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Quantitative Architecture of the Brachial Plexus and Surrounding Compartments, and Their Possible Significance for Plexus Blocks

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Background: **Nerve injury after regional anesthesia of the brachial plexus (BP) is a relatively rare and feared complication that is partly attributed to intraneural injection. However, recent studies have shown that intraneural injection does not invariably cause neural injury, which may be related to the architecture within the epineurium. A quantitative study of the neural components and the compartment outside BP was made.**

Methods: **From four frozen shoulders, high-resolution images of sagittal cross-sections with an interval of 0.078 mm were obtained using a cryomicrotome to maintain a relatively undisturbed anatomy. From this data set, cross-sections perpendicular to the axis of the BP were reconstructed in the interscalene, supraclavicular, midinfraclavicular, and subcoracoid regions. Surface areas of both intraepineurial and connective tissue compartments outside the BP were delineated and measured.**

Results: **The nonneural tissue (stroma and connective tissue) inside and outside the BP increased from proximal to distal, being significant between interscalene/supraclavicular and midinfraclavicular/subcoracoid regions ($P < 0.001$ for tissue inside BP, $P < 0.02$ for tissue outside BP). The median amount of neural tissue remained approximately the same in the four measured regions (41.1 ± 6.3 mm^2; range, 30–60 mm^2). The ratio of neural to nonneural tissue inside the epineurium increased from 1:1 in the interscalene/supraclavicular to 1:2 in the midinfraclavicular/subcoracoid regions.**

Conclusion: **Marked differences in neural architecture and size of surrounding adipose tissue compartments are demonstrated between proximal and distal parts of the brachial plexus. These differences may explain why some injections within the epineurium do not result in neural injury and affect onset times of BP blocks.**

NERVE injury after regional anesthesia is a feared complication that can cause immediate or subacute short-term neurologic deficits and pain sensations, which can last for weeks or even months.[1] Data about the incidence of nerve injury with the use of peripheral nerve blocks show a relatively small incidence, ranging from 0.02%[2,3] to 0.2%[4] for distal block (axillary nerve block) and from 0.03%[3] to 0.4%[5] in short- and severe long-term neurologic complications of proximal blocks (interscalene nerve block). In animal studies, persistent neurologic complications range from 0 to 5% after brachial plexus blocks, depending on the technique used.[6] Intraneural injection of the local anesthetic is believed to be a mechanism that might cause nerve injury, especially after intraneural injections that are associated with high pressures.[7] Therefore, the use of electrical neurostimulation to evoke motor responses in the proximity of the nerves without puncturing them has been advocated.

A further enhancement is ultrasound-guided local anesthetic injection, which enables visualization of the individual brachial plexus constituents and is assumed to be more effective and less time-consuming, with potentially fewer complications.[8,9] Even with the precise visualization afforded by use of ultrasound, most experts recommend avoiding injection within the epineurium.[10] However, recent findings about ultrasound-guided axillary block with visually confirmed intraepineurial injection of the local anesthetic showed that it does not invariably cause neural injury.[11] In fact, there were no long-term neurologic deficits. This raises questions about the architecture of the brachial plexus within the epineurium and how variations in this architecture might explain how injections within the epineurium do not invariably lead to neural injury. Further knowledge of the physical amount of neural tissue and its ratio to nonneural tissue inside the epineurium would, therefore, provide new insights.

Furthermore, differences in neural architecture might shed some light on differences in onset time and local anesthetic volumes that exist in daily practice between proximal and distal brachial plexus blocks. A successful block of the upper extremity depends, among others, on the type, amount, concentration, lipophilicity, place of injection, and anatomical distribution of the local anesthetic and of the lipid content of the nerve tissue and surrounding extraneural tissue. In general, proximal blocks (interscalene and supraclavicular) are thought to have a faster onset than distal blocks (infraclavicular and axillary), but there are few data and little consensus. Further, it is difficult to compare proximal and distal techniques because nervous structures are blocked that differ in organization and topographic arrangement.

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We hypothesized that distal compartments surrounding the brachial plexus are larger and contain more fat and stroma within the epineurium in the brachial plexus. This architecture would lead to a potentially larger volume of distribution in the distal sites for any local anesthetic injected. To evaluate this hypothesis, we determined the size of the compartments surrounding the brachial plexus at the sites mentioned above in human cadavers. Quantitative data of the brachial plexus based on high-resolution cross-sectional images[12] were acquired in four regions: interscalene, supraclavicular, infraclavicular, and subcoracoid.

Materials and Methods

Cryomicrotomy was used because it allowed us to conduct a detailed histologic examination while preserving the original relatively undisturbed neurovascular topography of the brachial plexus.[12] Advantages of this method are that dimensions and surfaces can be measured and that topographic relations remain unaltered, which is not the case when dissection is used.[12] Further, cryomicrotomy would provide better insight into the dimensions and location of the various tissues that are bypassed when inserting a needle. This was combined with high-resolution photography.

After institutional review board approval (University Medical Center Utrecht, Utrecht, The Netherlands), four shoulders of three different cadavers (table 1) were obtained from the Department of Functional Anatomy of the University Medical Center Utrecht. The shoulders contained the regions between the scalene muscles and the coracoid process. The shoulders were frozen in carboxymethylcellulose gel at −25°C. Using a heavy-duty sledge cryomicrotome (PMV 450; LKB Instruments, Stockholm Sweden), consecutive sagittal sections (interval, 0.078 mm) of each specimen were obtained. The surface of each section was photographed (Nikon D1X; Nikon Corporation, Chiyoda-ku, Tokyo, Japan) at a resolution of 300 pixels/inch. The exact dimensions of the part of the specimen that appeared on the photographed image were noted. In total 1,100–1,500 images per specimen were collected. Thereafter, the coronal and axial planes were reconstructed using Enhanced Multiplanar-reformatting Along Curves software (E-MAC Group, Department of Information and Computing Sciences, University of Utrecht, Utrecht, The Netherlands). Therefore, per shoulder, three digital data sets were obtained, each set comprising 8.8 gigabytes. *Via* synchronous display of all planes using an Interactive Image Sequence Viewer program (N. Moayeri and G. J. Groen, Utrecht, The Netherlands)[13] and E-MAC, the individual roots, trunks, cords, and nerves were visualized and identified. *Synchronous* refers to a feature of both programs to run movie-like animations of consecutive images in one of the planes, while at the same time, the level of the section is shown as a moving line in the two other planes.

Table 1. Baseline Characteristics of the Cadavers

Specimen	Age, yr	Sex	Weight, kg	Height, cm	BMI, kg/m^2
LI,RI	62	F	73	168	25.9
RII	45	F	85	178	26.8
RIII	73	M	92	188	26.0

BMI = body mass index; L = left specimen; R = right specimen.

In each digital data set of the shoulder, the interscalene, supraclavicular, midinfraclavicular, and subcoracoid regions were documented. Per region, five locations were chosen: a center site, *i.e.*, midpoint, and locations 5 and 10 mm medial and lateral to each midpoint. If clear identification was not possible in the sagittal images, concomitant views of the brachial plexus in the two other planes were used to visualize and indicate the exact location and anatomy. In the interscalene region, the midpoint was the first sagittal image where the trunks of the brachial plexus emerged between the anterior and middle scalene muscles. As midpoint of the supraclavicular region, the site was chosen at which the brachial plexus lay immediately superior to the first rib. The midinfraclavicular midpoint was the middle of the distance between the suprasternal notch and the most ventral part of the acromial apophysis. To identify this point during sectioning, a 22-gauge needle was inserted in the vertical plane, perpendicular to the back and just inferior to the clavicle. The needle was removed, the cannula was left *in situ*, and the sagittal images containing the cannula formed the midpoint. Finally, as midpoint of the subcoracoid region, the most ventral point of the coracoid process was chosen. For each shoulder, in all 20 locations, separate reconstructions of the brachial plexus were made strictly perpendicular to the axis of the plexus (fig. 1A). Therefore, a total of 80 digital perpendicular reconstructions were created. In each of the images, by using E-MAC, the epineurial surface area was delineated, after which pixel counting revealed the surface area in mm^2. When the continuity of the epineurium was not fully visible in one image, a rapid sequential display of consecutive images was used to identify the epineurium. In the same manner, the individual neural fascicles with their perineurium were identified, and their total surface area was calculated as neural tissue (fig. 1B).

The borders of the tissue compartments surrounding the brachial plexus were identified using muscular borders or the first distinct fascial layer within the fat. In the interscalene region, the borders were formed by the anterior and middle scalene muscles, and in the subcoracoid region, the borders were formed by the minor pectoral and subscapular muscles. In the supraclavicular and midinfraclavicular regions, the first distinctive fascial

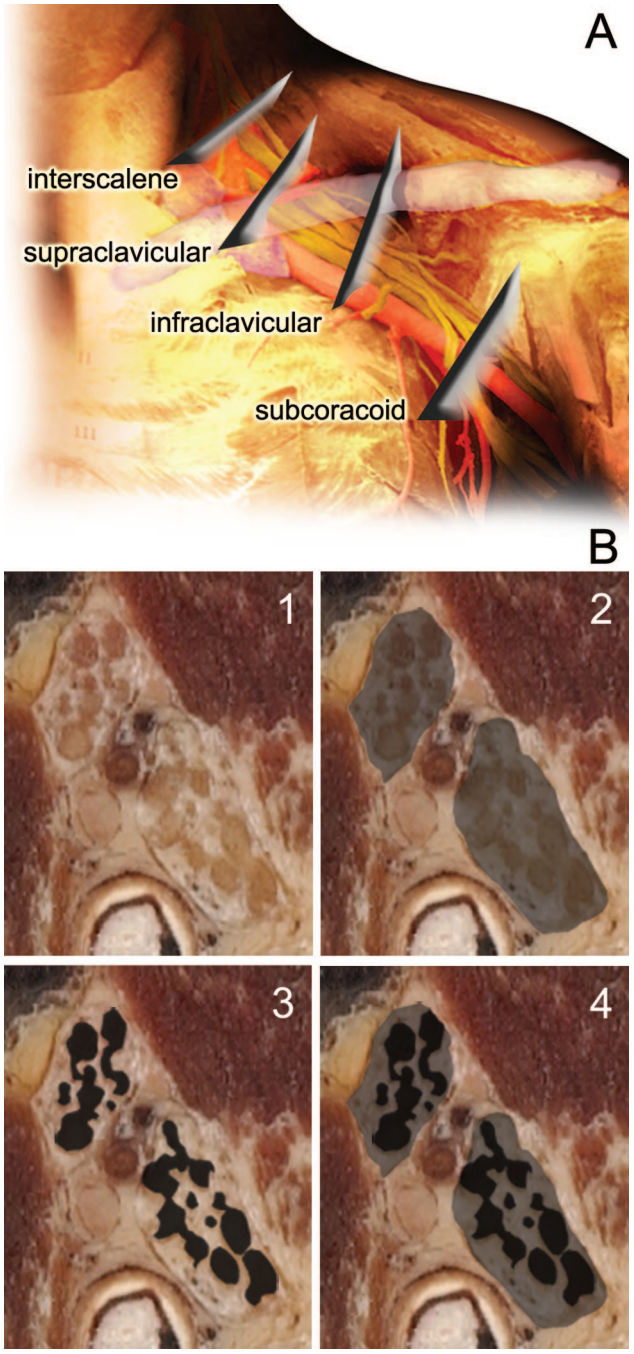

Fig. 1. (*A*) Reconstructed image of the brachial plexus showing the interscalene, supraclavicular, midinfraclavicular, and subcoracoid regions. (*B*) Representative perpendicular reconstructions demonstrating how measurements were conducted. Original reconstructed image (*1*), measured intraepineurial tissue (shaded in *gray*; *2*), measured neural tissue including perineurium and nerve fascicles (*black spots*; *3*), and combined image showing both measurements superimposed on the same image (*4*).

layer outside the epineurial layer of the brachial plexus lay within a large mass of adipose tissue. The area within this connective tissue compartment was demarcated and calculated.

In each region, the surface areas of fascicles/nerves and epineurium were subtracted from each other. The medians and the SDs of all values in the same region (midpoint and 5 and 10 mm medial and lateral to the midpoint) in all shoulders were calculated, including the areas surrounding the epineurium.

Some measurements were not included in the final analysis. The reason for this is that one or two regions medial or lateral to the midpoint in the interscalene and subcoracoid regions in the reconstructed perpendicular images did not contain the entire image of the brachial plexus. Differences in cross-sectional areas between the regions were determined by a two-sided Student *t* test. For statistical significance, a value of $P < 0.05$ was chosen.

Results

Figure 2 shows each region of the brachial plexus in detail. All values below are presented as median ± SD, unless stated otherwise. The median amount of neural tissue remained approximately the same throughout the brachial plexus (41.1 ± 6.3 mm^2; range, 30–60 mm^2) and did not show a significant difference between the four regions (fig. 3A). The values for the interscalene, supraclavicular, midinfraclavicular, and subcoracoid regions, respectively, were 40.7 ± 3,8 mm^2 (range, 32–45 mm^2), 45.0 ± 5.2 mm^2 (range, 37–57 mm^2), 38.5 ± 4.4 mm^2 (range, 33–48 mm^2), and 40.3 ± 9.4 mm^2 (range, 30–60 mm^2). The nonneural tissue inside the epineurium increased from proximal to distal. The median surface areas were 46.7 ± 9.5 mm^2 (range, 25–62 mm^2), 47.2 ± 7.4 mm^2 (range, 36–64 mm^2), 75.4 ± 16.3 mm^2 (range, 49–94 mm^2), and 76.0 ± 23.1 mm^2 (range, 50–123 mm^2) for the interscalene, supraclavicular, midinfraclavicular, and subcoracoid regions, respectively. Differences in values between interscalene/supraclavicular and midinfraclavicular/subcoracoid regions were significant ($P < 0.001$).

The ratio of neural to nonneural tissue in the epineurium is shown in figure 3B. In the interscalene, supraclavicular, midinfraclavicular, and subcoracoid regions, the percentages of neural tissue inside the epineurium were, respectively, 45 ± 4% (range, 41–57%), 48 ± 4% (range, 42–58%), 34 ± 6% (range, 29–48%), and 34 ± 3% (range, 30–39%). These differences were significant ($P < 0.001$).

The area of the connective tissue compartment surrounding the brachial plexus increased in all shoulders from proximal to distal (fig. 3C). The values per region showed the same pattern. The areas increased from 201.5 ± 38.5 mm^2 (range, 152–275 mm^2) to 222.7 ± 63.33 mm^2 (range, 159–396 mm^2), to 689.6 ± 181.7 mm^2 (range, 354–946 mm^2), and to 706.3 ± 148.4 mm^2 (range, 587–1,058 mm^2) for the interscalene, supraclavicular, midinfraclavicular, and subcoracoid regions, respectively. The increase in area between interscalene/

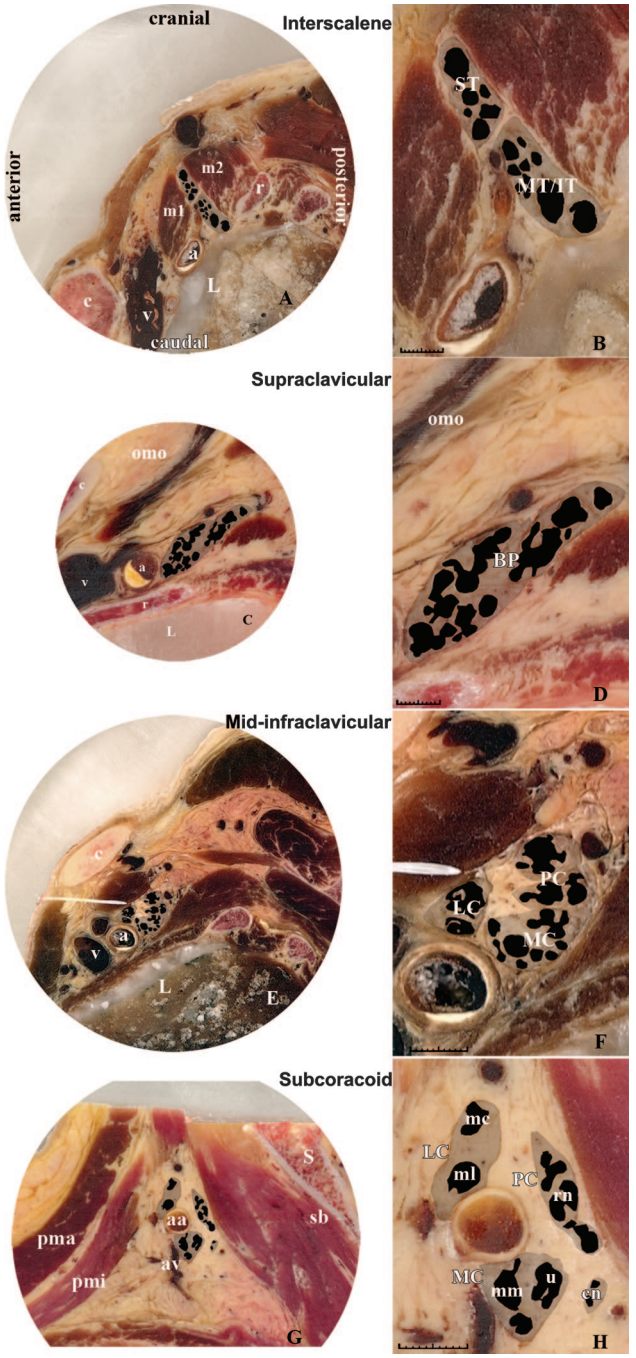

Fig. 2. Overview of the investigated areas (*left column*), with details of the measured neural contents (*right column*, *black spots*) and epineurial areas (*gray fields*). Interscalene (*A* and *B*), supraclavicular (*C* and *D*), midinfraclavicular (*E* and *F*), subcoracoid (*G* and *H*). aa/av = axillary artery/vein; a/v = subclavian artery/vein; BP = brachial plexus; c = clavicle; cn = cutaneous nerves; L = lung with plural cavity; m1/m2 = anterior/middle scalene muscle; mc = musculocutaneous nerve; ml/mm = lateral/medial root of median nerve; ST/MT/IT = superior/middle/inferior trunk; omo = omohyoid muscle; PC/MC/LC = posterior/medial/lateral cord; pma/pmi = major/minor pectoral muscle; r = first rib; rn = radial nerve; S = scapula; sb = subscapular muscle; u = ulnar nerve. *Bar* represents 10 mm.

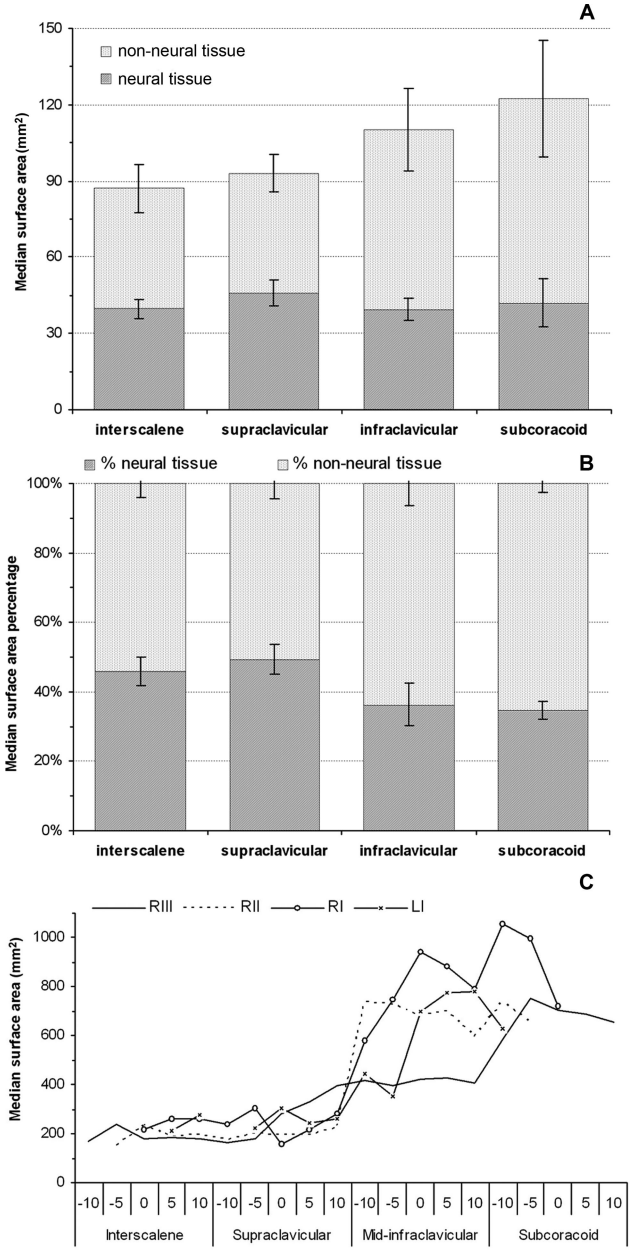

Fig. 3. Measured areas in the interscalene, supraclavicular, midinfraclavicular, and subcoracoid regions of all shoulders (L = left; R = right). (*A*) Absolute values (mm²) of neural and nonneural (connective) tissue inside the epineurium (median ± SD). (*B*) Relative values (percentage) of neural *versus* nonneural tissue inside the epineurium (median ± SD). (*C*) Absolute values (mm²) of adipose/connective tissue compartment surrounding the brachial plexus depicted per shoulder, from the most proximal (location − 10 interscalene) to the most distal area (location + 10 subcoracoid).

supraclavicular and midinfraclavicular/subcoracoid regions was significant ($P < 0.02$).

Discussion

The current study demonstrates in relatively undisturbed anatomy that differences exist in neural architec-

ture between the various parts of the brachial plexus, which may have implications for the understanding of the plexus blocks. The cryomicrotome technique we used is considered as the standard for allowing histologic examination of large areas of relatively undisturbed anatomy. It provides detailed information, which is currently superior to that of computed tomography, magnetic resonance imaging, or ultrasound.[12,14] Furthermore, the high-resolution images and reconstructions we made with an interval of less than 0.1 mm allowed us to accurately demarcate and measure the contents of the brachial plexus within and outside the epineurium.

The neural tissue inside the epineurium is formed by single nerve fibers enveloped by endoneurium, which are organized in bundles (fascicles) surrounded by perineurium. Although the perineurium *per se* is connective tissue as well, in this study we consider the perineurial tissue and its contents equivalent to neural tissue. The proximal (interscalene and supraclavicular) regions show a more solid, oligofascicular pattern. More distal, the fascicles show a more "scattered," polyfascicular pattern, which is in keeping with the work of Bonnel and Rabischong,[15,16] who showed, from proximal to distal, an increase in the number of fascicles and decrease in diameter of fascicles. Because the perineurium, in contrast to the epineurium, is a tough and mechanically resistant tissue,[17] it is unlikely that a blunt needle will penetrate it easily. These findings may explain why penetration of the epineurial layer does not always lead to observed neural damage.[11] It can further explain that, when a needle hits the perineurium, paresthesias are elicited, and they disappear after a small redirection of the needle. The polyfascicular configuration and relative increase in nonneural tissue more distally may explain why these events occur without clinical neurologic sequelae. Although results from two recent reports did show a higher incidence of neurologic dysfunction in proximal *versus* distal nerve blocks, the absolute number of complications is too low to draw definite conclusions about its etiology.[3,18]

Injection inside the perineurium is associated with high injection pressures and leads to fascicular injury and neurologic deficit, whereas injection inside the epineurium results in low initial pressures with return of normal motor function.[7] Further support for this phenomenon are the findings from a recent study in rats, in which intraneural injections of ropivacaine at concentrations routinely used in clinical practice seemed to have no deleterious effect on sciatic nerve motor function.[19] Therefore, it is tempting to say that an intraneural injection will not invariably cause neural damage as long as one stays out of the perineurium.

The neural tissue content of the brachial plexus remained approximately the same throughout the plexus. It varied between 38.5 and 45 mm^2, but the ratio of neural to nonneural tissue decreased from a proximal value (interscalene/supraclavicular) of approximately 1:1 to a distal value (midinfraclavicular/subcoracoid) of approximately 1:2. Our data confirm the results of histologic studies of 21 dissected brachial plexuses.[15,16] The 1:2 ratio we found is in keeping with these studies,[15,16] and also with the work of Slingluff *et al.*[20] However, the absolute and relative increases in nonneural tissue from the proximal (interscalene) to the distal (subcoracoid) parts of the plexus are not in keeping with that report.[20] In that dissection study, the amount of nonneural tissue remained approximately the same, and in fact showed a slight decline from proximal to distal (67% to 65.4%).[20] The observed differences in the proximal parts are most probably explained by the differences in techniques used, *i.e.*, undisturbed anatomy *versus* dissection. Furthermore, also the size of the compartment of adipose tissue outside the epineurium increased between interscalene and subcoracoid regions.

We speculate that the anatomical findings we described may have correlations with the onset time of brachial plexus blocks. Most practitioners attempt to inject local anesthetic outside the epineurium when they perform brachial plexus anesthesia. We have shown that there is a larger mass of fat outside the plexus in the more distal regions of the plexus, which might serve as a reservoir for lipophilic local anesthetics. Therefore, the time needed to reach the neural tissue might be prolonged because less local anesthetic is available to diffuse across the epineurium to block the neural tissue. At the same time, from proximal to distal, the neural tissue is surrounded by an increasing amount of epineurial connective tissue. Therefore, the local anesthetic will physically need more time to reach the fascicles if it is not injected in the vicinity of the fascicles. Also, the diffusion rate of the connective tissue will differ if it contains different substances. The aforementioned factors would lead to a slower onset time and a requirement for larger doses of local anesthetic in the distal plexus. This is, in fact, what most practitioners observe in clinical practice. Unfortunately, no clinical studies have been published up to now comparing onset time of the same local anesthetic between proximal and distal brachial plexus blocks. Because we did not include injection of stained solutions in cadavers or study the spread of local anesthetics in patients, these assumptions must be confirmed in further studies. Finally, one should take into account that the minimal local anesthetic concentrations commonly used to achieve reliable block require up to 40 ml of injected local anesthetic. This large dose may mask the differences that could be expected between proximal and distal approaches to the brachial plexus.

The limitations of this study make it necessary to use caution in extrapolating the data to the clinical field. The number of specimens used is very small, partly because of the elaborate work in obtaining, processing, and re-

constructing the large number of images. However, in our opinion, the data seem to be reliable because no large differences in measured values were observed between the specimens and because the observed increments appeared in all. The current study also does not take into account the elasticity of tissue in living patients. In ultrasound studies, an expansion of the epineurium of the brachial plexus components is observed after local anesthetic injection inside the epineurial layer.[11]

Further studies using the same manner of analysis after injection of stained solutions in the four brachial plexus approaches are recommended, as well as clinical imaging studies with local anesthetics to confirm our findings *in vivo*.

In conclusion, in relatively undisturbed anatomy using cryomicrotomy, differences in neural architecture and size of surrounding adipose tissue compartments have been demonstrated between proximal and distal parts of the brachial plexus. The observed differences may explain why injections within the epineurium do not always result in neural injury and may also be a factor in determining the onset time and quality of blocks performed at different levels along the course of the brachial plexus.

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