compared with short or long bevelled cannulae. Herein selected electrophysiological and histomorphological properties were the primary outcome parameters. Whether the needle diameter influences nerve integrity comparable to needle tip shape remains unknown.

To facilitate informed decision-making in clinical practice, such experiments should mimic the real-life scenario as close as possible. Therefore, pigs were used as the experimental model, since nerve diameter, anatomic sites, and basic physiology are comparable with human beings, enabling the use of standard equipment for regional anaesthesia. Moreover, specific requirements with respect to the experimental setting and the preparation of the specimen have to be fulfilled. First, the location of perforation must be reproducible during post-processing; thus the nerves were labelled atraumatic after needle penetration. Secondly, nerves were left in situ for 48 h to allow insult-related physiological and pathophysiological processes such as a regional inflammatory response. Thirdly, specimens need to be handled with extreme caution to avoid the introduction of artifacts eventually misinterpreted as nerve injury. For example, Figure 3 demonstrates a representative artifact which occurred during specimen post-processing rather than the experimental intervention.

Given the challenge of artifact-free post-processing, the unique focus on the presence or absence of visible direct nerve injury seems insufficient. Appreciating such difficulties, we therefore analysed parameters of inflammation related to the visible (and labelled) nerve injury. Any nerve injury without signs of regional inflammation as defined by the proposed score must therefore be regarded as artifact (injury without leucocytes=artifact). Of note, signs of regional inflammation are detectable no earlier than 24 h after the insult. Thus, one must wait with any specimen processing at least 24–48 h after the intervention without losing nerve integrity.

According to Mueller and colleagues and Eliav and colleagues, nerve-trauma-associated inflammatory responses were found after 48 h and the relative number of monocytic cells to leucocytes increased. In addition to inflammatory cell accumulation, haematoma and myelin damage were found as evidence of severe nerve injury. Acknowledging these investigations, we prolonged
anaesthesia for 48 h in our experiments with subsequent tissue harvesting.

Although no inflammatory response as a proof of nerve injury after direct penetration was observed in previous studies, this observation may be limited by direct tissue harvesting, thereby preventing any inflammatory response in vivo. This notion is emphasized by infrequent signs of myelin damage and haematoma in our experiments with small-diameter cannulae, comparable with the nerve perforation with small-diameter pencil-point needles as executed by Hirasawa and colleagues. Nevertheless, even 24 G pencil point canulas resulted in a pronounced inflammatory response. Neither axonal nor myelin alterations but inflammatory response was observed in almost all nerve specimen within the 24 G needle group. With regard to the applied methodology, we cannot conclude whether an isolated neuroinflammation (i.e. without any visible nerve injury) could eventually lead to neurological symptoms as well. Considering our experiments, a needle nerve perforation with 24 G needles does not result in structural nerve damage. This may in part explain why patients did not develop neurological deficits after intraneural needle placement in several clinical studies. Nevertheless, Eliav and colleagues demonstrated in rats that aseptic inflammation of a peripheral nerve is capable of provoking pain sensation that may be unrelated to apparent axonal damage. For instance, aseptic inflammatory responses caused by neurotrauma may alter neurological function induced by toxic mediators released via macrophages as described by Kiefer and colleagues and Moalem and Tracey. This finding serves to emphasize that macrophage accumulation itself may lead to neurological impairment independent of any structural lesion, for example, haematoma or myelin alterations.

According to our data, intraneural haematoma occurs frequently, especially after perforation with large-diameter needles. This alone may trigger the development of neurological deficits in patients, since several authors described neurological deficits and axonal loss subsequent to intraneural haematoma. The present investigation has a number of methodological limitations that need to be discussed. First, in our experimental setting, we utilized an open brachial plexus model. Although a percutaneous set-up might have been desirable for a variety of reasons (e.g. closer to clinical practice, abdication of surgery), a number of restrictions would have applied. Herein the challenge to execute and subsequently identify a defined nerve perforation illustrates important difficulties for a controlled and reproducible study design. Secondly, with respect to the development of local inflammation, data from Mueller and colleagues have demonstrated peak inflammation ~72 h after nerve trauma. Thus, given our experimental setting with an
observation period of 48 h, we may have missed the maximum of post-traumatic neuroinflammation. However, considering the surgical trauma and the experimental setting with indwelling catheters and long-term intubation and mechanical ventilation, the risk for nosocomial infection was not negligible. Moreover, Mueller and colleagues11 reported significant signs of inflammation after nerve trauma already after 48 h in almost all animals. We felt comfortable with a setting allowing for insult-related inflammation without too high a risk of nosocomial infection and therefore chose an observation period of 48 h.

Thirdly, we lack any functional assessment of nerve integrity, for example, electromyography or post-interventional assessment of the animals. However, the focus and aim of this study was to describe consequences of direct nerve perforation with respect to visible damage and pathophysiological reactions of nerves and surrounding tissue. Whether the described alterations necessarily translate into functional consequences indeed needs confirmation. Future experimental trials may focus on isolated nerve stretching compared with nerve perforation and the influence of regional inflammatory response combined with functional, neurological consequences. The applied methodology itself was robust leading to reproducible results among the animals. According to our controls, no systemic influences—that is, narcotics, antibiotics, or surgical procedure—on nerve integrity were found.

In conclusion, this study demonstrates that nerve perforation by large-diameter needles in pigs can elicit a more pronounced nerve injury compared with small-diameter cannulae. According to our histological findings, post-traumatic inflammation rather than structural damage of nerve tissue is the most reproducible sign of nerve injury after needle nerve perforation, irrespective of the applied needle diameter. Thereby, it seems advisable to use small-diameter needles for peripheral nerve blocks (e.g. in peripheral catheter techniques) in order to minimize the potential for nerve injury.

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