

Received: 2015.07.20  
Accepted: 2015.10.11  
Published: 2016.02.05

# Effect of Collateral Sprouting on Donor Nerve Function After Nerve Coaptation: A Study of the Brachial Plexus

Authors' Contribution:  
Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
Funds Collection G

ABCDEF 1 **Paweł Reichert**  
BD 2 **Zdzisław Kiełbowicz**  
BD 3 **Piotr Dzięgiel**  
BCD 3 **Bartosz Puła**  
BCD 4 **Marcin Wrzosek**  
BDF 5 **Aneta Bocheńska**  
BD 1 **Jerzy Gosk**

1 Department of Traumatology, Clinic of Traumatology and Hand Surgery, Wrocław Medical University, Wrocław, Poland  
2 Department of Surgery, The Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Wrocław, Poland  
3 Department of Histology and Embryology, Wrocław Medical University, Wrocław, Poland  
4 Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Wrocław, Poland  
5 Centre of Veterinary Medicine JU-UAK, The University of Agriculture, Cracow, Poland

**Corresponding Author:** Paweł Reichert, e-mail: pawelreichert74@gmail.com  
**Source of support:** Departmental sources

**Background:** The aim of the present study was to evaluate the donor nerve from the C7 spinal nerve of the rabbit brachial plexus after a coaptation procedure. Assessment was performed of avulsion of the C5 and C6 spinal nerves treated by coaptation of these nerves to the C7 spinal nerve.


**Material/Methods:** After nerve injury, fourteen rabbits were treated by end-to-side coaptation (ETS), and fourteen animals were treated by side-to-side coaptation (STS) on the right brachial plexus. Electrophysiological and histomorphometric analyses and the skin pinch test were used to evaluate the outcomes.

**Results:** There was no statistically significant difference in the G-ratio proximal and distal to the coaptation in the ETS group, but the differences in the axon, myelin sheath and fiber diameters were statistically significant. The comparison of the ETS and STS groups distal to the coaptation with the controls demonstrated statistically significant differences in the fiber, axon, and myelin sheath diameters. With respect to the G-ratio, the ETS group exhibited no significant differences relative to the control, whereas the G-ratio in the STS group and the controls differed significantly. In the electrophysiological study, the ETS and STS groups exhibited major changes in the biceps and subscapularis muscles.

**Conclusions:** The coaptation procedure affects the histological structure of the nerve donor, but it does not translate into changes in nerve conduction or the sensory function of the limb. The donor nerve lesion in the ETS group is transient and has minimal clinical relevance.

**MeSH Keywords:** **Peripheral Nerve Injuries • Spinal Nerves • Suture Techniques**

**Full-text PDF:** <http://www.medscimonit.com/abstract/index/idArt/895397>

 3266

 4

 6

 33



## Background

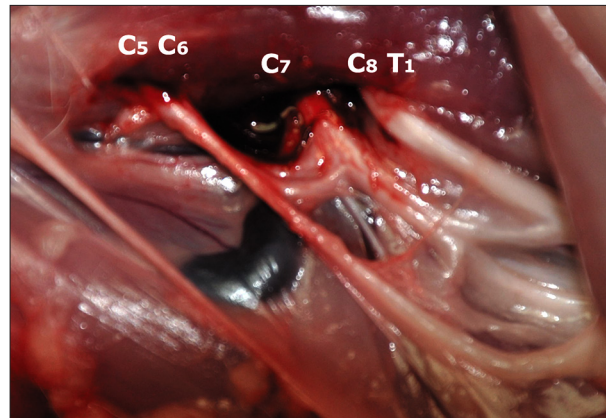
Avulsion injuries of the brachial plexus cause the most devastating palsies of the affected upper extremities. The prognosis of such injuries is grave, and functional results are limited. Muscle denervation can cause permanent muscular atrophy and eventual functional disability [1].

The treatment for brachial plexus avulsion lesions involves the use of neurotization procedures because neither direct repair nor interpositional nerve grafting can be performed for these irreparable preganglionic lesions [2,3]. New treatment guidelines for total root avulsion enhance the combination of various types of neurotization with ipsilateral intraplexus and extraplexus nerve donors, contralateral cervical seventh (CC7) root donor, and primary or secondary free functioning muscle transfer [2,3]. A wide variety of potential donor nerves are available, including the intercostal nerves [4], the spinal accessory nerve [5], the phrenic nerve [6], partial ulnar nerve [7], partial median nerve [8], pectoral major [9], thoracodorsal nerve [10], and the partial ipsilateral [11], the hemi [12] or total CC7 nerve roots [13,14]. Unfortunately, donor function is lost when the above procedures are performed.

The search for novel therapeutic methods has led to the return of coaptation, the effectiveness of which has been confirmed by both experimental and clinical studies [15–17]. Collateral sprouting of the distal stump of a damaged nerve to an uninjured donor nerve is gaining popularity as a method for nerve regeneration. The process is induced by molecular changes in the microenvironment in which the nerve lesion occurred, sustained by Wallerian degeneration, interruption of the normal neuronal turnover and local inflammatory response. [17]. One of the most pressing unsolved questions is the origin of the regenerating axons after end-to-side neurotization [17].

The controversy over coaptation is associated with the risk of nerve donor injury. The window in the epineurium or the perineurium of the donor nerve improves the effectiveness of end-to-side nerve repair. However, creating an epineurial or perineurial window is controversial due to the risk of donor nerve damage [18].

The aim of the present study was to evaluate the histopathological and functional changes in the donor nerve from the C7 spinal nerve of the rabbit brachial plexus after a coaptation procedure. Assessment was performed of avulsion of the ventral branches of the C5 and C6 spinal nerves treated by coaptation of these nerves to the C7 spinal nerve on the rabbit brachial plexus. Due to the injury at the spinal nerve level, the short, wide trunk of the spinal nerves, and technical difficulties in performing neurotization in humans, end-to-side and side-to-side coaptations were evaluated.



**Figure 1.** Macroscopic structure of the rabbit's right brachial plexus. C5, C6, C7, C8, and T1 – ventral branches of the C5, C6, C7, C8, and T1 spinal nerves.

## Material and Methods

### Experimental model

The experiments were approved by the Second Local Animal Ethics Committee at the University of Life Sciences in Wrocław. The study was conducted on 22-week-old White New Zealand rabbits (*Oryctolagus cuniculus*) (22 females and 6 males) that weighed an average of 3–3.5 kg.

All surgical procedures were performed with a lateral approach to the left brachial plexus (Figure 1). The ventral branches of spinal nerves C5 and C6 were exposed and incised at the spinal canal level. A 1-cm gap between the C5 and C6 spinal nerves and cranial trunk (C5, C6) was excised. An epineurial window on the cranial side of C7 was performed for end-to-side coaptation. For side-to-side coaptation, an additional epineurial window on the side of the cranial trunk was performed. The distal stump of the cranial trunk (C5 and C6) was sutured to the window by two stitches (Ethilon 10-0, Ethicon) in either the ETS or STS group. Twenty-eight right (contralateral) brachial plexuses were not treated (controls). After 20 weeks, the left and right brachial plexuses were exposed to obtain the sampled nerves. Fourteen rabbits were treated by ETS, and fourteen animals were treated by STS.

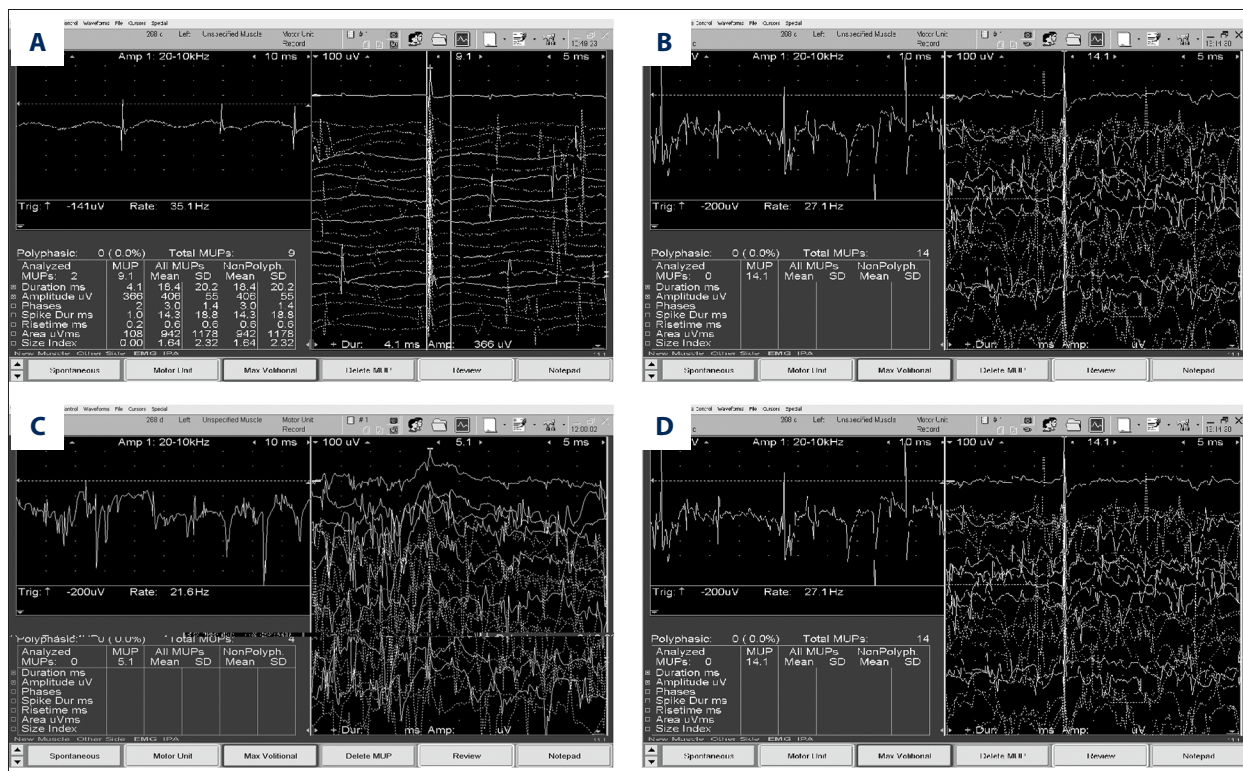
The same anesthesia protocol was used for all rabbits. Premedication was performed with medetomidine (Cepetor) at a dose of 150 µg/kg body weight, butorphanol (Torbugesic) at a dose of 0.2 mg/kg body weight and ketamine (Bioketan) at a dose of 35 mg/kg body weight. General anesthesia was performed with propofol, which was continuously administered intravenously (IV) at a dose of 0.1 mg/kg/min. During the operation, the analgesic effect was supported by fentanyl at a dose of 2–3 mcg/kg. After the procedure, buprenorphine (Vetergesic) was administered intramuscularly (IM) at a dose of 20 mcg/kg TID. The animals received meloxicam (Metacam)

**Table 1.** Muscle innervation of the rabbit thoracic limb.

Segment of the spinal cord	C5-C6	C6-C7	C5-C7	C7, C8, and T1
Nerve	Subscapular	Musculocutaneous	Subscapular	Radial
Muscle	Subscapularis	Biceps brachii	Teres major	Triceps brachii

**Table 2.** Modified semi-quantitative numerical scale for EMG evaluation of the degree of muscle denervation.

Scale	
0	No pathological potentials
1	Very rare denervation potentials
2	Sporadic pathological activity recorded in two or more places
3	Frequent pathological activity recorded, regardless of the needle electrode position
4	Abundant pathological activity recorded, regardless of the needle electrode position

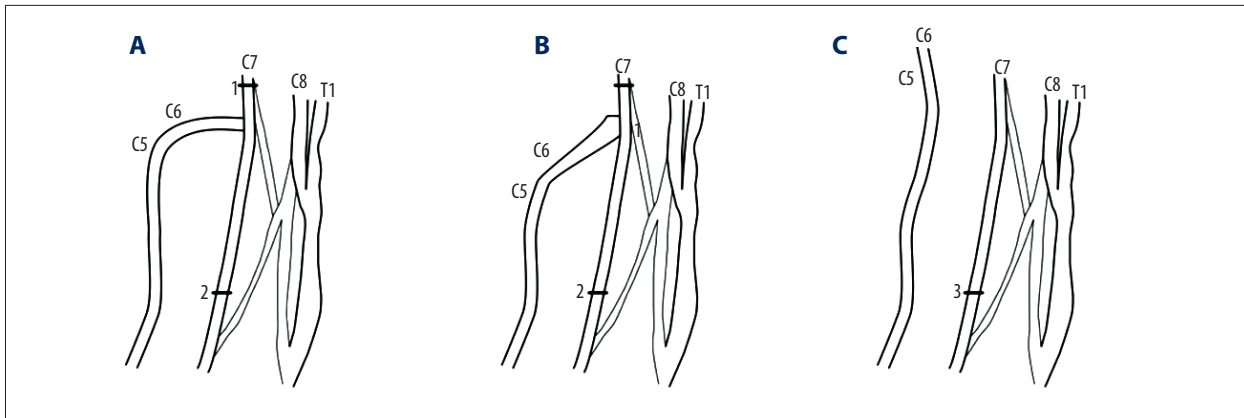


**Figure 2.** (A) An example of grade 0 (EMG triceps brachii, no. 8, ETS group); (B) An example of grade 1 (EMG teres major muscle, no. 10, STS group); (C) An example of grade 2 (EMG biceps brachii muscle, no. 6, ETS group); (D) An example of grade 3 (EMG subscapularis muscle, no. 2, STS group).

at a dose of 0.2 mg/kg body weight for two days after surgery. The animals underwent operations in the lateral recumbent position. The mean surgical time was approximately 28 min. Adequate care was taken to minimize the pain and discomfort during and after the operation. Animals were euthanized by intravenous pentobarbital injection (Morbital).

### Electrophysiological analysis

All of the animals were anaesthetized with IM injections of medetomidine (dose: 0.5 mg/kg), butorphanol (0.1 mg/kg) and ketamine (25 mg/kg) for the electromyographic evaluation.



**Figure 3.** C5, C6, C7, C8, and T1 – ventral branches of the C5, C6, C7, C8, and T1 spinal nerves. The numbers 1, 2 and 3 indicate the collection site of the analyzed nerves. **(A)** End-to-side coaptation (ETS); **(B)** Side-to-side coaptation (STS); **(C)** Control group.

**Table 3.** Dermatomes innervated by nerves derived from C7.

Nerve roots	Nerve	Skin branches	Innervation area
C6-C7	Musculocutaneous	Cutaneous nerve of the forearm, medial	Medial aspect of the forearm
C7-C8	Ulnar	Cutaneous nerve of the forearm, dorsal	Dorsal aspect of the forearm
C7, C8 T1	Radial	Cutaneous nerve of the forearm, lateral	Lateral aspect of the arm just above elbow

The electromyographic examination was performed at an ambient temperature of 22°C using the Nicolet Viking Quest portable system (version 11.0) electrodiagnostic equipment. A standard electromyographic concentric needle electrode (used as an active and recording electrode) and a subdermal, monopolar ground electrode were used in the study. The following muscles were analyzed to evaluate the nerve donor function: subscapularis, biceps brachii, teres major and triceps. The muscle innervation by nerves derived from the corresponding spinal nerves and segments of the spinal cord was assessed (Table 1).

A modified semi-quantitative numerical scale by Kimura was adopted for this assessment [16]. Higher values indicate a lower degree of muscle innervation (Table 2, Figure 2).

**Histomorphometric analysis of the sampled nerves**

The proximal C7 trunk was compared with the region distal to the coapted area (samples 1 and 2), and the C7 trunk distal to the coapted area was compared with the contralateral healthy C7 nerve at the same level (samples 2 and 3) and subjected to histomorphometric analysis (Figure 3).

The following parameters were analyzed: axon, myelin sheath and nerve fiber diameters; G-ratio and nerve area; number of axons; and myelin fiber density (mm<sup>2</sup>). The obtained nerve specimens were immersion-fixed in 2.5% glutaraldehyde for 12 h at 4°C and then washed with cacodylic buffer (Serva, Heidelberg,

Germany). Specimens were then post-fixed for 1 h in 1% osmium tetroxide (dissolved in cacodylic buffer) and subsequently washed with cacodylic buffer. Finally, the specimens were dehydrated in alcohols and embedded in Epon (Chempur, Piekary Slaskie, Poland). Power Tome XL (RMC Products, Tucson, AZ, USA) was used to cut the fixed nerve specimens into 0.6-µm thick sections, which were then stained with toluidine blue (Serva) and mounted using Euparal (Roth, Mannheim, Germany). The stained nerve cross-sections were examined under a BX41 light microscope that was equipped with the computer-assisted image analysis program Cell<sup>o</sup> (Olympus, Tokyo, Japan). For the analysis, non-overlapping photomicrographs at 630× magnification were taken manually. In each nerve cross-section, minimal diameters of the axons and nerve fiber (axon and adjacent myelin) were measured. Only circular fibers were measured, allowing us to calculate the myelin sheath thickness of the nerve fibers using the following formula: nerve fiber diameter – axon diameter. The degree of myelination was assessed using the G-ratio (the ratio between the minimal diameters of axons and nerve fibers) [17]. The myelin sheath density and myelinated fiber density were also calculated. At least 200 nerve fibers were analyzed per sampled nerve.

**The skin pinch test**

The skin pinch test was used to establish the return of sensory function. The animal's skin was gently pinched by forceps until the first signs of discomfort to the animal, such as



picking up the limbs, head turning or trembling skin, were observed. We focused on dermatomes innervated by nerves derived from C7 (Table 3). For this study, we prepared a 4-point grading scale: 0 – no response; 1 – mild response, in which the animal exhibited a very weak reaction; 2 – moderate response, in which the animal exhibited a reaction in response to the stimuli; and 3 – a clear/significant response to the stimuli.

### Statistical analysis

The data were analyzed by Prism 5.0 (GraphPad, La Jolla, CA, USA) statistical software. To compare the significance of the changes between the groups, Student's *t*-test for independent samples was performed. The Mann-Whitney U test was used for the non-parametric values. The distribution of the data was tested by the Shapiro-Wilk test. The dissimilarities between the groups were scrutinized using the non-parametric Kruskal-Wallis test with Dunn's post hoc analysis. The differences were considered significant at  $p < 0.05$  for Student's *t*-test and at  $p < 0.01$  for the Mann-Whitney U test.

## Results

### Histomorphometric analyses of the sampled nerves

Bar graphs present the results of the histomorphometric analyses of the sampled nerves (axon, myelin sheath and nerve fiber diameters and the G-ratio) (Figure 4). In the control group (sample 3), the nerve area was  $1.1304 \text{ mm}^2$ , the number of axons was  $362 \pm 2.83$ , and the myelin fiber density was  $3078.23 \pm 96.17 \text{ fiber/mm}^2$  for C7. In the ETS group (sample 2), distal from the coaptation, the nerve area was  $1.1267 \text{ mm}^2$ , the number of axons was  $322 \pm 3.15$ , and the myelin fiber density was  $2977.23 \pm 79.17 \text{ fiber/mm}^2$ . In the STS group (sample 2), distal from the coaptation, the nerve area was  $1.1255 \text{ mm}^2$ , the number of axons was  $312 \pm 4.16$ , and the myelin fiber density was  $2864.84 \pm 89.21 \text{ fiber/mm}^2$ .

A comparison of the tissue before coaptation (proximal) (Figure 5A) and 2 cm from the coaptation (distal) (Figure 5B) revealed statistically significant differences in the axon ( $MW < 0.0001$ ,  $p < 0.01$ ), myelin sheath ( $MW < 0.0001$ ,  $p < 0.01$ ) and fiber ( $MW < 0.0001$ ,  $p < 0.01$ ) diameters in both the ETS and STS groups. Conversely, comparing the G-ratio for the ETS proximal with the ETS distal group did not indicate any prominent differences ( $MW = 0.78$ ,  $p < 0.01$ ), whereas the STS proximal group significantly differed from the STS distal group ( $MW = 0.0001$ ,  $p < 0.01$ ).

A comparison of the sampled nerves 2 cm from the coaptation in the ETS and STS groups with the control group (Figure 5C) revealed significant differences in the following parameters:

axon ( $MW < 0.0001$ ,  $p < 0.01$ ), myelin sheath ( $MW < 0.0001$ ,  $p < 0.05$ ) and fiber diameters ( $MW < 0.0001$ ,  $p < 0.01$ ). The G-ratio did not differ significantly between the ETS group and the controls ( $MW = 0.1817$ ,  $p < 0.01$ ), but statistically significant differences in the G-ratio were observed between the STS group and the controls ( $MW = 0.0064$ ,  $p < 0.01$ ).

### Electrophysiological study

Changes in each muscle are presented graphically (Figure 6A, B). The ETS and STS groups significantly differed from the controls with respect to the following muscles: subscapularis ( $t < 0.001$ ,  $p < 0.05$ ), biceps ( $t < 0.001$ ,  $p < 0.05$ ) and teres major ( $t = 0.005$ ,  $p < 0.05$ ). However, there were no differences in the triceps (in the ETS group, we did not observe any changes). There were no differences between the ETS and STS groups (Figure 6C).

### Skin pinch test

On the lateral side, the sensory function recovery was the fastest and was associated with innervation from C8 and T1 (Table 4).

## Discussion

Previous studies have evaluated donor function using coaptation on the level of individual peripheral nerves. In our study, we evaluated the donor function at the level of the spinal nerves. The risk of damage to the donor is related to collateral sprouting from C7 in response to avulsion of the ventral branches of the C5 and C6 spinal nerves treated by coaptation of these nerves to the C7 spinal nerve. Collateral sprouting in end-to-side coaptation of the ventral branches of the C5 and C6 spinal nerves to the C7 spinal nerve in the rabbit brachial plexus was confirmed by electrophysiological, histomorphometric and behavioral results. To objectively analyze these results, all of the branches connecting the C5, C6, and C7 nerves below the site of coaptation were cut.

Electrophysiological study involves the use of EMG. Electromyography studies revealed denervation elements, such as fibrillations, positive sharp waves and repetitive complex discharges. These types of discharges are evidence of denervation or muscle atrophy. The electrodiagnostic study revealed that the largest lesions were found in the biceps brachii muscle, which is innervated by the musculocutaneous nerve, whose fibers are derived from the C7 and C6 cervical nerves. No lesions were noted in the triceps muscle innervated by the radial nerve, which originates from the C7, C8, and T1 spinal nerves. No muscles are innervated solely by the C7 spinal nerve; nevertheless, we made an assumption that the biceps brachii muscle and radial nerve are sufficient for C7 evaluation (despite C5 and C6 damage).

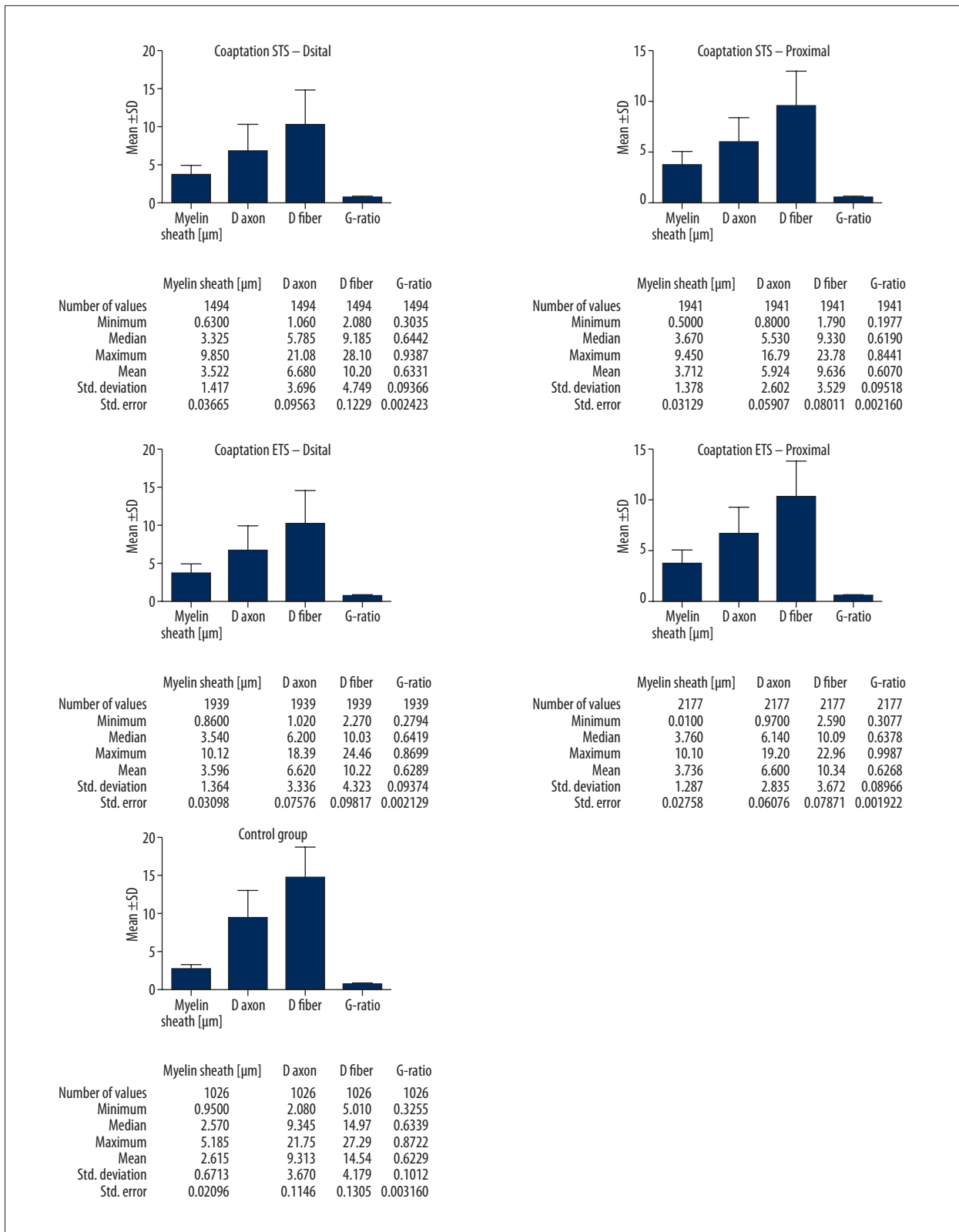
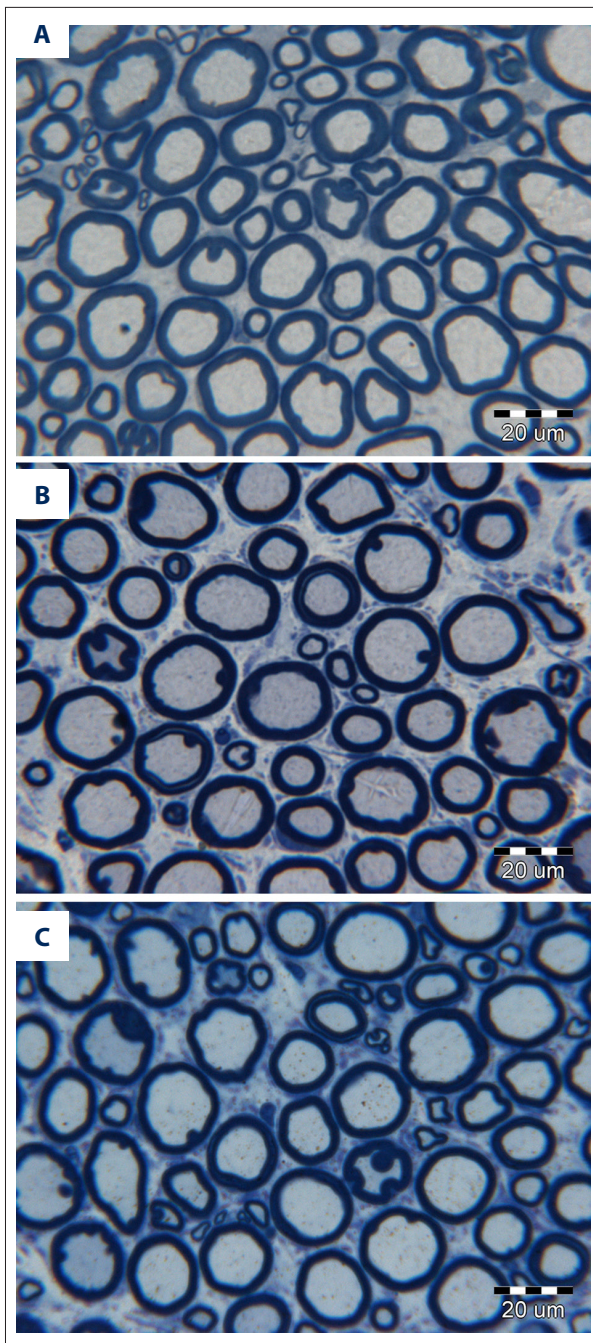


Figure 4. Bar graphs presenting histomorphometric analyses of the sampled nerves.

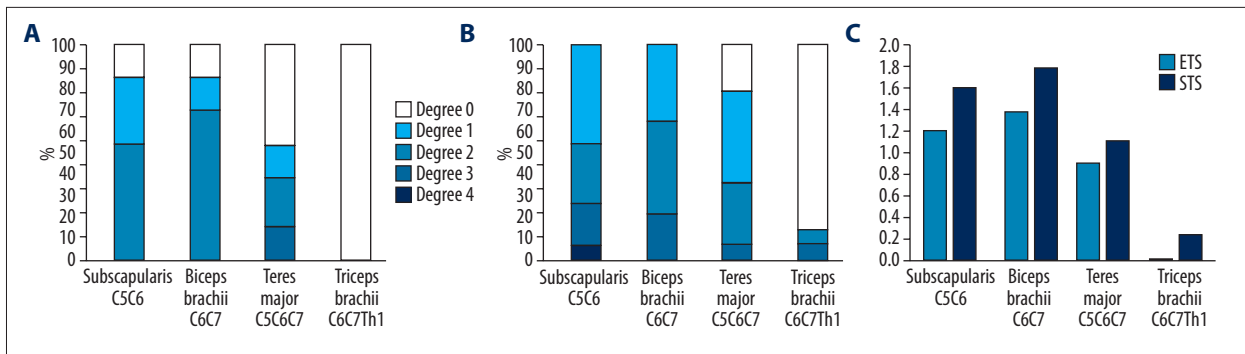


**Figure 5.** (A) Microscopic image of the sampled nerve before coaptation in the ETS group (toluidine blue staining); (B) Microscopic image of the sampled nerve 2 cm from the coaptation in the ETS group (toluidine blue staining); (C) Microscopic image of the sampled nerve in the control group (toluidine blue staining).

Our research results indicate that sensory function returns 2 weeks after surgery and is fully restored within 3 weeks. No significant deficiency in sensory function was noted. In a study of damaged rat sural nerves, sensory function returned

completely by 36 weeks after the end of the study [22]. In our studies, the results indicate that the sensory function of the donor is impaired for a brief period. The skin pinch test is not an adequate tool to assess subtle changes, and damaged senses may have been overlooked in this study. With respect to the histomorphometric examination, the differences were statistically significant and did not translate into nerve conduction. All changes in the axon, myelin sheath and fiber diameter were statistically significant. We also noted differences between the G-ratio for side-to-side and end-to-side coaptation compared with the control group, although the differences were statistically significant only for the STS group; overall, the differences in the G-ratio were less prominent than the other histomorphometric parameters. A G-ratio greater than 0.6 for the peripheral nerve indicates normal nerve conduction [20,21]. The histological examination of other parameters changed according to the construction of the nerve and was dependent on the level of sample collection. In a study of reconstruction of the peroneal nerve, we examined the number of axons relative to the cross-section of the nerve donor [23]. These tests were performed on the peripheral nerve motor branch. The results for the peroneal nerve were  $0.255 \pm 0.111$  mm for the cross-section, with an average of  $3363 \pm 1997$  axons [23]. In our study, we focused on mixed nerves. The number of axons and nerve areas were denoted. Based on these results, the nerve area was  $1.1304$  mm<sup>2</sup> and the number of axons was  $362 \pm 2.83$ , which is sufficient to obtain good sensory results.

Donor nerve lesions are believed to be transient and to regress with time [22]. Functional recovery after peripheral nerve injury depends on the survival of the affected neurons and their capacity to regenerate the injured axons, as well as reinnervation of target tissues. The microenvironment in the nerve segment distal to the injury site, undergoing metabolic and structural changes, was a decisive factor in the regeneration of injured axons and functional reinnervation of the denervated target tissues [24]. Skin sensation tests have been used as the primary neurological outcome examination [25]. Sensory exams have been used to classify injury severity [26,27]. After an ETS procedure in rats (tibial nerve to peroneal nerve), signs of nerve damage were observed within two weeks. A five-fold increase in the percentage of denervated muscle fibers was noted relative to the control group, but the properties of the operated and healthy contralateral limb were comparable after six months [26]. Lundborg demonstrated Wallerian degeneration in the donor nerve after a peroneal-to-posterior tibial coaptation procedure in rats 90 days after surgery [28]. This process did not influence the limb function. In the medical literature, there are reports referring to lesions in the sensory and motor peripheral nerves, but there are few studies defining the effect of collateral sprouting on the donor nerve for mixed nerves. Kovacic noted that motor neurons are more easily damaged



**Figure 6.** Distribution of pathological changes in the EMG. (A) ETS group, (B) STS group, (C) Degree of denervation of particular muscles in the ETS and STS groups.

**Table 4.** Assessment of the return of sensation in the dermatomes.

Dermatomes	1 Week after surgery		2 Weeks after surgery		3 Weeks after surgery		4 Weeks after surgery	
	ETS	STS	ETS	STS	ETS	STS	ETS	STS
C6 C7 Medial aspect of the forearm	0.21±0.42	0.14±0.36	1.35±0.49	1.28±0.46	2.64±0.49	2.35±0.49	3	3
C7 C8 Dorsal aspect of the forearm	0.35±0.49	0.28±0.46	1.64±0.49	1.42±0.51	3	3	3	3
C7 C8 T1 Lateral aspect of the arm just above elbow	0.92±0.26	0.85±0.36	1.92±0.26	1.78±0.42	3	3	3	3

than sensory neurons, which is likely due to their higher sensitivity. Our study confirms this theory; the returned sensory function was greater than the motor function [29].

Currently, the most widely used treatment for C5 or C6 avulsion is neurotization of the accessory nerve to the suprascapular nerve, the ulnar nerve fascicles to the motor branch biceps, and triceps branches to the axillary nerve [30]. In this case, the long-term triceps strength is not diminished relative to the preoperative values after sectioning of the triceps' motor branches; however, strength equal to the normal contralateral side is not achieved during recovery [30]. Additionally, there are no apparent consequences of removing fascicles of the ulnar nerve with respect to grasp or pinch strength [30].

In the case of C7 donor site complications, transection of the posterior division of C7 produces a weakness of muscles innervated by the radial nerve, especially the triceps and the wrist and finger extensors, although paralysis is not observed. However, these muscles regain their original strength by internal sprouting. [2]

Ipsilateral C7 nerve root transfer may cause injury to the thoracodorsal nerve, which receives motor fascicles from the cervical nerves largely from C7. The result of damage to the

thoracodorsal nerve is weakness or paralysis of the latissimus dorsi muscle, which it innervates [31].

The above examples describe the results of the contribution of the C7 fasciculus, which can represent a good donor but can cause some damage to the nerves. We believe the coaptation procedure may avoid nerve damage and that collateral sprouting of C7 can restore function.

The sensory component of the C7 nerve root contributes less than 25% to the innervation of any given major upper extremity nerve: less than 1%, 6%, 16% and 19% to the musculocutaneous, ulnar, radial, and median nerves, respectively. When using the C7 nerve root as a donor, the central sensory deficit will be less than 25% [32]. This is entirely within the ability of each major nerve to compensate and is likely a reason why C7 is a suitable donor for brachial plexus injury [32]

Another important aspect of the procedure is using an appropriate portion of the C7 from the donor, as the requirements depend on the type of injury. For example, musculocutaneous nerve regeneration in neurotization with the posterior division or the anterior division of C7 is significantly superior to that with the anterolateral fascicles of the anterior division or the phrenic nerve [33].



This study demonstrates that nerve topography is very important and may depend on the final outcome. Therefore, coaptation with various individual segments requires further evaluation.

Donor assessment after coaptation treatment at the level of the spinal nerves is particularly difficult due to the absence of selective innervation of muscles and dermatomes for each spinal nerve. The only study directly evaluating nerve changes included histological examination, but in some cases, the structural changes do not necessarily translate into nerve conduction function. Therefore, in our opinion, only the cumulative data from histology, electromyographic examination and sensory recovery allow the appropriate conclusions to be drawn.

Because the results of treating peripheral nerve injuries remain unsatisfactory, a more selective approach with respect to the spinal nerves, spinal trunks, or the peripheral nerves, as well as to the location of the injury and the method of repair and reconstruction, must be identified. A possible solution is coaptation, but the consequences of using spinal nerves as donors are unknown. We believe that confirming the absence of disrupted nerve conduction at the level of the spinal nerves will facilitate further research of coaptation defects at these spinal nerve levels. We hope that this technique combined with

neurotrophic procedures will provide an alternative to the previously used brachial plexus surgical techniques. However, we are aware that the primary issue is the translation of laboratory results into clinical practice.

## Conclusions

The coaptation procedure affects the nerve donor's histological structure, but this impact does not translate into detrimental effects at the level of nerve conduction or sensory function of the limb. This procedure is safe for donor nerves when ETS is performed. The donor nerve lesion is transient after ETS and has almost no clinical relevance.

## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

## Acknowledgments

We thank Bartosz Witkowski who provided medical writing service on behalf of Wrocław Medical University.

## References:

1. Bonham C, Greaves I: Brachial plexus injuries. *Trauma*, 2011; 13: 353–63
2. Tu YK, Tsai YJ, Chang CH et al: Surgical treatment for total root avulsion type brachial plexus injuries by neurotization: a prospective comparison study between total and hemicentral C7 nerve root transfer. *Microsurgery*, 2014; 34: 91–101
3. Doi K, Muramatsu K, Hattori Y et al: Restoration of prehension with the double free muscle technique following complete avulsion of the brachial plexus. Indications and long-term results. *J Bone Joint Surg Am*, 2000; 82: 652–66
4. Chuang DC, Yeh MC, Wei FC: Intercostal nerve transfer of the musculocutaneous nerve in avulsed brachial plexus injuries: evaluation of 66 patients. *J Hand Surg Am*, 1992; 17: 822–28
5. Bertelli JA, Ghizoni MF: Results and current approach for Brachial Plexus reconstruction. *J Brachial Plex Peripher Nerve Inj*, 2011; 6: 2
6. Gu YD, Ma MK: Use of the phrenic nerve for brachial plexus reconstruction. *Clin Orthop*, 1996; 323: 119–21
7. Leechavengvongs S, Witoonchart K, Uerpaiojkit C et al: Combined nerve transfers for C5 and C6 brachial plexus avulsion injury. *J Hand Surg Am*, 2006; 31: 183–89
8. Cho AB, Paulos RG, de Resende MR et al: Median nerve fascicle transfer versus ulnar nerve fascicle transfer to the biceps motor branch in C5–C6 and C5–C7 brachial plexus injuries: nonrandomized prospective study of 23 consecutive patients. *Microsurgery*, 2014; 34: 511–15
9. David S, Balaguer T, Baque P, Lebreton E: Transfer of pectoral nerves to suprascapular and axillary nerves: an anatomic feasibility study. *J Hand Surg Am*, 2010; 35: 92–96
10. Soldado F, Ghizoni MF, Bertelli J: Thoracodorsal nerve transfer for elbow flexion reconstruction in infraclavicular brachial plexus injuries. *J Hand Surg Am*, 2014; 39: 1766–70
11. Yin HW, Jiang S, Xu WD et al: Partial ipsilateral C7 transfer to the upper trunk for C5–C6 avulsion of the brachial plexus. *Neurosurgery*, 2012; 70: 1176–82
12. Sammer DM, Kircher MF, Bishop AT, Bertelli JA, Ghizoni MF: Results and current approach for Brachial Plexus reconstruction. *J Brachial Plex Peripher Nerve Inj*, 2011; 6: 2
13. Pan F, Wei HF, Chen L, Gu YD: Different functional reorganization of motor cortex after transfer of the contralateral C7 to different recipient nerves in young rats with total brachial plexus root avulsion. *Neurosci Lett*, 2012; 531: 188–92
14. Wood MB, Murray PM: Heterotopic nerve transfers: recent trends with expanding indication. *J Hand Surg Am*, 2007; 32: 397–408
15. Lykissas MG: Current concepts in end-to-side neurotization. *World J Orthop*, 2011; 2: 102–6
16. Gao W, Liu Q, Li S et al: End-to-side neurotization for nerve repair and function rehabilitation. *J Surg Res*, 2015; 197: 427–35
17. Tos P, Colzani G, Ciclamini D et al: Clinical applications of end-to-side neurotization: an update. *Biomed Res Int*, 2014; 2014: 646128
18. Geuna S, Papalia I, Tos P: End-to-side (terminolateral) nerve regeneration: a challenge for neuroscientists coming from an intriguing nerve repair concept. *Brain Res Rev*, 2006; 52: 381–88
19. Kimura J: *Electrodiagnosis in diseases of nerve and muscle: Principles and Practice*, 3<sup>rd</sup> edition, Oxford University Press, 2001
20. Rushton W: A Theory of the effects of fibre size in medullated nerve. *J Physiol*, 1951; 115: 101–22
21. Chomiak T, Hu B: What is the optimal value of the g-ratio for myelinated fibers in the rat CNS? A theoretical approach. *PLoS One*, 2009; 4: e7754
22. Kovacic U, Sketelj J, Bajrovic FF: Sex-related difference in collateral sprouting of nociceptive axons after peripheral nerve injury in the rat. *Exp Neurol*, 2003; 184: 479–88
23. White CP, Cooper MJ, James RB, Levis CM: Axon counts of potential nerve transfer donors for peroneal nerve reconstruction. *Can J Plast Surg*, 2012; 20: 24–27
24. Bajrovic FF, Sketelj J, Jug M et al: The effect of hyperbaric oxygen treatment on early regeneration of sensory axons after nerve crush in the rat. *J Peripher Nerv Syst*, 2002; 7: 141–48
25. Bracken MB, Shepard MJ, Collins WF et al: A randomized controlled trial of methylprednisolone or naloxone in the treatment of acute spinal cord injury. *N Engl J Med*, 1990; 322: 1405–11

26. Cederna PS, Kalliainen LK, Urbanchek MG et al: "Donor" muscle structure and function after end-to-side neuroorrhaphy. *Plast Reconstr Surg*, 2001; 107: 789–96
27. Lundborg G, Zhao Q, Kanje M et al: Can sensory and motor collateral sprouting be induced from intact peripheral nerve by end-to-side anastomosis? *J Hand Surg Br*, 1994; 19: 277–82
28. Marino RJ, Barros T, Biering-Sorensen F et al: International standards for neurological classification of spinal cord injury. *J Spinal Cord Med*, 2003; 26: S50–56
29. Bajrovic F, Kovacic U, Pavcnik M, Sketelj J: Interneuronal signalling is involved in induction of collateral sprouting of nociceptive axons. *Neuroscience*, 2002; 111: 587–96
30. Bertelli JA, Ghizoni MF: Reconstruction of C5 and C6 brachial plexus avulsion injury by multiple nerve transfers: spinal accessory to suprascapular, ulnar fascicles to biceps branch, and triceps long or lateral head branch to axillary nerve. *J Hand Surg Am*, 2004; 29: 131–39
31. Lu W, Xu JG, Wang DP, Gu YD: Microanatomical study on the functional origin and direction of the thoracodorsal nerve from the trunks of brachial plexus. *Clin Anat*, 2008; 21: 509–13
32. Li R, Machol JA IV, Liu X et al: C7 nerve root sensory distribution in peripheral nerves: a bold functional magnetic resonance imaging investigation at 9.4 T. *Muscle Nerve*, 2014; 49: 40–46
33. Xu JG, Wang H, Hu SN, Gu YD: Selective transfer of the C7 nerve root: an experimental study. *J Reconstr Microsurg*, 2004; 20: 463–70