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# Peripheral nerve surgery: Neurosurgery beyond technology

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# Peripheral nerve surgery: Neurosurgery beyond technology

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# Editorial: Peripheral nerve surgery: Neurosurgery beyond technology

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KEYWORDS

peripheral nerve, surgery, technology, functional restoration, quality of life

Editorial on the Research Topic
Peripheral nerve surgery: Neurosurgery beyond technology

Neurosurgery is a relatively young specialty, and it is heavily riding on the wheels of technology for the past few decades. Both surgeons and staff has shown great understanding and involved all kinds of technological advances, in visualization, imaging, navigation, monitoring etc …. Peripheral nerve surgery, as an integral and indispensable part of neurosurgery has undergone significant development over the past 50 years, primarily with the introduction of microscope and microsurgical techniques and it is taking advantage of improved preoperative and intraoperative diagnostic procedures. Still, there are injuries and diseases which pathophysiology is not yet sufficiently understood, and these complex patients are multidisciplinary challenges. The developments force constant re-evaluation of the techniques and outcomes, and new trends in peripheral nerve surgery represent the advances from a variety of specialties (1).

Solving controversies and dilemmas in peripheral nerve surgery depends on the surgeon, his choice of methods, and his experience, but the main point is that the treatment should not be worse than the disease itself. In recent years we have been relying on the essence of nerve surgery hidden in the acronym—KISS (keep it simple surgeon!). The history and clinical examination remain the foundations of diagnosis in peripheral nerve surgery, however preoperative confirmation using a variety of diagnostic procedures is mandatory. The indications for surgery should be kept clear, avoiding complications, but accepting and dealing with them, when they occur (2).

Basic nerve surgery can be done with simple, basic instruments. Nevertheless, advances in understanding nerve fiber regeneration and possible factors that affect regeneration and improve outcomes, as well as the technological innovations have greatly expanded the indications and improved the results (3).

This Research Topic offers a modern comprehensive approach to peripheral nervous system surgery and consists of nine original research articles and one review paper.

## Basic research

The study by Rochkind S. et al. investigated the innovative Guiding regenerative gel and antigliotic guiding regenerative gel fillings for nerve conduits, prepared with FDA approved agents, and expected to provide an alternative to an autologous nerve graft and enabling reconnecting massive nerve gaps in rabbit model of chronic peripheral nerve injury with massive loss defect that simulates the human condition of chronic injury with large gap. They concluded that the application of and antigliotic guiding regenerative gel led to a stronger nerve recovery and may be an alternative to autologous grafts Rochkind et al. (2021).

Dehu Tian group used an electrospun poly-e-caprolactone-amnion nanofibrous membrane comprising an amnion membrane and nonwoven electrospun poly-e-caprolactone to wrap the sciatic nerve repair site in the rat model of a sciatic nerve transection, and noted effective improvement of nerve regeneration through promotion of Schwann cell proliferation, axon regeneration, limiting muscle denervation and fibrosis after nerve repair, leading to the improved functional recovery Bai et al. (2022).

Another paper from my group, by Lepić et al. reviews the potential role of the diet, nutrients, and supplementation for the outcome augmentation in surgical treatment of peripheral nerve injuries. It emphasizes the importance of standardized diet, micro- and macronutrients intake, and supplementation protocols within the multidisciplinary approach to achieve best possible results and improve nerve regeneration and functional recovery Lepić et al. (2022).

## Clinical evaluation

The aim of the study by Dazhi Yang group from was to compare the clinical characteristics of diabetic carpal tunnel syndrome between 276 patients with neuropathic and non-neuropathic pain The light touch, electrophysiological test results, and psychological factors were found to be related to the neuropathic pain occurrence, found in the majority of patients with diabetic carpal tunnel syndrome Liu et al. (2022).

## Advanced preoperative planning

The pre-spinal route of contralateral C7 nerve transfer developed by Prof. Wendong Xu helps realize the direct anastomosis of the bilateral C7 nerves. However, there are still no less than 20% operations requiring nerve graft, which leads to unfavorable prognosis. This study aimed to explore the optimized pre-spinal route with MRI to further improve the prognosis. According to these data, the middle route was optimally applied to 50 patients, where the rate of nerve transplantation was only 4%, and no such serious complications as vertebral artery and brachial plexus injury occurred. Conclusion According to the 50 patients' low rate of nerve transplantation and their absence of serious complications, the middle route was the optimal Zhao et al. (2022).

## Contemporary surgical management

The two papers from my group are focused on the radial nerve injuries associated with humeral shaft fractures. These lesions are a great burden to everyone involved: the patient, the orthopedic surgeon, work and economical, as well as the social status and institutions, and last but not least the neurosurgeon. The first paper focuses on the etiology, epidemiology and characteristics of patients suffering to these injuries with the findings confirming the previous claim, as the patients are young working population, and the treatment is a great challenge, as presented in the second paper (Rasulić et al. (2022), Rasulić et al. (2021)). The results of surgical management remain diverse. In this paper we presented the outcomes and analyzed the patient, clinical, and surgical procedure related characteristics and factors that may influence the outcome overall.

## New tehnologies

The paper by Qiangqiang Liu of Jiwen Xu group presented a novel minimally invasive robot-assisted percutaneous balloon compression technique for trigeminal neuralgia with short term outcomes in six consecutive patients. Despite requiring a longer time for preoperative preparation, robot-assisted technique allowed for a high degree of accuracy and safety, shortening the learning curve for surgeons unfamiliar with the technique, calling for the further research and development of the percutaneous balloon compression technique as a viable treatment option for trigeminal neuralgia Liu et al. (2022).

## Outcome and quality of life

Quality of life and even the functional recovery of the injured lower extremity nerves are rarely evaluated and the results of our study on peroneal nerve found an apparent advantage of neurolysis, over nerve repair, over tendon transfer procedure, diminishes when all aspects of quality of life are considered, emphasizing individual approach to achieve optimal results in all groups of patients Rasulić et al. (2022).

## Conclusion

As multidisciplinary as the peripheral nerve surgery is, I hope that this Research topic will be helpful to neurosurgeons, vascular and plastic surgeons, anesthesiologists, neurologists, radiologists, neurophysiologists, as well as all physicians involved in the diagnosis and treatment of patients with injuries and diseases of peripheral nerves.

When this Research Topic was proposed, I had a vision to present a broad collection of papers covering all aspects of peripheral nerve surgery, with an insight into modern knowledge about different problems and pathologies, with particular emphasis on the practical understanding of functional recovery, in the light of technology and technological advances, emphasizing the role of surgery beyond technology.

## Author contributions

Prepared the editorial in all aspects.

## Acknowledgments

I am grateful to all the colleagues who contributed to this Research Topic for academic and professional exchange of experiences as an author, reviewer, or editor.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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ORIGINAL RESEARCH
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# Reviving Matrix for Nerve Reconstruction in Rabbit Model of Chronic Peripheral Nerve Injury With Massive Loss Defect

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**Background and Aims:** The aim of this study was to investigate the innovative guiding regenerative gel (GRG) and antigliotic GRG (AGRG) fillings for nerve conduits, prepared with Food and Drug Administration (FDA)-approved agents and expected to provide an alternative to autologous nerve graft and to enable reconnection of massive nerve gaps in a rabbit model of chronic peripheral nerve injury with massive loss defect that simulates the human condition of chronic injury with a large gap.

**Methods:** The components and dosimetry for GRG and AGRG formulations were investigated *in vitro* on nerve cell culture and *in vivo* on 10-mm reconstructed sciatic nerves of 72 rats using different concentrations of agents and completed on a rabbit model of delayed (chronic) complete peripheral nerve injury with a 25-mm gap. Forty rabbits underwent delayed (9 weeks after complete injury of the tibial portion of the sciatic nerve) nerve tube reconstruction of a gap that is 25 mm long. GRG and AGRG groups were compared with autologous and empty tube reconstructed groups. Rats and rabbits underwent electrophysiological and histochemical assessments (19 weeks for rats and 40 weeks for rabbits).

**Results:** Application of AGRG showed a significant increase of about 78% in neurite length per cell and was shown to have the most promising effect on neuronal outgrowth, with total number of neurites increasing by 4-fold. The electrophysiological follow-up showed that AGRG treatment is most promising for the reconstruction of the tibial portion of the sciatic nerve with a critical gap of 25 mm. The beneficial effect of AGRG was found when compared with the autologous nerve graft reconstruction. Thirty-one weeks post the second surgery (delayed reconstruction), histochemical observation showed significant regeneration after using AGRG neurogel, compared with the empty tube, and succeeded in significantly regenerating the nerve, as well as the autologous nerve graft, which was almost similar to a healthy nerve.

**Conclusion:** We demonstrate that in the model of delayed peripheral nerve repair with massive loss defect, the application of AGRG led to a stronger nerve recovery and can be an alternative to autologous nerve graft.

**Keywords: antigliotic guiding regenerative gel, artificial peripheral nerve, peripheral nerve injury, guiding regeneration gel, nerve regeneration**

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## INTRODUCTION

Peripheral nerve injury (PNI) occurs in about 2.8% of all trauma patients and can cause disability and a significant decrease in quality of life (1). A growing number of traffic and work accidents, natural disasters, and military activity often result in PNIs, causing lifelong dysfunction associated with loss of sensory and motor functions, and in some cases intractable pain, and requiring long-term peripheral nerve rehabilitation treatments. There are about 300,000 cases of PNIs per year (2). The annual incidence rate of nerve injuries is reported to be 13.9/100,000 inhabitants per year (3). In the USA alone, 50,000 nerve graft procedures are performed annually (4), accounting for seven billion USD in expenses. This indicates that improved treatment strategies for PNIs may not only improve the situation for the patients but also significantly reduce costs for the society.

The current standard of care for PNIs includes the gold standard autografts, Food and Drug Administration (FDA)-approved hollow conduits, and decellularized nerve allografts (5). The gold standard for the reconstruction of nerve damage is non-immunogenic nerve grafts (6) that have been harvested from the same patient. Depending on the extent of the nerve injury or the distance to overcome, a complete reconstruction can be difficult or even impossible due to the limited extent of the grafting material. Furthermore, autologous nerve graft (ANG) may result in a painful neuroma formation at the donor site with loss of the donor nerve function (7).

Nerve guidance conduits represent a biomaterial-based scaffolding to aid in nerve repair and regeneration to bridge nerve defects and guide axon regeneration to the appropriate distal target. Currently, there are 11 FDA-approved conduits for treatment of PNI produced with biomaterials, of both natural and synthetic origins. The advantages of the nerve guiding conduit in comparison to the ANG are the simplicity of the procedure, a significant decrease in time of surgery, and no sensation loss or cosmetic defect as a result of donor site intervention. On the other hand, the main disadvantage of the nerve guiding conduit is the inability to bridge nerve loss that is more than 2–2.5 cm long.

Guiding regenerative gel (GRG), as previously reported by Rochkind and Nevo (8), was developed with the aim of enabling reconstruction of injured peripheral nerve with massive loss defect by using commercial nerve guiding conduits. GRG is a special milieu that increases nerve growth and promotes recovery, aiming, ultimately, at restoring the function of an affected nerve. The major advantages of the GRG lay in its composition, including the three most important and essential elements needed in the initial period of the adjustment and integration of the implant in its new surrounding: (1) antioxidants, found to exhibit high anti-inflammatory activities; (2) synthetic laminin peptides, which act as a scaffold for the nerve fibers to grow along; and (3) hyaluronic acid (HA), which is highly hydrated and contributes to the success of survival, growth, and regeneration of nerve fibers by protecting them from drying.

An *in vivo* study (3 months) on peripheral nerves with massive nerve loss showed that GRG loaded into a commercial collagen tube enabled massive growth of myelinated axons and continuation of axonal sprouting through the tube to the distal part of the nerve in a 15-mm-long gap in the sciatic nerve in rats, which is not possible when bridging with an empty tube. No significant difference was found between GRG and the "gold standard" treatment (nerve autograft) study groups, emphasizing that the GRG enables optimal axonal regeneration. In an additional functional study (9), we evaluated the efficacy of GRG in restoring function to paralyzed limb following a massive nerve loss defect of 15 mm. Three groups were studied: ANG and an implantation of empty tube, with and without GRG. After 6 months of follow-up, we found the group with tubes filled with GRG to be superior to the current gold standard treatment by its ability to regain function, where an empty tube was unable to support any movement.

While the rat model remains the first choice for *in-vivo* testing, it has been postulated that the disproportionate number of studies using rats may in fact skew treatment outcomes and lead to inappropriate evaluation of risks and benefits (10). One example of why a larger animal model may need to be chosen is the limit in nerve gap length that can be studied. While the rat model has effectively been used for short nerve gap model, nerve regeneration over longer gap lengths is far more challenging, with the mode of reconstruction playing a determining factor in recovery (11, 12). This is especially true as the critical gap length for humans of near 3 cm is reached. As such, larger animal models have become more widely considered and further examined for clinical translations, especially when gaps longer than 1.5 cm (13–15) are being evaluated. Of these, a rabbit model is most widely used. To date the sciatic nerve injury is the most well-documented nerve injury model in rabbits with 45% of studies carried out using the sciatic nerve (13–15). There are distinct advantages to utilizing rabbits as the chosen animal model (16). Furthermore, using the rabbit for a peripheral nerve model has allowed for the testing of injuries more than 2 cm, with documented cases on the facial, sciatic, peroneal, median, radial, and ulnar nerve (17, 18).

The aim of this study was to investigate the modified GRG and new combination of AGRG fillings for nerve conduits, prepared with FDA-approved agents, and expected to provide an alternative to an ANG, by supporting and enhancing axonal regeneration, enabling reconnecting massive nerve gaps. The components and dosimetry for new GRG and AGRG formulations were investigate on *in-vitro* nerve cell culture, *in-vivo* rats model and completed on rabbit model of the delayed (chronic) PNI with massive loss defect that represents the human condition (chronic, large gap).

## MATERIALS AND METHODS

### *In vitro* Study—Spine Primary Culture

One-month Sprague-Dawley rats were euthanized anesthetized with Ketamine-xylazine solution (100 and 10 mg/kg, respectively). Then, the spinal cord was removed, placed in a sterile 10 mm petri dish with Hank's Balanced Salt Solution (HBSS) medium (without calcium and magnesium; Biological Industries Ltd., Israel) buffered with 2% HEPES (Biological Industries Ltd., Israel), and kept on ice. The meninges were

stripped away, and the spine was dissected into small pieces and collected into a 15 ml centrifuge tube with 40% TrypLE (Biological Industries Ltd Israel), in HBSS. The tube was agitated horizontally at room temperature for 20 min. Then, the tube was centrifuged for 5 min at 200 g. The supernatant was discarded and 2 ml of fresh Complete Culture Media (Biological Industries Ltd., Israel) were added. The cells were dissociated by pipetting up and down 10 times, first in a normal Pasteur pipette, and then 10 times in a pipette with a tip fire polished to nearly half the normal diameter. Clumps were left to stand for 5 min and then the supernatant was collected into a new 15 ml centrifuge tube. The cells were then seeded on a coverslip in Complete Culture Media (containing 5% horse Serum; Biological Industries Ltd., Israel) for 24 h. After 1 day, the Complete Culture Media was replaced to Incomplete Culture Media (Biological Industries Ltd., Israel), containing Dulbecco's Modified Eagle Medium (DMEM; Biological Industries Ltd., Israel) with 2% B27 (Rhenium, Israel) and 1% Glutamax (Rhenium, Israel), and the tested compounds were added and the study was finalized at day 10. All assays were run in triplicated and repeated at least twice.

## Preparation of GRG/AGRG

A stock solution of 25 ml/mg synthetic laminin peptide consisting of 16 amino acids (synthesized at Bachem, Switzerland) was aseptically prepared by diluting the laminin in 100% dimethyl sulfoxide (DMSO; Sigma-Aldrich), filtered with 0.22-μm filter, and divided into aliquots, and stored at −20°C. For a final concentration of 10 μg/ml, the stock solution was diluted in DMSO to obtain a solution at a concentration of 5 mg/ml. For the *in vitro* studies, 4 μl of the solution was added to 2 ml of incomplete culture media; for the *in vivo* studies, 4 μl of the solution was added to 2 ml of phosphate-buffered saline (PBS; Biological Industries Ltd., Israel).

DL-α-Tocopherol (Merck Millipore, Israel) was diluted with 1 ml of 100% DMSO, generating a stock solution at a concentration of 450 mM with 60% DMSO. The stock solution was used to achieve the different tocopherol concentrations used in the experiments (for 10 and 3 mM, the stock solution was used; for 1 mM, the stock solution was diluted 1:3 with 60% DMSO to achieve a solution at a concentration of 150 mM; for 0.3 mM, the stock solution was diluted 1:10 with 60% DMSO, giving a concentration of 45 mM). Then, to reach a final concentration of 0.3, 1, and 3 mM, 6.67 μl of each solution was added to every 1 ml of either culture medium (for the *in vitro* studies) or PBS (for the *in vivo* studies). To reach a final concentration of 10 mM, 22.23 μl of the stock solution was added to every 1 ml of PBS (for the *in vivo* studies).

HA (0.4%) of high molecular weight (1.67 MDa) (Lifecore Biomedical, USA) was prepared aseptically with incomplete culture media for the *in vitro* studies and with PBS for the *in vivo* studies and stored at 4°C.

The final GRG formulation for the *in vivo* rat study was prepared as 0.4% HA solution with the addition of laminin at a final concentration of 10 μg/ml and tocopherol at final concentrations of 0.1, 1, 3, and 10 mM. The final DMSO concentration was about 0.6%.

Additionally, 10 μg/ml of Copaxone (glatiramer acetate; Teva Pharmaceutical Industries Ltd.) was added to the GRG hydrogel to test an additional benefit in neuronal outgrowth. A stock solution of 20 mg/ml was used, and 0.5 μl was dissolved either with 1 ml of medium in the *in vitro* study or with 1 ml of PBS in the *in vivo* study, to reach a final concentration of 10 μg/ml.

The final AGRG formulation for the *in vivo* rabbit study was prepared as 0.4% HA solution with the addition of laminin at a final concentration of 10 μg/ml, tocopherol at a final concentration of 3 mM, and Copaxone at a final concentration of 10 μg/ml. The final DMSO concentration was about 0.6%.

## *In vitro* Analyses

A full scan and imaging of three coverslips per treatment group were taken. Imaging was done using a BX43 Olympus microscope driven by the standard "CellSens" software by Olympus. Images were taken under 20X objective using a DP74 camera (Olympus). To estimate the neurite length, an ImageJ plugin—"NeuronJ"—was used. Pictures from different areas were taken at various time points from at least three wells per treatment group. The following readouts were measured using ImageJ software with the NeuronJ plugin: (1) mean neurite length per cell, total number of neurites; (2) total number of cells; (3) total number of neurites, mean neurite length per cell; (4) mean number of neurites per cell; and (5) mean number of bifurcations per cell.

## Animals and Surgical Procedure

All animal experiments were approved by the Council for Experiments of Animal Subjects at the Israeli Ministry of Health and adhered strictly to the Animal Care guidelines. The animals were housed under standard conditions [room temperature 20–24°C; a relative humidity (RH) of 30–70%; a 12:12 h light:dark cycle; 15–30 air changes per hour in the study room]. Food and water were provided *ad libitum*.

### Rat Acute PNI Model

Seventy-two male Wistar rats, weighing 250–300 g, were anesthetized using an intraperitoneal injection of 10% ketamine (35 mg/kg) and 2% xylazine (8 mg/kg) mixture. Then the animals were placed on the surgery table. The area of the surgery was shaved, washed with ethanol and Polydine solution, and then covered with a sterile sheet to ensure sterile conditions. The operation on the sciatic nerve was carried out on the left hind limb. Rats were put in a prone position, with the hind limbs abducted, and the skin over the lateral and caudal aspects of the limb up to the lumbar midline was sheared. An incision of about 4–5 cm in length was made along the fusion line of the muscles. The fascia was sharply divided, and the muscles were bluntly retracted to enable access to the sciatic nerve. With a microscope, the sciatic nerve was exposed and was transected proximally and distally, removing 10 mm of length using a microsurgical razor. Prior to transection closure, Marcaine 0.5% (Vetmarket, Israel) was applied. All groups underwent neural reconstruction with either ANG or a NeuraGen® tube (Integra LifeSciences, USA) (the groups are described in **Table 1**). Then the nerve was reconstructed as follows:

**TABLE 1 |** Rat acute PNI experimental design.

| Treatment | *n* |
|---|---|
| 1. Autologous nerve graft (ANG) | 12 |
| 2. NeuraGen® Nerve Guide | 12 |
| 3. NeuraGen® Nerve Guide filled with GRG (0.3 mM tocopherol) | 12 |
| 4. NeuraGen® Nerve Guide filled with GRG (1 mM tocopherol) | 12 |
| 5. NeuraGen® Nerve Guide filled with GRG (3 mM tocopherol) | 12 |
| 6. NeuraGen® Nerve Guide filled with GRG (10 mM tocopherol) | 12 |

**TABLE 2 |** Rabbit chronic PNI experimental design.

| Treatment | 1st surgery | 2nd surgery |
|---|---|---|
| 1. Autologous nerve graft (ANG)* | 8 | 8 |
| 2. NeuraGen® Nerve Guide | 11 | 10 |
| 3. NeuraGen® Nerve Guide filled with GRG | 11 | 11 |
| 4. NeuraGen® Nerve Guide filled with AGRG | 11 | 11 |

**This group was conducted separately, as part of a developmental experiment, but the experiment design was the same as in the efficacy experiments (groups 2–4).*

(1) *ANG* (group 1): The removed 10-mm nerve segment was inverted and implanted between proximal and distal parts of the nerve. Immediately afterwards, an end-to-end anastomosis was performed between the peripheral nerve segment and the proximal and distal parts of the left sciatic nerve, using 10-0 sutures. Cooptation of the nerve was carried out in order to preserve all of the fascicles within the epineural sac. The muscles were sutured using 3-0 Vicryl threads. The skin was closed using special metal staples.

(2) *NeuraGen® Nerve Guide tube* (groups 2–5): After removal of the 10-mm nerve segment, the proximal and distal ends of the nerve were fixed into the 15-mm NeuraGen® Nerve Guide tube (Integra LifeSciences, USA) pre-immersed in saline, creating a 10-mm gap between the two ends, and were microsurgically reconnected using 10-0 epineural sutures. In groups 3–5, before the second end of the nerve was sutured, the corresponding GRG treatment was injected into the NeuraGen® Nerve Guide (see **Table 1**). The external connective area between the tube and the nerve was covered by TISSEEL sealant (Baxter, USA). Then the muscles were sutured using 3-0 Vicryl threads, and the skin was closed using special metal staples.

## Rabbit Chronic PNI Model

### *Induction of PNI (First Surgery)*

Forty-one female New Zealand White rabbits, weighing 2.5–3 kg, were anesthetized using intramuscular injection of 10% ketamine (35 mg/kg) and 2% xylazine (5 mg/kg) mixture. Then, the rabbits were placed on the surgery table and connected to an anesthetic machine that delivered isoflurane (1.5–3%) and 100 oxygen mixture at a rate of 0.5–15 L/min. The area of the surgery was shaved, washed with ethanol and Polydine solution, and then covered with a sterile sheet to ensure sterile conditions.

The operation on the tibial portion of the sciatic nerve was carried out on the left hind limb. The rabbit was put in a prone position, with the hind limbs abducted, and the skin over the lateral and caudal aspects of the limb up to the lumbar midline was sheared. An incision of about 7 cm in length was made along the fusion line of the muscles. The fascia was sharply divided, and the two muscles (biceps femoris and semimembranosus) were bluntly retracted to enable access to the sciatic, peroneal, and tibial nerves. With a microscope, the tibial portion of the sciatic nerve was exposed. Marcaine 0.5% (Vetmarket, Israel) at a volume of 100 μl at each side of the nerve was applied epineurally to the dissected area. The tibial nerve was transected proximally and distally removed at 1 cm of its length. The ends of the transected nerve were sutured to the muscle to prevent possible sprouting of axons. Then the muscles were sutured using 3-0 Vicryl threads, and the skin was closed using special metal staples.

### *Repair of the PNI (Second Surgery)*

Nine weeks after the induction of the injury, 40 rabbits were re-anesthetized (one rabbit was culled after the first surgery due to ethical reasons; see **Table 2**), as described in the first surgery, and the initial PNI was repaired. All groups underwent neural reconstruction with either ANG or a NeuraGen® tube (Integra LifeSciences, USA) (**Table 2**). Then the nerve was reconstructed as follows:

(1) ANG (group 1): The right hind limb and the left hind limb were shaved, cleaned with soap and water, and then washed with ethanol and Polydine solution. With a microscope, the right tibial portion of the sciatic nerve was exposed. Marcaine 0.5% at a volume of 100 μl at each side of the nerve was applied to the dissected area. A tibial nerve segment of 2.5 cm was extracted using a microsurgical razor. Then, the muscles were sutured using 3-0 Vicryl threads, and the skin was closed using metal staples. After that, the 2.5-cm piece of the right tibial nerve was reversed and transplanted to the left limb, after exposing the transected tibial nerve in that limb. The ends of the previously transected nerve of the left hind limb were released, and a 4 mm portion from the proximal and distal ends was removed. Immediately thereafter, an end-to-end anastomosis with a 2.5-cm autologous graft was performed between the proximal and distal parts of the left tibial nerve, using 10-0 sutures. Cooptation of the nerve fascicles was carried out in order to preserve all the fascicles within the epineural sac. Then the muscles were sutured using 3-0 Vicryl threads. The skin was closed using metal staples.

(2) NeuraGen® Nerve Guide tube (groups 2–4): The left hind limb was shaved, cleaned with soap and water, and then washed with ethanol and Polydine solution. The operation was carried out by exposing the proximal and distal ends of the left tibial nerve and separating it from the muscles. The transected tibial nerve ends were released, and a 4-mm portion from each of the transected end was removed. The proximal and distal ends of the nerve, 2.5 mm each, were fixed into 3 cm of the NeuraGen® Nerve Guide tube, creating a 2.5-cm gap between the two ends, and microsurgically reconnected using 10-0 epineural sutures. In groups 3 and 4, before the second end of the nerve was sutured, the corresponding GRG/AGRG

treatment was injected into the NeuraGen® Nerve Guide (see **Table 2**). The external connective area between the tube and the nerve was covered by TISSEEL sealant (Baxter, USA). Then the muscles were sutured using 3-0 Vicryl threads, and the skin was closed using metal staples.

## Electrophysiological Assessment

Non-invasive electrophysiological evaluation was performed before the surgical procedure and again between surgeries in the chronic PNI model and following the repair PNI surgery (the second surgery in the chronic PNI model). The anesthetized animals were placed in a prone position on a heating pad that was only switched off for the short period of actual recording to keep their body temperature at $\leq$36.5°C. In the chronic PNI model, the rabbits were then connected to an anesthetic machine that delivered oxygen at a rate of 0.5–15 L/min. The recordings were performed in the operated left limb and the right limb using a Dantec® Keypoint® focus device (Natus Medical Inc., USA). Bipolar stimulating needle electrodes were placed at the sciatic notch and the paired recording needles at the gastrocnemius muscle in the rabbits and in the tibialis anterior muscle in the rats. The ground electrode was placed on the thigh on the side of stimulation. The sciatic nerve was stimulated by a bipolar stimulating electrode with a pulse of 0.1-ms duration. The stimulus intensity was increased gradually, up to 30% supramaximal level. Then, evoked compound muscle action potentials (CMAPs) were recorded. CMAP amplitude (baseline to negative peak of the M-wave) was measured and normalized to the value measured between the surgeries (chronic PNI model) and to the value measured at baseline (acute PNI model). In cases when animals did not show a CMAP, the amplitude was set to 0.

## Histological and Immunohistochemical Evaluation

Thirty one weeks post the second surgery, the tibial portion of the sciatic nerve was harvested and cross-sectioned into three pieces: proximal to the injury, middle (the injury area), and distal to the injury. The tissues were fixed in 10% formalin and processed and embedded in paraffin blocks. Finally, 132 paraffin blocks of the tibial portion of the sciatic nerve of 40 animals were evaluated (12 paraffin blocks from the healthy right hind of four rabbits).

Embedded tissues in paraffin blocks were sectioned at ∼5-μm thickness, two slides per block; put on a glass slide; and stained with hematoxylin and eosin (H&E, Rhenium, Israel) and immunohistochemistry with myelin basic protein (IHC:MBP, Zotal, Israel). The stained slides were subjected to histological evaluation. Then, pictures were taken using a microscope (Olympus BX60, serial no. 7D04032) at a magnification of X4 with the microscope's camera (Olympus DP73, serial no. OH05504). Picture acquisition was performed only on pathological changes and of representative animals. Image analysis was done with the Image Pro Plus version 6.3 software (Media Cybernetics, USA). An area of interest (AOI) and spatial calibration were applied to each image. Then an RGB histogram threshold was used to depict the brown stain, and the area and area ratio (%) of each threshold were measured.

We performed two stains separately: H&E to assess the quality of the sample and IHC:MBP to evaluate the number of intact motor fibers, neuron fibers, and myelination. Representative pictures were taken by a pathologist. The relative areas of myelin fibers were calculated using the digital morphometric method with IHC:MBP-stained samples (means ± SEM of the different groups were calculated).

## Statistical Analyses

GraphPad Prism version 6.07 (GraphPad Software, USA) was used to perform statistical analyses of the data recorded in this study. To detect significant differences, one-way ANOVA followed by Tukey's multiple comparisons, one-way ANOVA followed by Holms test, and one-way ANOVA followed by Dunnett's multiple comparisons (electrophysiological assessment and *in vitro* study) were applied. For the immunohistochemistry analyses, one-tailed and two-tailed Student's *T*-tests were applied. The $p$ value for statistical significance was set to $p < 0.01$, $p < 0.05$, or $p < 0.01$. All results are presented as percentages or mean ± SEM indicated in the respective tables or figures. For the statistical analyses of electrophysiological evaluation (CMAP amplitude) and immunohistochemistry analyses, animals had to be excluded due to ethical reasons.

# RESULTS

## *In vitro* Study

The *in vitro* studies were conducted to show the effect of the GRG and AGRG on the neuronal outgrowth. For this study we used a similar GRG formula, as we previously reported (8), but we decided to substitute the antioxidant substance (superoxide dismutase 1; SOD1) to a substance that is clinically approved. Thus, we conducted an *in vitro* study to find the best antioxidant substance and it concentration. Following this experiment, we chose tocopherol at a concentration of 3 mM to substitute the SOD1 in the GRG formula, since it showed the best outgrowth in the spine primary neurons (data not shown). Afterwards, we conducted an additional *in vitro* study to evaluate the effect of several compounds, each in combination with GRG (i.e., AGRG) to study the neurite outgrowth in spinal primary neurons of adult rat. Treatment with GRG+copaxone 10 μg/ml showed to most promising effect on the neuronal outgrowth (data not shown).

Due to the used of DMSO to solve some of the compounds in the GRG formula (see method and materials), we performed a control *in vitro* study to test the effect of 1% DMSO, which is the highest concentration used, on neurite outgrowth of the spine primary neurons. No significant change in the mean neurite length per cell was seen following the addition of 1% DMSO (data not shown).

Consequently, the AGRG formula contains HA 0.4%, tocopherol 3 mM, laminin 10 μg/ml and copaxone 10 μg/ml.

**Figure 1** and **Table 3** show that treatment with GRG resulted in an increase of more than 50% in neurite outgrowth vs. DMED-treated cells. Adding 10 μg/ml of Copaxone to the GRG (AGRG) resulted in a significant increase of about 78% in the mean neurite length per cell, when compared with the GRG formulation only (**Table 3** and **Figure 1**). The mean neurite length per cell following treatment with GRG was 260.77 ± 40.68

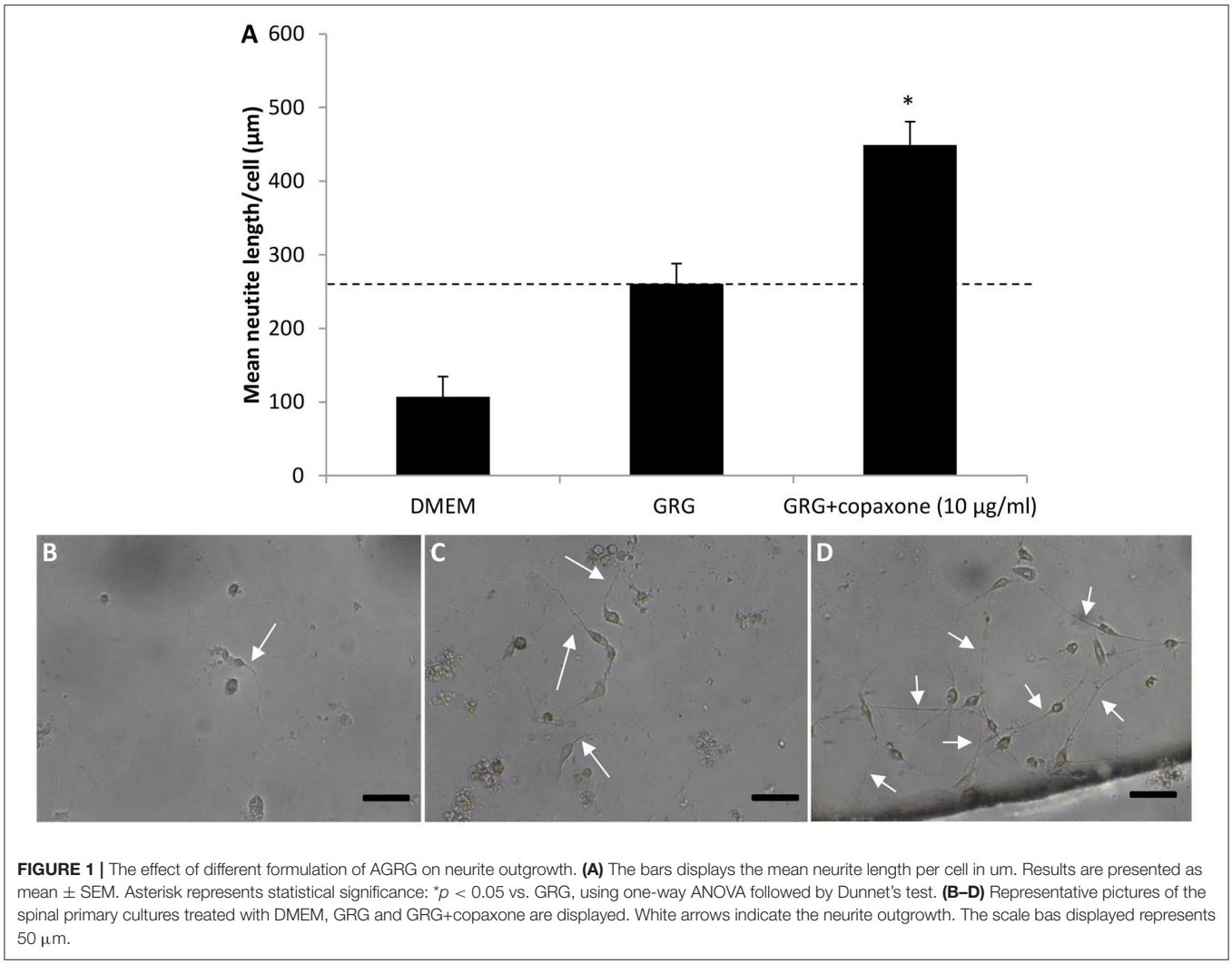


**FIGURE 1 |** The effect of different formulation of AGRG on neurite outgrowth. **(A)** The bars displays the mean neurite length per cell in um. Results are presented as mean ± SEM. Asterisk represents statistical significance: *$p < 0.05$ vs. GRG, using one-way ANOVA followed by Dunnet's test. **(B–D)** Representative pictures of the spinal primary cultures treated with DMEM, GRG and GRG+copaxone are displayed. White arrows indicate the neurite outgrowth. The scale bas displayed represents 50 µm.

**TABLE 3 |** Effects of AGRG different formulations on neurite outgrowth.

| Treatment | Total number of neurites | Total number of cells | Mean neurite length/cell (µm) | Mean number of neurites/cell | Mean number of bifurcations/cell |
|---|---|---|---|---|---|
| DMEM | 15 | 7 | 107.38 ± 27.16 | 2.08 ± 0.27 | 0.33 ± 0.33 |
| GRG | 46 | 14 | 260.77 ± 40.68 | 3.29 ± 0.34 | 2.13 ± 0.70 |
| AGRG | 64 | 186 | 449.13 ± 31.66* | 2.96 ± 0.18 | 1.92 ± 0.23 |

*$p < 0.05$ vs. GRG, using one-way ANOVA followed by Dunnet's test.*

vs. 449.13 ± 31.66 µm per cell following treatment with AGRG (**Figure 1** and **Table 3**; $p < 0.05$, using one-way ANOVA followed by Dunnett's test). Analyzing the total number of neurites shows that AGRG treatment resulted in an increase of proximally 4-fold vs. GRG treatment (186 vs. 46, respectively). Treatment with higher concentrations of Copaxone did not result in further increase in total neurite growth. The number of neurons also showed an increasing trend following AGRG treatment (GRG: 14; AGRG: 64). The number of neurites per cell and the number of bifurcation per cell were not significantly different when comparing the GRG treatment vs. the AGRG treatment. Interestingly, treatment with Copaxone alone (without GRG), at a concentration of 10 µg/ml, showed an increase of 66.02% in the mean neurite length per cell, compared with the GRG formulation only (data not shown).

## *In vivo* Studies

Following finalization of the GRG and AGRG formulations in an *in vitro* assay, we decided to conduct an *in vivo* study on rats to see which tocopherol concentrate ion is most efficient. Thus, we conducted an acute PNI model in rats, with a nerve deficit of 10 mm. The rats were treated as described in **Table 1** and were

followed up for a period of 5 months. During this period, the rats underwent electrophysiological assessments, clinical scoring, and functional recovery.

**Figure 2** displays the left hind limb's normalized amplitude (to baseline) of the CMAPs measured from the tibialis anterior muscle. Generally, the normalized amplitude values increased for the injured left hind limb during the entire study period. Ten weeks after surgery, we observed a slight recovery in all groups; in the ANG group, the recovery was significantly higher ($0.20 \pm 0.04$; $p < 0.01$, using one-way ANOVA followed by Tukey HSD test). Toward the end of the study, on week 16, the normalized amplitude of the ANG treatment was $0.33 \pm 0.05$, significantly higher than the normalized amplitude of the NeuraGen® Nerve Guide tube ($0.15 \pm 0.03$; $p < 0.05$, using one-way ANOVA followed by Holms test), although this difference was not detectable at week 19. Treatment with GRG containing 1 mM tocopherol showed significantly lower normalized amplitude when compared with the ANG treatment [$p < 0.01$ (week 13) and $p < 0.05$ (weeks 16 and 19), using one-way ANOVA followed by Tukey HSD test]. From week 16, there is no significant difference between the ANG treatment and treatment with GRG containing 0.1, 3, or 10 mM tocopherol. These findings suggest that treatments with GRG containing 0.1, 3, and 10 mM tocopherol are as beneficial as the ANG treatment, repairing a 10-mm gap of the sciatic nerve.

Combining the findings of both the *in vitro* and the *in vivo* studies, we set the tocopherol concentration of 3 mM on the GRG formula. Then, we conducted a chronic PNI model on rabbits with a critical gap of 25 mm in the tibial portion of the sciatic nerve, to assess the effect of the GRG and AGRG hydrogels on nerve reconstruction. The rabbits were treated as described in **Table 2** and were followed up for a period of 31 weeks after treatment (**Figure 3A**). During this period, the rats underwent electrophysiological assessments (until week 23; **Figure 3B**) and clinical scoring.

Following transection of the tibial portion of the sciatic nerve and preservation of the peroneal portion, the CMAPs were measured from the gastrocnemius muscle. The signal during the entire study in the injured limb (left) was markedly lower than that of the right limb throughout the study, with an exception of the ANG treatment, in which the right hind limb was also injured (data not shown). However, observing the normalized amplitude of the left limb to the amplitudes values measured between surgeries (week−5; **Figure 3A**), the treatment with NeuraGen® Nerve Guide+AGRG show a significant higher value than that of the NeuraGen® Nerve Guide, at week 15 (**Figure 3B**; 6.20

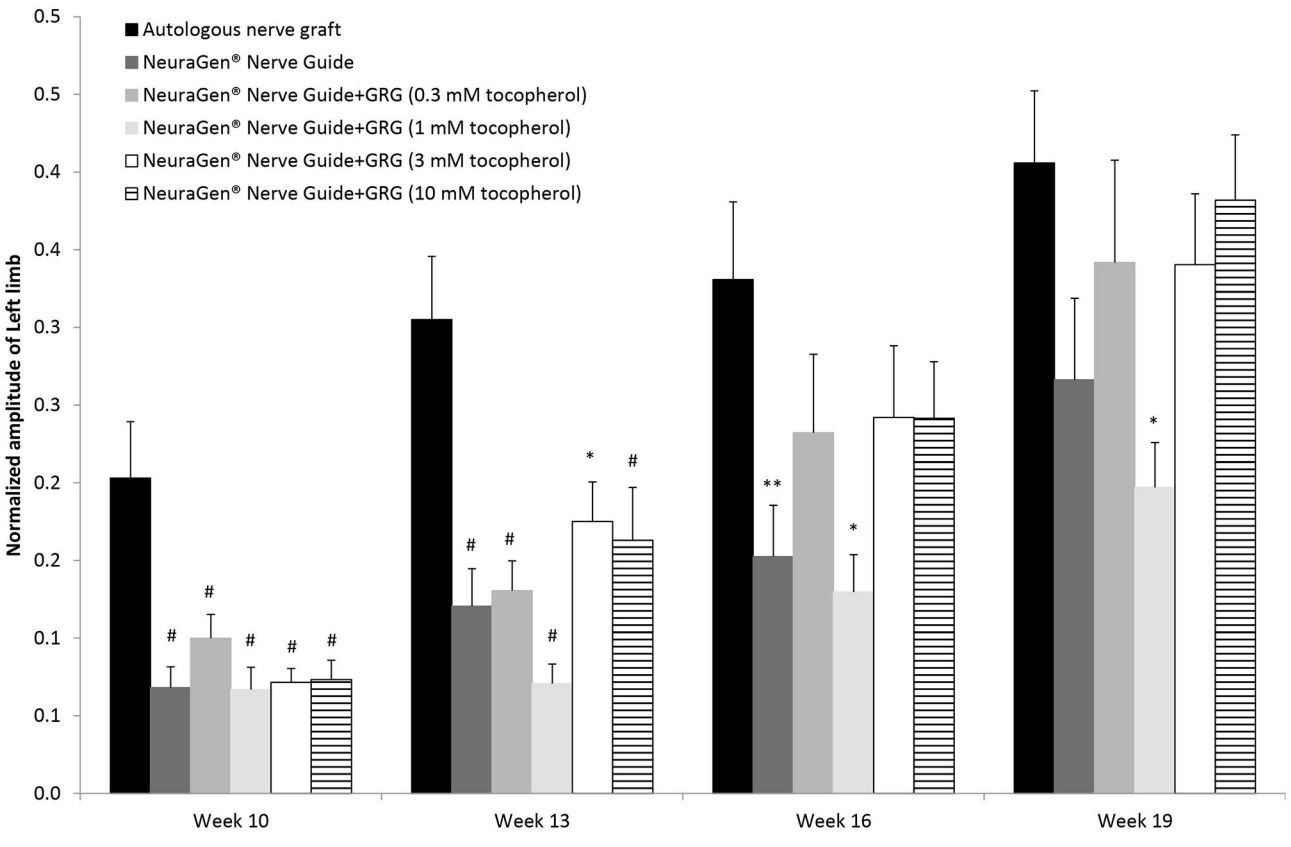


**FIGURE 2 |** CAMP normalized amplitude. The CMAPs amplitude values of the left limb measured at weeks 10, 13, 16 and 19 weeks after surgery, were normalized to the amplitude measured at baseline. Results are presented as mean ± SEM. Asterisk represents statistical significance: $^{*}p < 0.05$ vs. autologous nerve graft, using one-way ANOVA followed by Tukey's HSD test; $^{**}p < 0.05$ vs. autologous nerve graft, using one-way ANOVA followed by Holms test; $^{\#}p < 0.01$ vs. autologous nerve graft, using one-way ANOVA followed by Tukey HSD test.

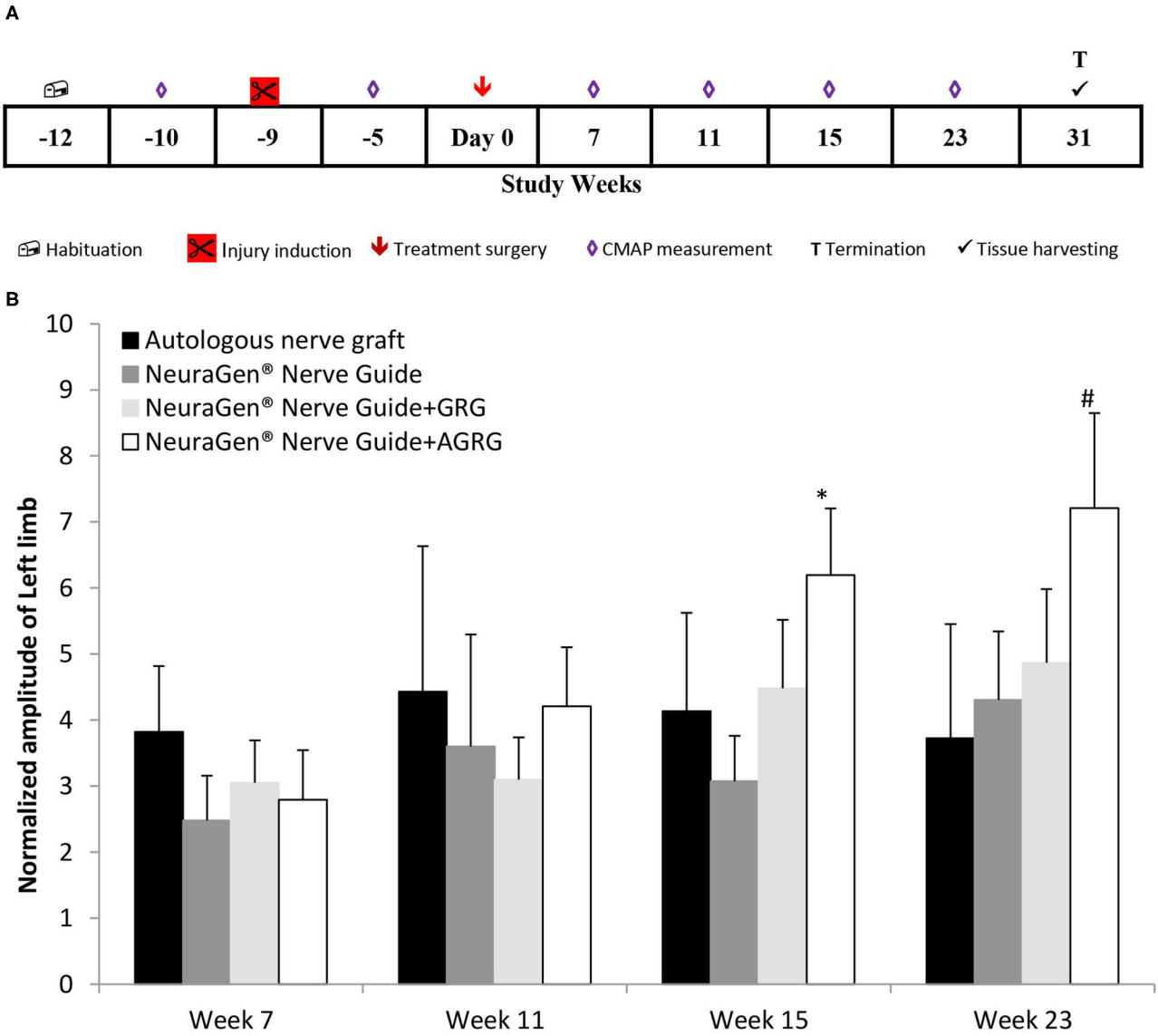


**FIGURE 3 |** Chronic PNI model. **(A)** Study design. The injury was induces 9 weeks prior the treatment surgery and the rabbits were followed up for 31 weeks post treatment. **(B)** Left limb (injured) CAMP normalized amplitude. The amplitude values of the CMAPs measured at weeks 7, 11, 15 and 23 weeks after treatment, were normalized to the amplitude measured 5 weeks prior the treatment surgery (after the injury inducing and before the treatment surgery). Results are presented as mean ± SEM. Asterisk represents statistical significance: $^{*}p < 0.05$ vs. NeuraGen® Nerve Guide, using one-way ANOVA followed by Dunnett's test; $^{\#}p < 0.05$ vs. week 7, using one-way ANOVA followed by Tukey HSD test.

± 1.01 vs. 3.08 ± 0.69, respectively; $p < 0.05$, using one-way ANOVA followed by Dunnett's test). This finding was also observed at week 23, when NeuraGen® Nerve Guide+AGRG showed the highest result in comparison to the other treatments. Although this finding is not statistically significant at week 23, the trend continues to show that AGRG treatment is the most promising for reconstruction of the tibial portion of the sciatic nerve with critical gap of 25 mm. It is important to emphasize that the baseline values of the CMAPs, measured from both hind limbs, were within the normal range (data not shown; right hind limb: 17.24 ± 0.88 mV; left hind limb: 18.06 ± 0.90 mV).

Upon harvest the tibial portion of the sciatic nerve for immunohistochemistry analysis at weeks post-treatment, we assessed the quality of the samples by preforming H&E staining. The H&E staining showed that most proximal cross sections were unaffected or contained a mild vacuolization of the nerve's fibers and a very mild lymphocytic infiltration. In the distal sections were mostly mildly affected with fibers vacuolization (data not shown).

Then, we stained the samples with myelin-based protein (MBP) to evaluate the nerve reconstruction. The MBP mean relative area values of the proximal sections are similar, as the healthy section, regardless the treatment (**Figures 4**, **5**

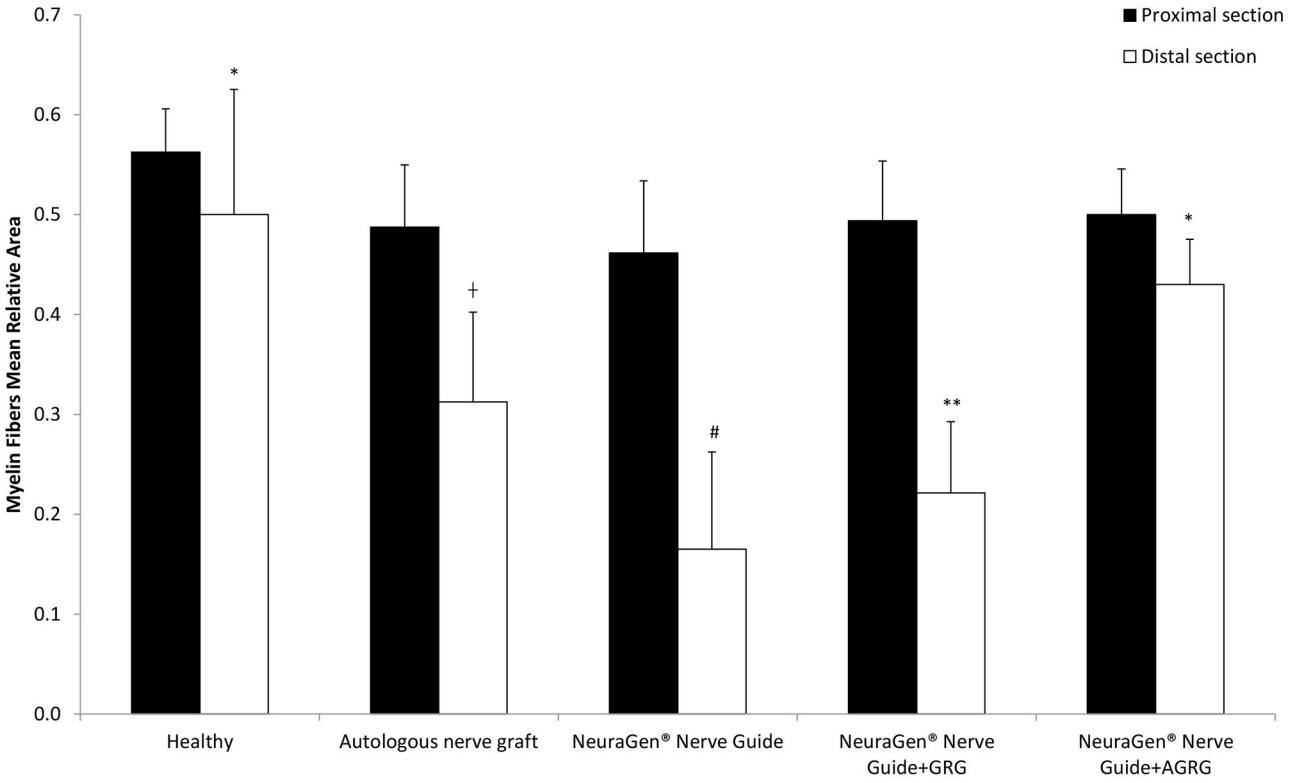


**FIGURE 4 |** MBP staining to assess regeneration. **(A)** The graph display the mean relative area (mean ± SEM) of the MBP in Prox and Dist sections. Asterisk represents statistical significance: $^{*}p < 0.1$ using two-tailed Student's *T*-test vs. NeuraGen® Nerve Guide; $^{**}p < 0.01$ using two-tailed Student's *T*-test vs. proximal section; $^{\#}p < 0.05$ using two-tailed Student's *T*-test vs. proximal section; $^{\dagger}p < 0.1$ using one-tailed Student's *T*-test vs. proximal section.

and **Table 4**). When observing the regeneration process of the distal sections (**Figures 4**, **5** and **Table 4**), there is a significant regeneration of the AGRG treatment, compared with the NeuraGen® Nerve Guide treatment (**Figures 4**, **5** and **Table 4**; $^{*}p < 0.1$, two-tailed Student's *T*-test).

According to the distal sections findings we can conclude that the AGRG treatment succeeded to significantly regenerate the injured lesion, as good as the ANG and healthy groups, after 31 weeks post-treatment months from treatment; while the NeuraGen® Nerve Guide treatment show a mild regeneration process.

## DISCUSSION

The ultimate goal of present study on GRG/AGRG is to improve the functional performance and quality of life of patients affected by PNI with massive loss defect, which represents a major cause for morbidity and disability in affected patients and may cause substantial costs for the society in a global perspective.

The current clinical gold standard for peripheral nerve reconstruction, when larger nerve gaps exist (20 mm or longer in humans), is an autologous sensitive nerve graft (autograft). The reconstruction of a segmental nerve loss poses a significant surgical challenge in order to achieve better results and lower donor morbidity. Reinnervation with cutaneous sensitive nerves is not always satisfactory, as motor fibers need to be included into the bridging nerve grafts (19). In addition, nerve harvesting and subsequent donor site morbidity lead to functional loss, as well as to an increased risk of neuroma formation, paresthesias, and higher costs associated with a second surgical site (20). Moreover, long nerve gap lengths have been among the most difficult injuries to repair, demonstrating slow rates of regeneration and often incomplete recovery. Thus, further development of novel concepts to accommodate longer nerve deficits must be encouraged.

One of the promising solutions already in clinical practice is artificial nerve conduits. The most significant advantage to using commercial nerve conduits is to avoid sacrificing the patient's functional nerve for an autograft. The procedure is simpler, there is a significant decrease in time of surgery, and there is no sensation loss or cosmetic defect in the leg. These are the advantages of using nerve conduits in comparison to ANG, explaining the efforts invested in optimizing this solution worldwide. Experimental research with simple nerve guiding conduits showed unsuccessful bridging of relatively long gaps of 15 mm in the rat (21), of ∼30 mm in rabbits, and of 30 mm in primates (22–24). Results from clinical studies are often comparable to autografts in the treatment of lesions with nerve defects of <3 cm. These models do not assure nerve regeneration in more extensive lesions. Therefore, the disadvantage of commercial nerve conduits is the inability to bridge more than 2–3-cm-long nerve loss. Another methodology is based on a decellularized cadaveric nerve (allograft), which is prepared through a process of detergent decellularization,

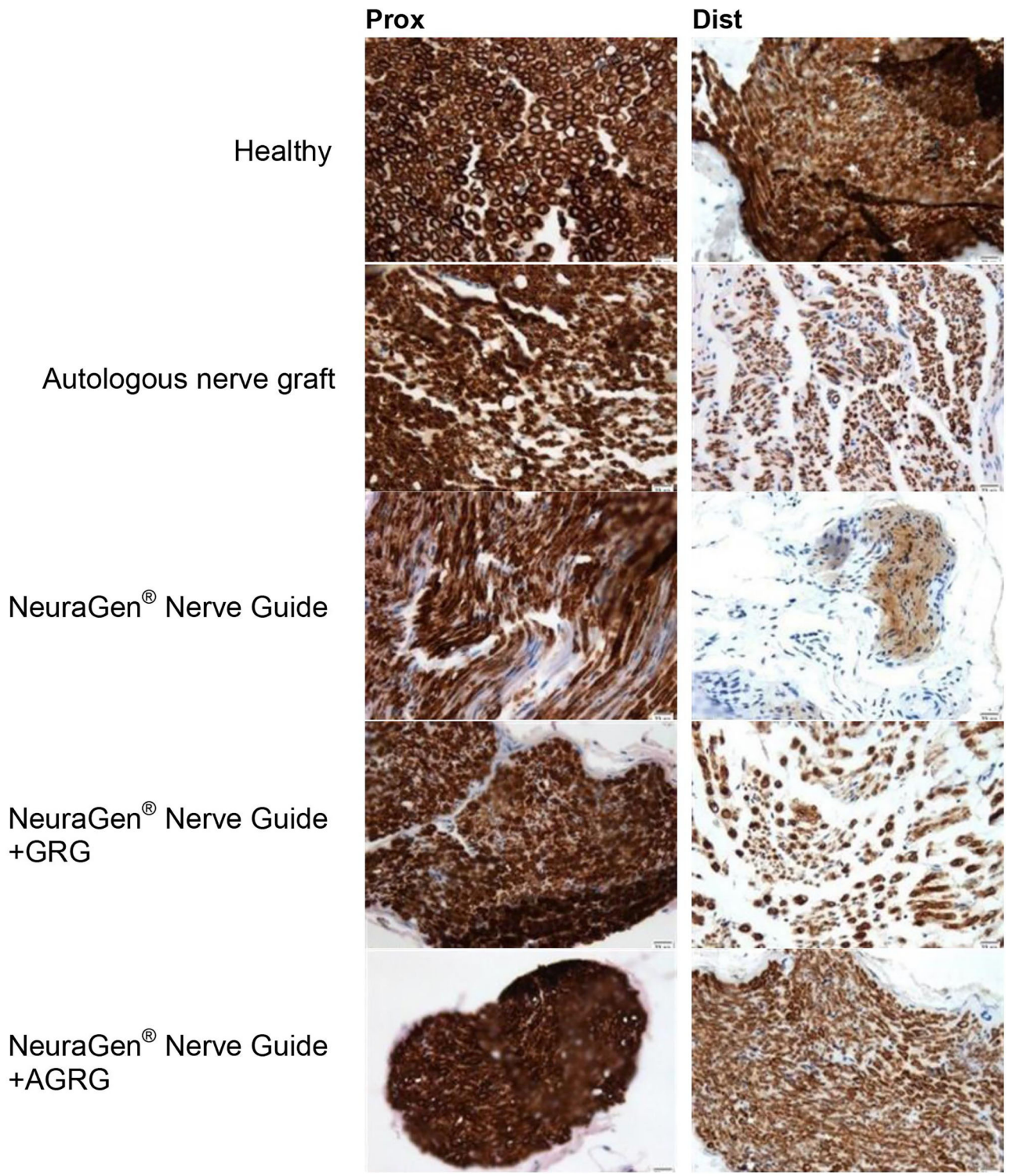


**FIGURE 5 |** Representative histologic pictures of the MBP staining. The proximal and distal sections of the tibial portion of the sciatic nerve of each treatment are displayed. Pictures were taken at magnification of ×20.

enzyme degradation, and gamma irradiation sterilization (25). Acellular nerve allografts rather than fresh allografts do not need immunosuppression and appear to be effective based on clinical studies (26). The decellularization methods reported in the literature give rise to a series of disadvantages, such as an increased risk of contamination, technical incompatibility

**TABLE 4 |** MBP assessment.

| Treatment | Prox | Dist |
|---|---|---|
| Healthy | 0.56 ± 0.04 | 0.50 ± 0.13* |
| Autologous nerve graft | 0.49 ± 0.06 | 0.31 ± 0.09$^{+}$ |
| NeuraGen® Nerve Guide | 0.46 ± 0.07 | 0.17 ± 0.10$^{\#}$ |
| NeuraGen® Nerve Guide filled with GRG | 0.49 ± 0.06 | 0.22 ± 0.07** |
| NeuraGen® Nerve Guide filled with AGRG | 0.50 ± 0.05 | 0.43 ± 0.05* |

*The values of the MBP mean relative area are presented as mean ± SEM. Asterisk represents statistical significance: *$p < 0.1$ using two-tailed Student's T-test vs. NeuraGen® Nerve Guide; **$p < 0.01$ using two-tailed Student's T-test vs. proximal section; $^{\#}p < 0.05$ using two-tailed Student's T-test vs. proximal section; $^{+}p < 0.1$ using one-tailed Student's T-test vs. proximal section.*

(27), and compromised tissue functionality after gamma-ray sterilization (28). Other attempts to improve nerve regeneration is developed with conduit luminal scaffolds, from collagen and laminin hydrogels to synthetic and collagen filaments and channels (20, 29–33). However, these modifications have not produced results better than those of the autograft and therefore do not offer a substantial benefit over the autograft at this time (31, 34, 35).

Although nerve conduit has advantages, in comparison to the ANG, the nerve conduit's inability to bridge a gap of over 2–3 cm of nerve loss prevents its widespread application in clinical practice for reconstruction of peripheral nerves. Therefore, the repair and regeneration of peripheral nerve injuries with massive loss defects still remain a major clinical issue for the relatively new fields of regenerative medicine and biomaterials and tissue engineering.

We started to investigate the possibility of increasing nerve regeneration through a long-distance gap by using a composite neurotube in 2004 (36) and created a GRG matrix (8) that would serve as a vehicle to axonal growth and surviving and therefore enable the reconstruction of peripheral nerves with massive loss defect.

Our current study suggests that the modified procedure of using a commercial nerve conduit filled with a newly developed AGRG formulation for nerve reconstruction may be successfully used in clinical practice for treatment of PNI with massive loss defect. We base our statement on the positive effect we received in the treatment of a rabbit model of delayed (chronic) PNI that represents the most common human condition of delayed PNI with a gap of more than 2 cm. We used delayed nerve repair because in clinical practice, it often occurs and is indicated in complex cases of severe local soft tissue and/or bony injuries associated with a significant area of nerve injury and a ragged nerve transection (37).

In the present study, GRG formulation was modified, and a novel combination of AGRG prepared with FDA-approved agents was investigated *in vitro* on the neuronal outgrowth. Application of AGRG (GRG+Copaxone) showed a significant increase of about 78% in neurite length per cell and was shown to have the most promising effect on neuronal outgrowth (**Figure 1** and **Table 3**). In addition, the total number of neurites increases by 4-fold when compared with the GRG formulation only.

For the finalization of the GRG and AGRG formulations, different concentrations were added in an *in vitro* assay. We decided to conduct an *in vivo* study on rats to investigate which tocopherol concentration is most efficient. We found that the GRG+tocopherol treatments are as beneficial as the 10-mm ANG. Then, we conducted a study on a rabbit model of delayed (chronic) PNI with a critical gap of 25 mm (a model that imitated the human condition of delayed repair and large gap) to assess the effect of the GRG and AGRG hydrogels on nerve recovery. Nine weeks after injury, the nerve was repaired.

The electrophysiological follow-up showed that AGRG treatment is the most promising for reconstruction of the tibial portion of the sciatic nerve with a critical gap of 25 mm (**Figure 3**). Moreover, a surprising finding was the beneficial effect of AGRG when compared with the autologous nerve reconstruction.

Thirty-one weeks post the second surgery (delayed reconstruction), histochemical observation showed significant regeneration after using AGRG hydrogel, compared with the empty tube (**Figures 4**, **5** and **Table 4**). Based on to the distal sections findings, we can conclude that the AGRG treatment succeeded to significant nerve regeneration nerve, as well as the ANG and healthy groups.

*In conclusion*, we demonstrate that in our injury model of a delayed nerve repair with massive nerve loss defect, the application of AGRG led to a stronger nerve recovery than other reconstructive strategies in the past.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The animal study was reviewed and approved by Council for Experiments of Animal Subjects at the Israeli Ministry of Health.

## AUTHOR CONTRIBUTIONS

SR co-invented the matrix, conceived and designed, planned the experiments, and surgery. MA planned the experiments and conducted the electrophysiological assessment. SM conducted the *in vitro* and *in vivo* experiments and data analysis. ZN co-invented the matrix. All authors contributed to the article and approved the submitted version.

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# Surgical Treatment of Radial Nerve Injuries Associated With Humeral Shaft Fracture—A Single Center Experience

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Radial nerve injuries are often associated with humeral shaft fractures. The results of treatment of these injuries, by contemporary surgical approaches, remain diverse. In this paper we presented the outcomes and analyzed the patient, clinical, and surgical procedure related characteristics and factors that may influence the outcome overall, in 77 patients treated at Clinic for Neurosurgery, Clinical Center of Serbia during a 20 years period. The nerve injuries were verified by US and EMNG. The majority of patients were treated by neurolysis or sural nerve grafting, while only few were treated by direct suture. The final recovery was evaluated by muscle strength assessment and classified using MRC. We analyzed extension of the wrist, extension of the fingers including the thumb, and abduction of the thumb. There was a significant statistical difference in MRC grade following the treatment. The total rate of useful functional recovery was achieved in 69 (89.61%) out of all studied patients, out of whom 20 (28.99%) achieved excellent recovery, 26 (37.68%) achieved good recovery and 23 (33.33%) achieved fair recovery. Only 8 (10.39%) out of all studied patients achieved poor recovery. The injured nerves, that were preserved in continuity, acquired by a low-energy trauma, and treated earlier than the 6 months were associated with better functional outcome following the surgery. In addition, there was a trend of better functional improvement with aging, keeping in mind that the old were subjected to lower energy trauma. The expectant management followed by surgery of radial nerve injury associated with humeral shaft fracture should be around 3 months, and the surgical nerve repair should not be performed later than the 6 months after injury. The energy of trauma may be a factor predicting patient's final recovery following the treatment.

**Keywords: radial nerve injuries, humeral shaft fracture, surgical treatment, outcome, neurolysis, grafting**

## INTRODUCTION

The fractures of the humeral shaft make up about 1–3% of all skeletal fractures, and belong to the group of the most common bone injuries (1–4). The incidence increases with age and may be associated with significant in-patient mortality and health care utilization costs (2, 5–7). In addition, the patients remain unable to return to work for a long period even after the surgery (8, 9), which is a significant socioeconomic issue (10).

Due to the close topographic ties between nervous, bony and vascular tissues (1, 11), the injuries of peripheral nerves are often associated with these injuries (12–14), and radial nerve injuries occur in between 2 and 18% of cases with humeral shaft fracture (15–19). This high rate of combined injuries is probably due to their close anatomic relation in the spiral groove (sulcus nervi radialis - SNR) at the posterior side of the humeral shaft, as well as due to the rigidity of the radial nerve while piercing the lateral intermuscular septum after exiting the groove (20–22). Despite the fact that the fracture repair is usually successful (23, 24), the injury to the radial nerve can leave permanent functional disability of the hand (wrist drop) and sequentially the arm as a whole (20). This loss of hand function is found to be a horrifying experience for the majority of patients (25), and the fact that most of the patients contribute significantly to the household and the community further exacerbates their own and their families suffering (26–28) and presents a big socioeconomic issue (25, 29, 30).

The expert opinions on the timing and necessity of the surgery for associated radial nerve injuries are divided. Some studies suggest that these lesions have a high rate of satisfactory spontaneous recovery (15, 16, 31), but it may take more than a year for the most of the patients to return to work (1, 32–34). Early exploration is only indicated in open fractures (15, 35), while the primary nerve repair is only indicated if the nerve has a clean-cut margin, both of which are rare when the nerve is injured by the bone fragments (32).

Based on the contemporary surgical approaches, and a vast personal experience, a clear strategy was developed to treat these patients, and we treated 77 patients during the last 20 years. Beside the outcomes, we aimed to analyze the patient, clinical, and surgical procedure related characteristics and factors that may influence the outcome overall.

## MATERIALS AND METHODS

### Patients

We retrospectively analyzed hospital records in the period from January 1st, 2001 until December 31st, 2020 and found 147 patients with isolated radial nerve lesion, out of whom 77 met below mentioned criteria.

### Inclusion Criteria

- Patients surgically treated during a 20 years period (January 1st, 2001–December 31st, 2020)
- Minimal follow up of 1 year
- Unilateral non-pathological humeral shaft fracture
- Unilateral radial nerve palsy due to humeral shaft fracture or as a consequence of orthopedic management of the fracture

### Exclusion Criteria

- Compressive neuropathy
- Radial nerve injury without associated humeral shaft fracture
- Patients with previous history of peripheral nerve sheath tumor (PNST), demyelinating disorders, or neuropathy due to vasculitis or diabetes mellitus that have acquired humerus fracture and were sent to our clinic for examination
- Patients treated by artificial nerve graft

Before meeting the patients, we made a detailed review of their medical records, and formed a database. All data were re-checked and supplemented in subsequent contacts with the patients.

### Clinical Features

Prior to the surgery, all patients underwent a physical and a complete diagnostic evaluation. Humeral shaft fractures were verified by radiography, while nerve lesions were verified by ultrasonography (US) and neurophysiology, usually the electromyoneurography (EMNG).

To enquire a potential link between patient's characteristics and nerve recovery following the surgery, we considered the age, gender, smoking habits and presence of associated diseases.

**TABLE 1 |** Combined scale for evaluating final recovery in study patients.

| | |
|---|---|
| Poor | M0, M1 and M2 for all muscle groups |
| Fair | M3 for extension of the wrist and fingers; M0, M1, and M2 for thumb abduction |
| Good | M4 and M5 for extension of the wrist and fingers; M3 for thumb abduction |
| Excellent | M4 and M5 for all muscle groups |

*Extension of the wrist, extension of the fingers including the thumb, and abduction of the thumb were examined.*

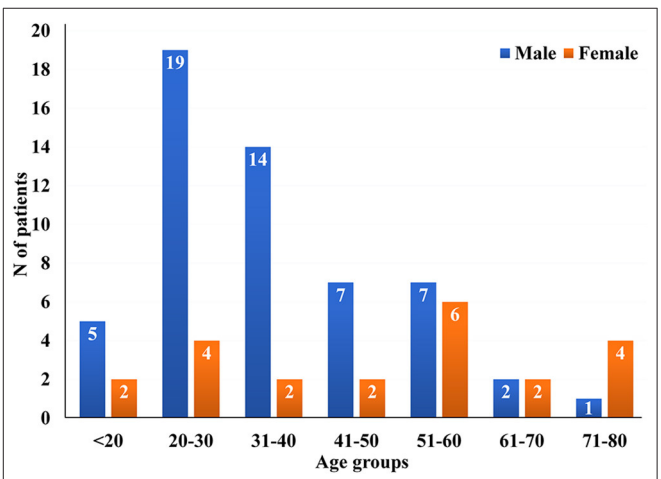


**FIGURE 1 |** Age and sex distribution among included patients.

**TABLE 2 |** Distribution of associated injuries in patients with one, two and multiple associated injuries.

| Types of associated injuries | *n* of associated injuries | | | |
|---|---|---|---|---|
| | **1 (*n* = 11)** | **2 (*n* = 9)** | **Multiple (*n* = 8)** | **Total (*n* = 14)** |
| **Long bone fractures** | 4 | 8 | 13 | 25 |
| Radius | 4 | 4 (p1*, p2, p8, p9) | 2 (p3, p7) | 10 |
| Ulna | / | 4 (p1, p2, p8, p9) | 4 (p2, p3, p7, p8) | 8 |
| Femur | / | / | 4 (p1, p2, p6, p8) | 4 |
| Fibula | / | / | 2 (p2, p8) | 2 |
| Clavicle | / | / | 1 (p4) | 1 |
| **Axial skeletal fractures** | 1 | 3 | 5 | 9 |
| Cervical spine | / | / | 3 (p1, p4, p6) | 3 |
| Thoracic spine | / | 1 (p4) | / | 1 |
| Ribs | 1 | 2 (p3, p4) | 1 (p6) | 4 |
| Pelvis bones | / | / | 1(p1) | 1 |
| **Joint luxation** | 4 | 3 | 1 | 8 |
| Elbow joint | 2 | 1 (p3) | 1 (p7) | 4 |
| Humeral joint | 2 | 2 (p5, p7) | / | 4 |
| **Nerve injuries** | 1 | 2 | 5 | 8 |
| Median nerve | / | / | 2(p1, p5) | 2 |
| Ulnar nerve | 1 | 2 (p5, p7) | 2(p2, p5) | 5 |
| Brachial plexus | / | / | 1(p3) | 1 |
| **Muscles and tendons injuries** | 1 | / | 1 | 2 |
| Subscapular muscle | 1 | / | / | 1 |
| Deltoid muscle | / | / | 1 (p4) | 1 |
| **Vascular injuries** | / | / | 1 | 1 |
| Brachial artery | / | / | 1 (p5) | 1 |
| **Abdominal injuries** | / | 2 | / | 2 |
| Spleen | / | 1(p6) | / | 1 |
| Mesentery | / | 1(p6) | / | 1 |

**p1–p9 in the brackets represent injury that occurred in same patient.*

The energy of the initial trauma was determined according to the etiology of injury (36): a low-energy trauma (fall from the standing position) and a high-energy trauma (falls from height, traffic accidents, and crushing injuries) and it was previously identified as a prognostic factor that may affect the final recovery (37).

For analyzing how preoperative nerve status affected patient's final recovery, we took into account nature of nerve injury, level of the nerve failure, and continuity of the nerve. Due to insignificant sensory disturbances following radial nerve injury, level of the nerve failure was evaluated by muscle strength assessment and graded according to British Medical Research Council muscle strength scaling system (MRC) (38). We analyzed extension of the wrist, extension of the fingers including the thumb, and abduction of the thumb. The final preoperative result for every single patient was achieved by summarizing MRC scores for all muscles tested.

Humeral shaft fractures were classified based on the level of the fracture line on the shaft (33): D1 (surgical neck fracture), D2 (proximal metaphysis fracture), D3 (fracture of the joint of the proximal and middle third of the body), D4 (fracture of the middle third of the body), D5 (fracture of the junction of the middle and distal third of the body), and D6 (distal metaphysis fracture). In order to analyze how associated injuries affected patient's final recovery, we took into account humerus fracture type and presence of other associated injuries.

## Treatment

The decision-making process and surgical strategy were determined according to the several principles.

Early surgical exploration was indicated in cases with open injuries, or iatrogenic cases with evident (or US confirmed) laceration or compression which were treated as soon as possible. When clear cut margins were present the patient underwent immediate direct suture.

In cases of traumatic nerve palsies associated with closed fracture of the humeral shaft, and iatrogenic nerve palsies without evident cause, a late exploration was indicated.

Following the failure of conservative treatment, after 3 months of expectance for EMNG signs of recovery to appear, the patients were referred to our institution for surgical evaluation and treatment.

**TABLE 3 |** Distribution of the study patients in reference to etiology of nerve injury, nature of nerve injury, nerve continuity and level of nerve failure.

| Nature of nerve injury | | Etiology of nerve injury | Nerve failure level | | Nerve continuity | |
|---|---|---|---|---|---|---|
| | | | Complete | Incomplete | Preserved | Interrupted |
| Primary injury | $n = 45$ (100%) | Traffic accident | 11 (24.4) | 5 (11.1) | 12 (26.7) | 4 (8.9) |
| | | Fall | 9 (20.0) | 1 (2.2) | 8 (17.8) | 2 (4.4) |
| | | Occupational accident | 11 (24.4) | 2 (4.4) | 7 (15.5) | 6 (13.3) |
| | | Other | 3 (6.7) | 3 (6.7) | 3 (6.7) | 3 (6.7) |
| | | Total | 34 (75.6) | 11 (24.4) | 30 (66.7) | 15 (33.3) |
| Secondary injury | $n = 32$ (100%) | Internal fixation | 20 (62.5) | 7 (21.9) | 23 (71.9) | 4 (12.5) |
| | | Osteosynthetic material removal | 5 (15.6) | / | / | 5 (15.6) |
| | | Total | 25 (78.1) | 7 (21.9) | 23 (71.9) | 9 (28.1) |

**TABLE 4 |** Distribution of the study patients in reference to the surgical procedure performed, nature of the nerve injury, level of the nerve failure and time to surgery.

| Surgical procedure | Nature of nerve injury (palsy) | | Nerve failure level (palsy) | | Time to treatment (months) | | | |
|---|---|---|---|---|---|---|---|---|
| | Primary *n* (%) | Secondary *n* (%) | Complete *n* (%) | Incomplete *n* (%) | 0–3 | 3–6 | 6–9 | >9 |
| Direct suture | 2 (4.4) | / | 2 (3.4) | / | 2 | / | / | / |
| Neurolysis | 30 (66.7) | 23 (71.9) | 35 (59.3) | 18 (100) | 7 | 35 | 3 | 8 |
| Grafting | 13 (28.9) | 9 (28.1) | 22 (37.3) | / | / | 12 | 8 | 2 |
| Total | 45 (100) | 32 (100) | 59 (100) | 18 (100) | 9 | 47 | 11 | 10 |

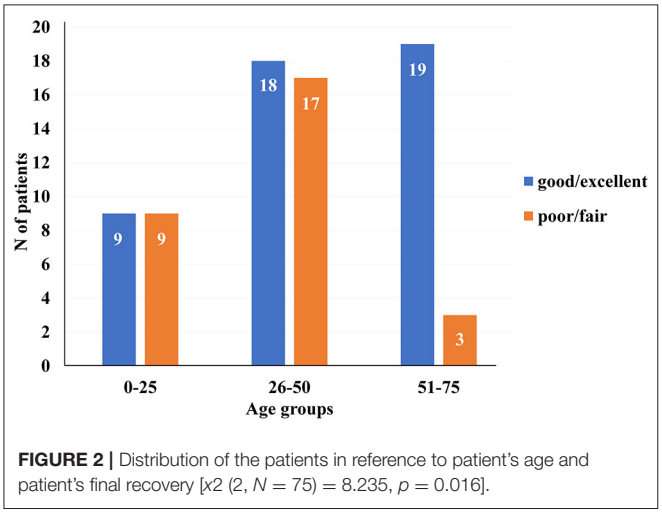


**FIGURE 2 |** Distribution of the patients in reference to patient's age and patient's final recovery [$x2$ (2, $N = 75$) = 8.235, $p = 0.016$].

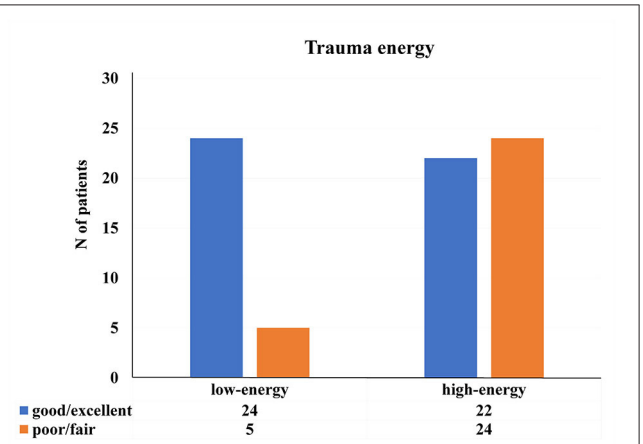


**FIGURE 3 |** Distribution of the patients regarding energy of the initial trauma and patient's final recovery [$x2$ (1, $N = 75$) = 9.152, $p < 0.01$].

This process had not changed much during the last 20 years, and there were no significant variations in treatment of this group of patients.

## Outcome Assessment

The final recovery was evaluated by muscle strength assessment and classified using MRC. The same muscles, tested preoperatively, were tested postoperatively, and the results were compared (total MRC score for all muscle groups tested). The modified scale of Highet and Holmes (**Table 1**), was used to classify the recovery, and fair or better results were deemed satisfactory (1, 32, 39).

In order to examine how treatment modality and timing affected patient's final recovery, we took into consideration surgical procedures performed as well as the time elapsed until the surgery.

## Statistical Analysis

All statistical procedures were performed using IBM SPSS v26.0. Parameters of interest were described using the methods of descriptive statistics: mean, median, range, absolute (N) and relative (%) frequencies. The normality of data was assessed using Shapiro-Wilk test. For analyzing the association between patient's groups and patient's final recovery we performed Fisher's

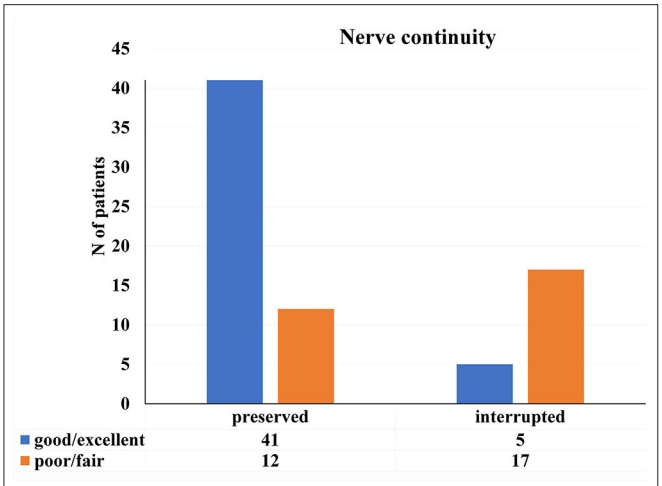


**FIGURE 4 |** Distribution of the patients in reference to nerve continuity and patient's final recovery [$x2$ (1, $N = 75$) = 19.565, $p < 0.01$].

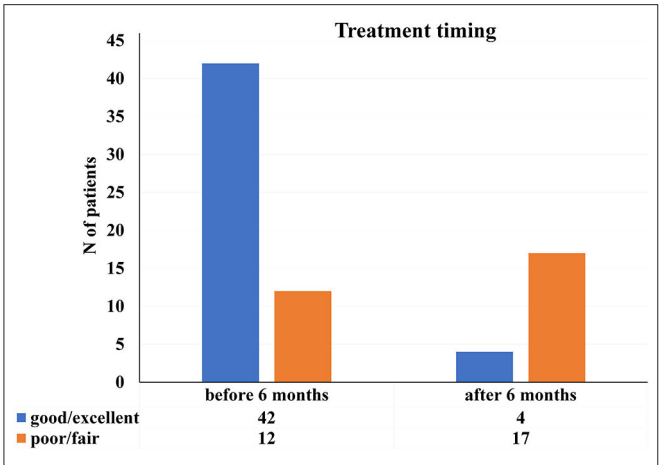


**FIGURE 5 |** Distribution of the study patients according to patient's final recovery and timing of the treatment [$x2$ ($N = 75$), $p < 0.01$].

exact and Chi-Square test. For comparing preoperative and postoperative measurements, we used Wilcoxon signed-rank test. The significance factor was set to be lower than 0.05. Due to low occurrence, the 2 patients treated by direct suture were excluded from the statistical analysis.

For easier statistical analysis of certain factors and characteristics, the patients were divided into the groups: age (0–25, 26–50, and 51–75 years old), nature of nerve injury (traumatic/iatrogenic), humerus fracture type (D3 and/or proximally/D4 and/or distally), treatment timing (before 6 months/after 6 months), final recovery (excellent or good/fair or poor).

## RESULTS

Total 55 (71.43%) male and 22 (28.57%) female patients were in the study group (**Figure 1**). The mean age was 39.39 ± 17.10, while their age ranged from 12 years old to the oldest patient of 75 years old. The mean and median age of male population were 35.38 ± 14.34 and 32.0 (12–32), while the mean and median age of female population were 49.41 ± 19.56 and 59.0 (18–75), respectively. More than a half of all studied patients—42 (54.55%) lived in urban places, while 35 (45.45%) of them lived in rural places.

Out of all studied patients, 31 (40.26%) were tobacco smokers. Twenty one patients (27.27%) had one associated disease, 4 (5.19%) had two, while 52 (67.53%) had none.

Concerning the energy of the initial trauma, 46 (59.74%) patients were subjected to injury by a high-energy trauma (male vs. female = 40:6), while 31 (40.26%) patients were subjected to injury by a low-energy trauma (male vs. female =15:16). The high-energy trauma was more common in the groups of patients aged 0–25 (70.0%) and 26–50 (74.29%), comparing to the group of patients aged 51–75 (27.27%). Furthermore, all patients with two or multiple associated injuries were subjected to injury by a high-energy trauma (**Table 2**).

Regarding humeral shaft fracture type, 13 (16.89%) patients had fracture at the proximal third/middle third junction (D3), 42 (54.55%) patients had fracture at the middle third (D4), 20 (25.97%) patients had fracture at the middle third/distal third junction (D5), and only 2 (2.59%) patients had fracture at the distal third (D6) of the humeral shaft.

Primary nerve injury occurred in 45 (58.44%) patients, while secondary (iatrogenic) nerve injury occurred in 32 (41.56%) patients (**Table 3**). Out of all studied patients, 59 (76.62%) acquired complete nerve palsy (M0 for all muscle groups), while only 18 (23.38%) acquired incomplete nerve palsy (M1–M3 for all muscle groups).

Most of the patients had the nerve preserved in continuity, and all these patients were treated with neurolysis procedures, while the patients with completely interrupted continuity were subjected to the nerve repair. Out of 53 patients with the nerve preserved in continuity, 35 (66.04%) were treated by external neurolysis, 10 (18.87%) were treated by longitudinal epineurotomy, and 8 (15.09%) were treated by circumferential epineurectomy and interfascicular neurolysis. Two patients (8.33%) had direct nerve suture immediately during the initial exploration of the cut nerve. Other 22 patients (91.67%) with interrupted continuity underwent grafting. There were no complications related to the nerve surgery. **Table 4** shows the distribution of the study patients in reference to the surgical procedure performed, nature of the nerve injury, level of the nerve failure, and time passed to the surgery.

The signs of motor recovery were accomplished in all studied patients. There was a significant increase in MRC grade following surgical treatment ($Z = -7.544$, $p < 0.01$). The total rate of useful functional recovery was achieved in 69 (89.61%) out of all studied patients, out of whom 20 (28.99%) achieved excellent recovery, 26 (37.68%) achieved good recovery and 23 (33.33%) achieved fair recovery. Only 8 (10.39%) out of all studied patients achieved poor recovery.

Regarding patient's characteristics such as gender ($p = 0.192$), smoking habits ($p = 0.150$), and presence of associated diseases ($p = 0.065$), there were no significant statistical differences in patients' final recovery. However, there was a significant difference with reference to patient's age (**Figure 2**). The excellent

**TABLE 5 |** Distribution of the patients in reference to age, treatment timing, nerve continuity and final recovery.

| *n* of patients = 77 | | Poor/fair | | | | Good/excellent | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | High-energy | | low-energy | | High-energy | | Low-energy | |
| | | p.c.* | i.c.** | p.c. | i.c. | p.c. | i.c. | p.c. | i.c. |
| 0–25 | <6 months | / | 2 | / | 2 | 5 | 2 | 2 | / |
| | >6 months | 1 | 4 | 2 | / | / | / | / | / |
| 26–50 | <6 months | 2 | 4 | 2 | / | 11 | 2 | 3 | / |
| | >6 months | 4 | 5 | / | / | / | / | 2 | / |
| 51–75 | <6 months | 1 | / | / | 1 | 4 | / | 12 | 1 |
| | >6 months | / | 1 | / | / | / | / | 2 | / |

*p.c., preserved continuity; **i.c., interrupted continuity.

and good results were more common in the group of patients that were aged 51–75.

As for the concern of energy of the initial trauma, there was a significant statistical difference in patients' final recovery (**Figure 3**), while, there were no significant differences regarding humerus fracture type ($p = 0.801$) and presence of other associated injuries ($p = 0.120$). The majority of patients subjected to injury by a low-energy trauma−24 (82.76%) achieved excellent or good results.

Regarding parameters such as nature of nerve injury ($p = 0.764$) and level of the nerve failure ($p = 0.982$), there were no significant differences in patient's final recovery following surgery. However, there was a significant difference with reference to continuity of the nerve (**Figure 4**). The majority of patients with the nerve preserved in continuity−41 (77.36%) achieved excellent or good results.

Regarding timing of the treatment, there was a significant difference in patients' final recovery between the groups treated earlier and groups treated later than the 6 months since the injury (**Figure 5**). The most of the patients treated earlier than the 6 months−42 (77.78%) achieved excellent or good results.

Distribution of the patients regarding age, treatment timing, nerve continuity and final recovery is presented in the **Table 5**.

## DISCUSSION

The vary fact that more than a half of radial nerve lesions treated at our clinic during the last 20 years were associated with humeral shaft fracture, indicates the importance of this particular entity.

Recently published studies (16, 18, 31, 34, 35, 37, 40), concerning the outcome in patients with radial nerve injury associated with humeral shaft fracture, have used different inclusion and exclusion criteria comparing to our study. Most of these studies included only conservatively treated patients (16, 18, 31), while some of them included only complete nerve failures (34, 35, 37), or only primary nerve injuries due to humerus fracture (18, 31). The studies that included both surgically and conservatively treated patients were mainly concentrated on determining the best indication for early nerve exploration and repair (34, 35, 40). Therefore, it was difficult to compare all these results with each other, as well as with the results of our study. However, we were able to compare the results of our study with the results of two other studies (1, 32), which have also included only surgically treated patients and have evaluated patient's final recovery using MRC muscle scale.

According to the published literature (41–44), the aging influences morphologic and functional features of the peripheral nerves, which may alter final regeneration and recovery of the nerves. However, according to our results, there was a trend of improved functional recovery with aging. Although apparently misleading, the older population is more cautious, and the trauma is usually a low-energy event (45, 46), therefore, the injury as well as eventual surgery is less extensive, and with better recovery potential. Contributing to this are the results of the study by Joseph et al. (47) which have revealed that age, as an independent factor, was not predictive of functional outcome after injury.

The most of our younger patients were subjected to injury by a high-energy trauma, which was associated with poorer final recovery. A poorer final recovery in patients subjected to injury by a high-energy trauma has also been shown in another study conducted over the same subject, and according to those authors (37) it may be caused by the extensive zone of tissue injury.

The quality of functional recovery was better in patients with the injured nerve preserved in continuity compared to the interrupted cases, which is in accordance with the results of previous studies (20, 48–50).

Regarding previous studies, concerning associated humeral shaft fractures and radial nerve injuries (1, 32, 35), in case of no indications for primary exploration, the expectant management of nerve injury followed by surgical treatment should last for 3–4 months, and the treatment should not be performed later than 5–6 months (51). We agree with these recommendations, and therefore, we treated most of our patients in the period between 3 and 6 months. We were not able to perform early exploration in all situations where it was indicated (1, 32, 35), because many of our patients lived in rural places, and it took more time for these patients to be referred to our institution, as local physicians were not always aware of recent indications for closed injuries. Regarding the 9 patients treated earlier than the 3 months since the injury, 2 of them had the nerve with clean-cut margins, which was an indication for primary nerve repair, 3 had the nerve compressed by a plate, and 4 others had an immediate radial nerve palsy following conservative treatment by other

specialists, confirmed by clinical and EMNG findings. However, some of our patients were treated later than the 6 months since the injury, which may be also due to different place of patient's residence, as well as due to different extent of patient's injury and number of other associated injuries. The cause of eventual later management of nerve injuries in the patients who lived in rural places might be due to difficulties for general practitioners to diagnose peripheral nerve injuries, and therefore it takes more time for those patients to be referred to our institution. The cause of eventual later management of nerve injuries in all patients may be due to polytrauma and delayed deployment of these patients from the institutions responsible for the care of bone fractures (1, 52).

The results of our study regarding the total rate of useful functional recovery are comparable with the results of previous studies (1, 32) that have used modified Highet's scale in order to qualitative describe patient's final recovery. Despite the fact that rate of useful functional recovery in patients with the nerve preserved in continuity was similar, the rate of useful functional recovery in patients with the nerve interrupted in continuity was lower in our study comparing to the results of aforementioned studies (1, 32). The differences in these results may be due to different length of the nerve gap, as well as due to different energy of the initial trauma.

Considering that, in their study, neither of these authors presented energy of the trauma, we emphasize the importance of presenting it and considering it as a prognostic factor that may predict patients' final recovery following surgery.

## CONCLUSION

The expectant management followed by surgery of radial nerve injury associated with humeral shaft fracture should be around 3 months, and the surgical nerve repair should not be performed later than the 6 months after injury. The energy of trauma may be a factor predicting patient's final recovery following the treatment.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Faculty of Medicine, university of Belgrade. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

All authors contributed to the preparation of this manuscript. The manuscript has been seen and approved by all authors.

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# Robot-Assisted Percutaneous Balloon Compression for Trigeminal Neuralgia: Technique Description and Short-Term Clinical Results

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**Objective:** Percutaneous balloon compression (PBC) is a minimally invasive treatment for trigeminal neuralgia (TG) with a favorable cost-effectiveness ratio, but this technique has a steep learning curve. This study presents our initial clinical experience of robot-assisted PBC using a neurosurgical robot on six consecutive patients with TG.

**Methods:** We fixed the patient's head with a skull clamp and connected it with the linkage arms of a Sinovation® neurosurgical robot, which was then registered using four bone fiducials by the robotic pointer. The puncture needle was positioned at the entry point on the skin using a robotic arm and advanced to the target point after the skin had been incised with a pointed surgical blade. This procedure was repeated for a second trajectory. A balloon was then advanced and inflated using 0.3 ml of a contrast agent. Upon injection of 0.6 ml contrast agent, the ganglion was kept compressed for 120 s. After removal of the balloon and puncture needle, compression of the face was performed to achieve hemostasis.

**Results:** All patients achieved immediate pain relief following PBC. No permanent or severe complications were registered, and there was no pain recurrence in any of the patients during the follow-up period.

**Conclusions:** Despite requiring a longer time for preoperative preparation, robot-assisted PBC provided a high degree of accuracy and safety, and it can also shorten the learning curve for surgeons unfamiliar with PBC. Robot-assisted surgical approaches should be further developed and adopted for PBC.

**Keywords: trigeminal neuralgia, percutaneous balloon compression, robotics, stereotactic neurosurgery, technique**

## INTRODUCTION

Trigeminal neuralgia (TG) is a facial pain disorder characterized by sharp and paroxysmal pain confined to areas innervated by the trigeminal nerve (1, 2). Because of the sudden and stabbing-like episodic pain that characterizes it, TG negatively affects patients' quality of life (1). Microvascular decompression (MVD) is recommended as the first therapeutic option because it provides pain relief for longer duration, but the fact that it involves major intracranial surgery makes it unsuitable for elderly or infirm patients (3). In addition, some patients are unwilling to undergo MVD or are unresponsive to surgical treatment. Alternative treatment options include percutaneous approaches such as percutaneous balloon compression (PBC), glycerol rhizolysis (GR), and radiofrequency ablation (RF) (4–7).

PBC of the gasserian ganglion, first described in 1983 by Mullan and Lichtor (8) is a minimally invasive treatment for TG that has recently regained popularity and attention because of its favorable cost-effectiveness.

Although PBC and other percutaneous procedures can be easily performed by experienced neurosurgeons with good results and considerably low complication rates, mastering of the technique involves a steep learning curve. Additionally, serious complications associated with PBC leading to severe morbidity and death have been reported (9). These complications are mostly related to needle misplacement in different foramina, incorrect foramen ovale puncture, repeated attempts at needle placement, or the use of a cannula of the wrong size. The identification of the foramen ovale using conventional monoplanar fluoroscopy may not always be adequate. In addition, natural anatomical variations encountered in 2–4% of patients may prevent the puncturing of the foramen (10). Several methods have been proposed to improve the success rate of the foramen ovale puncture, including intraoperative C-Arm, 3D-computed tomography (CT), neuronavigation, and personalized 3D-printed jig plates (11–13).

This study presents our initial clinical experience of robot-assisted PBC using a Sinovation® neurosurgical robot (Sinovation, Beijing, China).

## MATERIALS AND METHODS

### Study Population

Our study cohort consisted of six consecutive patients with TG who underwent PBC at the Clinical Neuroscience Center of the Ruijin Hospital in China between May and August 2021. All the participants provided their informed consent for inclusion in the study. TG diagnosis was verified using standard clinical criteria (presence of trigger points, neurologic examination of cranial nerve V function and presence of paroxysmal pain). Patients were also screened for drug resistance, defined as a failure in controlling pain using carbamazepine at a maximum dosage of 1,200 mg per day for 6 months.

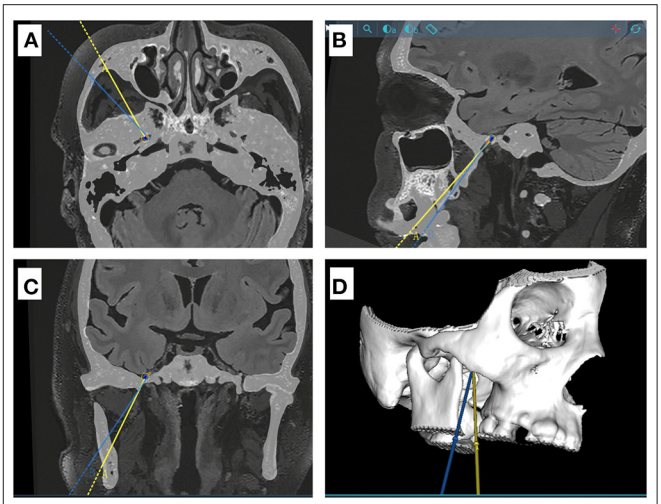


**FIGURE 1 |** Schematic diagram for two different puncture trajectories in a sample case. **(A–C)** CT and MR Flair sequence fusion images showing the projections of the two puncture trajectory A and B (yellow and blue line, respectively) in axial, sagittal and coronal positions. Trajectory B is located outside and below trajectory A. **(D)** three-dimensional model of the skull base.

### Preoperative Preparations

All patients underwent magnetic resonance scanning (United, uMR 890), which consisted of T1 magnetization-prepared rapid acquisition gradient echo, T2-weighted fluid-attenuated inversion recovery, and thin-slice head CT scanning.

We utilized stainless steel self-tapping bone-screws (Sinovation, Beijing, China) as bone fiducials. On the morning of surgery, five fiducials were screwed into the skull using a battery-powered auto driver after sterilization and infiltration of local anesthetic. We utilized the four bone fiducials for robot registration and another bone fiducial for verification. Head CT scans with 1-mm slice thicknesses, without intervals, were obtained.

### Surgical Plan

All image data were transferred to a Sinovation planning station (version 2.0.1.2; a portable computer; Sinovation, Beijing, China). Our Surgical plan included two trajectories. In trajectory A, the entry point was set on the face (2.5 cm lateral to the angle of the mouth), and the target point was set on the foramen ovale. In trajectory B, the target point was set on Meckel's cave, and the entry point was set based on the foramen ovale and Meckel's cave (**Figure 1**). The two trajectories would be optimized on the 3D view of the skull and the trajectory view based on the preoperative MR and CT images (**Figure 2**). A C-arm monoplanar fluoroscopy machine (GE, Wisconsin, USA) was set only for lateral imaging to check the correct position of the cannula and the balloon shape (**Figure 3**).

**Abbreviations:** BNI, Barrow Neurological Institute; CT, Computed tomography; DBS, Deep brain stimulation; GR, Glycerol rhizolysis; MVD, Microvascular decompression; PBC, Percutaneous balloon compression; RF, Radiofrequency ablation; SEEG, Stereoelectroencephalography; TG, Trigeminal neuralgia.

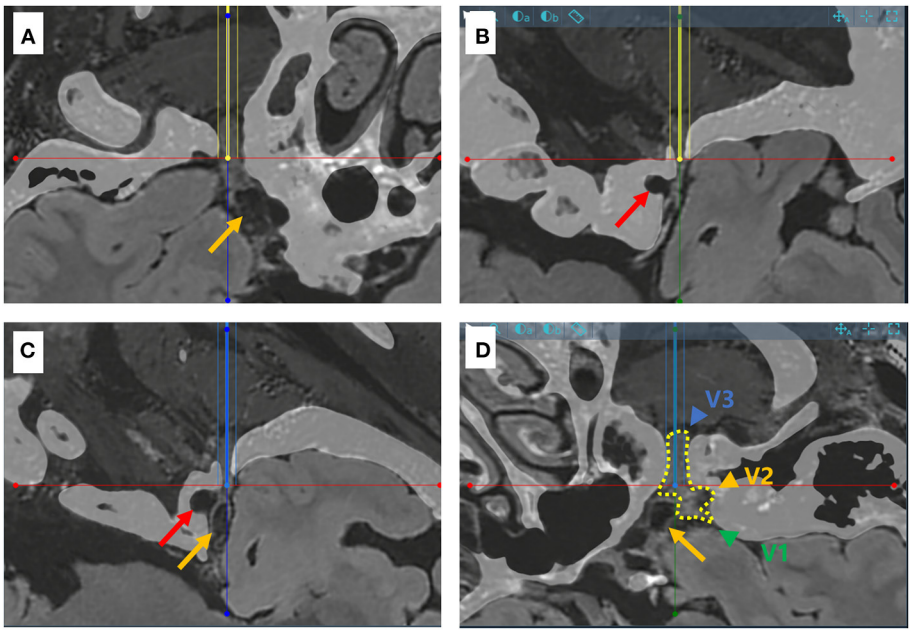


**FIGURE 2 |** Schematic diagram for the puncture targets of two different trajectories in a sample case. **(A,B)** CT and MR flair sequence fusion images showing the needle-path perspective for trajectory A (yellow line). The trajectory penetrates the foramen ovale smoothly, but not Meckel's cave (yellow arrow). The red arrow points to the internal carotid artery. **(C,D)** Needle-path perspectives for trajectory B (blue line), which effectively reaches Meckel's cave. The dotted line in yellow shows the three branches of the trigeminal nerve (V1, V2 and V3, green, represented by yellow and blue arrows respectively).

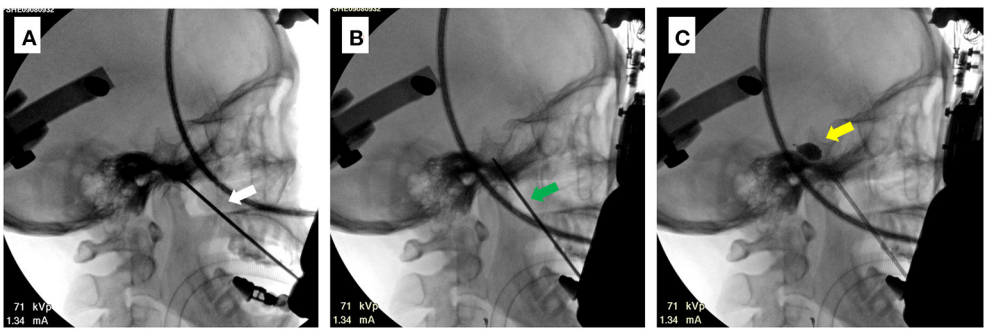


**FIGURE 3 |** Intraoperative C-arm images for a sample case (patient 6). **(A)** Image of completed trajectory A (white arrow). **(B)** Image of completed trajectory B (green arrow). The direction of trajectory can be observed to change. **(C)** Image of the balloon after injection of the contrast agent. The inflated balloon can be distinguished by its piriform appearance (yellow arrow).

## Robot-Assisted Percutaneous Balloon Compression

The surgery was performed under general anesthesia. The patient was placed in the supine position with a slight neck extension. We used the DORO QR3 skull clamp with three head fixation points to fix the patient's head and confirm that the centerline of the head is perpendicular to the C-arm monoplanar fluoroscopy machine. The skull clamp was then connected with the linkage arms of the Sinovation® neurosurgical robot. The surgical plan was uploaded to the Sinovation® neurosurgical robot. The Sinovation® neurosurgical robot was then registered using these four bone fiducials by the robotic pointer. The registration process was repeated if the registration error was above 0.3 mm. The robotic pointer was then utilized to touch the center of the fifth bone fiducial for visual verification of the registration error. Therefore, only registration errors below 0.3 mm were accepted.

Fourteen gauge puncture needles were chosen to exactly fit the size of the robotic holder (inner diameter 2.2 mm). The puncture needle was positioned at the entry point on the skin using a robotic arm. The puncture needle was advanced to the target point after the skin was incised with a pointed surgical blade. The location of the puncture needle was confirmed using an X-ray machine. The robotic arm was then adjusted

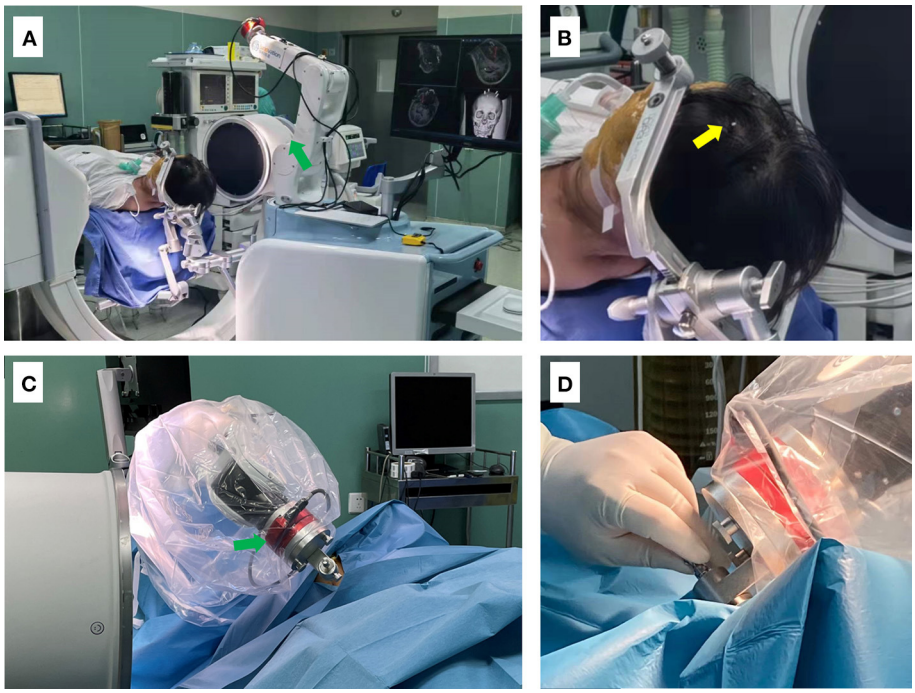


**FIGURE 4 |** Photographs depicting the setup for the surgical procedure. **(A)** The patient is shown placed in supine position after general anesthesia, with the head fixed using a DORO head frame. The C-arm was then placed in position, and the head frame was connected and fixed to the robot. **(B)** Enlarged view showing the position of bone markers (yellow arrow). **(C,D)** The robotic arm of the surgical robot (green arrow) is shown guiding and supporting the puncture needle.

to follow the second trajectory, and the puncture needle was again advanced to the target point. A fine puncture needle was then advanced, with its position confirmed by X-rays. Lidocaine was injected into the trocar to reduce cardiac responses. A balloon was then advanced, with the position of its tip confirmed by X-rays. After the balloon was inflated with a 0.3 ml contrast agent, its shape was checked. Upon injection of a total of 0.6 ml contrast agent, the balloon ended up adopting its piriform appearance. Subsequently, the ganglion was kept compressed for 120 s. After this, the balloon and the puncture needle were removed. Compression of the face was performed to achieve hemostasis, and the surgery was finally completed (**Figure 4**).

Patient data (age, sex, presence or absence of apparent neurovascular conflict, neurologic status before and after surgery, and preoperative and postoperative incidence of herpes labialis) were recorded, along with the number of puncture attempts, the number of adjustments, and surgery duration.

## Efficacy Evaluation

The postoperative follow-up was completed by nonsurgical doctors monthly, via telephone, text messages, outpatient service, or mail. Postoperative facial pain was measured using the Barrow Neurological Institute (BNI) Pain Intensity Score (14), and facial numbness was determined using the BNI facial numbness score (15).

# RESULTS

## Patient Data

A total of six consecutive patients with TG underwent frameless robot-assisted PBC at the Ruijin Hospital Luwan Branch from May to August 2021. All procedures were performed by a single surgeon (Q.Q. LIU). The mean age of the patients at surgery was 65 years (range: 59–75 years). The symptomatic period before undergoing PBC ranged from 2 to 15 years. Two patients had already undergone previous MVD surgery, and one patient had already undergone radiofrequency ablation. There were no postoperative complications such as bleeding or infection. The mean follow-up time was 4.5 (range: 3–6) months. A summary of the patients' characteristics and surgery results is presented in **Table 1**.

## Accuracy, Effectiveness, and Safety

Bone fiducials registration was successfully completed in all six patients, and the mean registration error was 0.14 mm. Twelve trajectories (trajectory A and B for each patient) were successfully reached without any block from the skull. The positions of cannulas were adequate, and pear-shaped balloons appeared in all instances after injection of 0.5–0.7 mL of contrast material.

All patients achieved immediate pain relief following PBC and were classified as having BNI Pain Intensity Score grade I. Five patients (83.3%) exhibited facial numbness and hypoesthesia, including four cases with BNI numbness score grade II and one with grade III. The sensory symptoms were transient and entirely

**TABLE 1 |** Summary of the patients' characteristics and surgery results.

| No. | Gender | Age (years) | Symptoms' duration (years) | Side | Distribution of symptoms | Previous surgery | BNI Pain Intensity Score | BNI facial numbness score | Other complications | Registration error (mm) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Female | 59 | 8 | Left | V3 | MVD | I | II | - | 0.17 |
| 2 | Male | 70 | 6 | Right | V2-3 | - | I | II | Facial muscles weakness | 0.13 |
| 3 | Female | 64 | 15 | Right | V2-3 | MVD | I | III | - | 0.14 |
| 4 | Male | 75 | 10 | Right | V3 | - | I | II | - | 0.17 |
| 5 | Female | 59 | 4 | Left | V3 | - | I | III | - | 0.09 |
| 6 | Female | 67 | 2 | Right | V3 | RF | I | II | Herpes labialis | 0.15 |
| Mean | | 65.6 | 7.5 | | | | | | | 0.142 |

**TABLE 2 |** Length and angles for the trajectories A and B.

| Patient | Direction | Trajectory A(degrees) | Trajectory B(degrees) | Arc adjustment(degrees) | Ring adjustment(degrees) | Length of trajectory A (mm) |
|---|---|---|---|---|---|---|
| 1 | Arc | 104.4 | 105.8 | −1.4 | | 73.8 |
| | Ring | 320.7 | 313.9 | | 6.8 | |
| 2 | Arc | 72.5 | 71.3 | 1.2 | | 74.5 |
| | Ring | 319.3 | 312.3 | | 7 | |
| 3 | Arc | 75.5 | 73.4 | 2.1 | | 73.2 |
| | Ring | 309.9 | 305.3 | | 4.6 | |
| 4 | Arc | 73.7 | 69.8 | 3.9 | | 79.1 |
| | Ring | 310.6 | 304.6 | | 6 | |
| 5 | Arc | 104.5 | 107.2 | −2.7 | | 72.2 |
| | Ring | 312.7 | 307.8 | | 4.9 | |
| 6 | Arc | 69.8 | 61.5 | 8.3 | | 70.8 |
| | Ring | 311.2 | 305.6 | | 5.6 | |
| mean | Arc | 16.237 | 19.5 | 3.27 | | 73.9 |
| | Ring | 314.07 | 308.25 | | 5.82 | |

resolved within 3 months following the operation. Transient masseter muscular weakness was encountered in one patient (16.7%) and entirely resolved within 2 months. No permanent or severe complications were encountered in this group of patients, including neural or vascular neural injuries, oral hematomas, diminished corneal reflexes, or infections. According to the last follow-up, no pain recurrence was reported within the relatively short follow-up period.

The average distance of the puncture route from the skin to the foramen ovale (trajectory A) for the six patients was 73.9 mm (range, 70.8–79.1 mm). Since trajectory B required pushing against the skin, we did not record the distance of this puncture route. To calculate the positional relationship between the two puncture routes at different angles, we calculated the position of the two puncture routes at the arc-angle and the ring-angle on the anterior commissure-posterior commissure plane, and then determined their positional relationship at different angles. Compared with trajectory A, trajectory B moved laterally by an average of 3.2 degrees at the arc-angle and moved downward by an average of 5.82 degrees at the ring-angle. The distance and the angles of the two trajectories are shown in **Table 2**.

## DISCUSSION

MVD is recommended as the first option for TG treatment because it has a considerably higher rate of initial pain-free outcomes and a lower rate of long-term recurrence than other percutaneous procedures. However, PBC offers the advantages of being minimally invasive, requiring a shorter operation time and being less frequently associated with serious complications. These features make PBC an attractive choice for TN treatment, especially among elderly and infirm patients (16–18).

Although rare, serious complications related to needle misplacement such as carotid cavernous fistulae and external carotid artery system fistulae, subarachnoidal hemorrhage, intraparenchymal hematoma, and blindness have been reported for PBC (9, 19, 20). Facial numbness, hypoesthesia, and mastication weakness were the most common postoperative complications. They might have been caused by repeat punctures or long compression times, and were usually resolved within weeks or a few months after surgery at the latest (16, 21). An accurate surgical puncture may therefore improve the surgical outcome and reduce complications (21).

Based on our experience, the PBC surgical technique is focused on two aspects: foramen ovale puncture and Meckel's cave puncture. PBC consists of three sequential stages, each with specific steps: (1) foramen ovale insertion, (2) Meckel's cave cannulation, and (3) compression of the retroganglionic rootlets.

Several authors have introduced various methods to improve the success rate and the speed of foramen ovale puncture. At present, most surgical units perform the surgery using C-arm fluoroscopy, but observation or identification of the foramen ovale using this method is still a challenge. The difficulty in the identification of the foramen ovale is even more pronounced in patients with anatomic variations at the skull base. Therefore, some authors have proposed the use intraoperative 3D CT-guided puncture to improve the success rate of foramen ovale puncture, especially in patients with a narrow foramen (12, 22, 23). This method can improve the accuracy of the puncture, but multiple puncture attempts and adjustments are still required, and the patient needs to undergo multiple CT scans. Neuronavigation was also used to achieve accuracy during puncturing, but this approach requires the surgeon to monitor real-time videos, and registration duration increases while accuracy decreases when compared with the use of robots. A personalized 3D-printed jig plate could be an effective tool for rapid foramen ovale puncture, with a duration of around 1 min (11). However, the angle and depth of the puncture needle still need to be manually adjusted and controlled to access Meckel's cave.

Meckel's cave is located in most cases above the foramen ovale in the line that connects the puncture entry point in the facial region and the foramen ovale. The tail end of the puncture needle needs to be pressed downwards and the needle further advanced by ∼1 cm to access Meckel's cave (24). This represents a second challenge for the surgeon, since mistakes can lead to puncture failure or damage to the nerves, blood vessels, or the cavernous sinus. Excessive penetration of the puncture needle might also cause damages to the brain stem, especially in patients with a broad foramen ovale. None of the previously mentioned approaches allows for accurate control of the depth and direction of the puncture needle. Therefore, the surgeon has to rely on surgical experience and anatomical knowledge to tackle these challenges, as well as attempt the puncturing multiple times (13).

Our study used robot-assisted technology to navigate the challenges described above. This technology was confirmed to be safe and effective when applied in the context of stereoelectroencephalography (SEEG) implantation, deep brain stimulation, and other neurosurgical operations. Its planning station can design the puncture trajectory preoperatively to accurately locate and puncture the foramen ovale and Meckel's cave, while its robotic arm can accurately control the depth and direction of the puncture needle.

We designed trajectories A and B for foramen ovale puncture and Meckel's cave puncture, respectively. Trajectory B with the target point set on Meckel's cave was created because the tip of the puncture needle would touch the temporal bone instead of reaching Meckel's cave if trajectory A was used for this purpose. Previous studies suggested that the tip of the puncture needle needs to be adjusted when advanced from the foramen ovale to Meckel's cave, but no specific data was provided (24, 25). Our study shows that the tail of the puncture needle had to be moved laterally by an average of 3.2 degrees at the arc-angle and moved downward by an average of 5.82 degrees at the ring-angle after the needle has reached the foramen ovale. When accessing Meckel's cave, the puncture needle had to be moved laterally to the medial line by 19.5 degrees at the arc-angle and by 308.25 degrees at the ring-angle. Despite the small sample size, these findings might already provide some guidance to surgeons attempting the procedure.

In this study, the target points were successfully accessed after adjusting once in all six patients, and no other adjustments were performed. Previous studies did not mention the number of puncture attempts required when the puncture needle was advanced from the foramen ovale to Meckel's cave. Our approach minimizes the risk of potential complications triggered by unsuccessful puncture attempts and reduces the patient's exposure time to radiation. Trajectory B could be adopted directly, but since the entry point is too low, there is a risk of penetration of the oral cavity by the needle.

The registration error was only 0.14 mm during the operation, which provides a firm guarantee for accurate puncture. Needle entry was not blocked by the bone in any puncture route, which suggests that the robotic arm followed the exact planned puncture route during the operation. Since intraoperative CT was not used, the accuracy in target point puncture cannot be verified. However, given the accuracy of previous electrode implantations, the probability of puncture needle misplacement is very low.

## Learning Curve

Although experienced neurosurgeons can easily perform PBC with good results and low chances of complications, the technique has a steep learning curve. Intraoperative CT, neuronavigation, and the use of a 3D-printed jig plate in the initial stages of learning have been reported to be useful during training (11, 13). In our study, the surgeon had previously performed SEEG and deep brain stimulation (DBS) electrode implantation in thousands of cases, but he had no previous experience performing PBC. We successfully achieved effective compression thanks to the accuracy provided by the robotic equipment and the visualization of the surgical trajectories. Our approach can therefore help many surgeons who are not familiar with PBC. The visualized design of the puncture routes, in particular, can improve the surgeon's anatomical understanding of the foramen ovale and Meckel's cave.

## Limitations

Some limitations of the present study are the small sample size and the relatively short duration of the follow-up periods. We are collecting more cases at present. Robot-assisted surgeries require longer preparation time and fixation of the patient's head with a skull clamp. To reduce scalp injuries caused by

bone screws, we are attempting the use of structured light 3D scanning for registration. In addition, we are also attempting to reduce the duration of the surgery by using a single puncture route.

## CONCLUSIONS

PBC is an effective and minimally invasive treatment for TG. This study proposes a novel surgical approach for PBC. Despite requiring a longer time for preoperative preparation than previous approaches involving direct puncture, our approach provides a high degree of accuracy and safety. Robot-assisted PBC can also shorten the learning curve substantially, especially for surgeons unfamiliar with PBC. Robots have been widely deployed for SEEG and DBS electrode implantations; likewise, robot-assisted surgical approaches should be further developed and widely adopted in PBC.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ruijin Hospital Luwan Branch Ethics Committee, Shanghai JiaoTong University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

QL: conceptualization, methodology, and funding acquisition. JW: writing - original draft and investigation. CW: software and investigation. WenzeC: writing - original draft and software. WenzhC: data curation and writing - original draft. XY: data curation and writing - original draft. ZM: data curation. CZ: writing - review & editing. JX: supervision and writing - review & editing. All authors contributed to the article and approved the submitted version.

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# Electrospun Polycaprolactone (PCL)-Amnion Nanofibrous Membrane Promotes Nerve Regeneration and Prevents Fibrosis in a Rat Sciatic Nerve Transection Model

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Functional recovery after peripheral nerve injury repair is typically unsatisfactory. An anastomotically poor microenvironment and scarring at the repair site are important factors impeding nerve regeneration. In this study, an electrospun poly-e-caprolactone (PCL)-amnion nanofibrous membrane comprising an amnion membrane and nonwoven electrospun PCL was used to wrap the sciatic nerve repair site in the rat model of a sciatic nerve transection. The effect of the PCL-amnion nanofibrous membrane on improving nerve regeneration and preventing scarring at the repair site was evaluated by expression of the inflammatory cytokine, sciatic functional index (SFI), electrophysiology, and histological analyses. Four weeks after repair, the degree of nerve adhesion, collagen deposition, and intraneural macrophage invasion of the PCL-amnion nanofibrous membrane group were significantly decreased compared with those of the Control group. Moreover, the PCL-amnion nanofibrous membrane decreased the expression of pro-inflammatory cytokines such as interleukin(IL)-6, Tumor Necrosis Factor(TNF)-a and the number of pro-inflammatory M1 macrophages, and increased the expression of anti-inflammatory cytokine such as IL-10, IL-13 and anti-inflammatory M2 macrophages. At 16 weeks, the PCL-amnion nanofibrous membrane improved functional recovery, including promoting nerve Schwann cell proliferation, axon regeneration, and reducing the time of muscle denervation. In summary, the PCL-amnion nanofibrous membrane effectively improved nerve regeneration and prevent fibrosis after nerve repair, which has good clinical application prospect for tissue repair.

**Keywords: nerve repair, amniotic membrane, poly-e-caprolactone, nerve regeneration, fibrosis, macrophage polarization**

## INTRODUCTION

Peripheral nerve injury caused by trauma is a very common clinical disease (1) and the main repair method is direct end-to-end repair by suturing the epineurium (2). However, complete recovery of nerve function is rarely achieved. Peripheral nerve injury may result in partial loss of sensory and motor functions, muscle atrophy, and in severe cases, poor limb function and complete paralysis, reducing the patient's quality of life and resulting in serious social and economic consequences (3, 4). Peripheral nerve regeneration is affected by many factors such as perineural and intraneural scarring, surgical suture method, and age (5, 6). Perineural and intraneural scarring at the repair site are important factors affecting axon regeneration, which reduce the growth rate of axons and limits the number of regrowing axons (7, 8). Therefore, inhibiting scarring proliferation at the repair site is an important measure for nerve function recovery (9, 10), which may allow the target muscle to regain innervation as soon as possible and avoid neuromuscular joint degeneration and muscle atrophy.

Drugs, autologous tissue, natural materials, and synthetic materials are currently used to wrap the nerve repair site, which act as physical barriers between the nerve repair site and surrounding tissues, reducing scarring proliferation and limiting intraneural scarring (11–14). All of these measures have had some success. However, drugs have disadvantages of fast biological absorption and short temporary barrier function. Autologous tissue is limited by insufficient donor sites and scope, and secondary injury to the donor (15). Natural or synthetic materials either have a strong inflammatory response, degrade quickly, or lack support strength and other disadvantages (16, 17). Therefore, the desired materials are biodegradable, with similar structure and function as a natural extracellular matrix, to support cell growth, guide tissue regeneration, and control active factor release to allow for neural sliding. These materials create a microenvironment that promotes axon regeneration and limits scarring formation at the nerve repair site.

An amniotic membrane is a thin membranous structure without blood vessels, lymph cells, or nerves, which has low immunogenicity, a variety of cytokines, and a large amount of collagen and elastin (18–20). An amniotic membrane has anti-inflammatory and anti-fibrosis properties (21), and its unique structure makes it an ideal choice for a nerve repair material. However, an amniotic membrane has a fast degradation rate, poor extrusion resistance, and low mechanical strength (22) that does not provide sufficient space for nerve regeneration. There are measures to improve the structure and properties of the amniotic membrane and overcome congenital defects in the amniotic membrane. Polycaprolactone (PCL) is a non-toxic biodegradable polyester with good histocompatibility and a long degradation time (23, 24). PCL has been used in skin reconstruction, bone tissue engineering, medicinal scaffolds, and medicinal membranes to prevent tendon adhesion (25–27). Electrospinning technology can continuously prepare nanofibers with the unique advantages of a biomimetic natural extracellular matrix. Nanofibers can greatly promote axon regeneration and prevent scarring formation (28).

In order to reduce adhesion and promote nerve repair following peripheral nerve injury surgery, we have invented a novel biomaterial named as PCL-amnion nanofibrous membrane. Our previous study demonstrated that electrospun PCL-amnion nanofibrous membranes obviously alleviate tissue adhesion following neural surgery and accelerate nerve regeneration in a rat model of sciatic nerve compression in order to determine whether this new type of biomaterial can exert beneficial effects in chronic nerve injury animal model (29). In the current study, we explore the effect of PCL-amnion nanofibrous membrane on nerve regeneration and scarring formation at the nerve repair for the recovery of nerve function in a rat sciatic nerve transection model in order to determine whether this new type of biomaterial can exert beneficial effects in acute nerve injury animal model.

## MATERIALS AND METHODS

### Preparation of the Electrospun PCL-Amnion Nanofibrous Membrane

According to the Declaration of Helsinki and approval from the medical ethics committee of the Third Hospital of Hebei Medical University, an amniotic membrane was provided by the Department of Obstetrics and Gynecology from June 1st of 2021 to June 30th of 2021. After written consent was signed, fresh amniotic membrane was harvested from healthy pregnant women that underwent Cesarean section. The fresh amniotic membrane was immediately washed with physiological saline and laid flat on a nitrocellulose membrane punch with the amniotic epithelial cells directed upwards. The amniotic membrane was prefrozen at −50 to 0°C for 1–2 h, and cooled in a condensation chamber with a cold trap temperature reaching−50 to −30°C. After the vacuum pump was operated for 6–8 h, the freeze-dried amniotic membrane was prepared. One gram PCL (average Mn-80,000; Sigma-Aldrich, USA) and 0.5 g gelatin (Porcine skin, Vetec reagent grade, Sigma-Aldrich, USA) were completely dissolved in 10 ml hexafluoroisopropanol (>99.5%, Shanghai Nortel Co., Ltd.) by electro-magnetic stirring, defoaming, and stirring using a magnetic bar for 15 h. The operating temperature was 25°C and the humidity was 60%; the polymer solution was placed in a 2 ml syringe pump with a blunt needle diameter of 0.7 mm. The solution was delivered at flow rate of Q = 1.0 mL/h and a voltage of 13 kV. As the solution was ejected from the needle, charged PCL nanofibers traversed a distance of 15 cm and were deposited on the two surfaces of the freeze-dried amnions. The PCL-amnion nanofibrous membranes were dried overnight in a vacuum (29). The PCL-amnion nanofibrous membranes were sterilized by cobalt 60 irradiation before use (30).

### Animals

Adult male Sprague–Dawley rats weighing 200–250 g were provided by Hebei Iviwo Biological Technology Co., LTD. The study was conducted under all protocols approved by the Animal Research Ethical Committee of the Third Hospital of Hebei Medical University and in line with the guidelines for animal care and use (Approval number: Z2021 - 008 - 1).

The rats were housed in a 12 h light/dark cycle at a temperature of 22.1°C and 65–70% humidity. Food and water were available *ad libitum*. All the rats were acclimated to the environment for 1 week prior to surgical procedures. All surgery was performed under general anesthesia with sodium pentobarbital (3%), and all efforts were made to ameliorate animal suffering.

## Experimental Design

A total of 64 rats were randomly divided into PCL-amnion nanofibrous membrane group ($n = 32$) and a Control group ($n = 32$). Two time points (4 and 16 weeks) were used to assess the nerve regeneration. The inflammation and nerve regeneration were examined at 4 weeks. At 4 weeks, forty rats were used for histology (twenty-four, $n = 12$ per group) and qRT-PCR (sixteen, $n = 8$ per group). At 16 weeks, functional nerve recovery was examined in 24 rats. Rats were used for sciatic function index (SFI) and nerve electrophysiological assessment. The CMAP amplitude and latency of the sciatic nerve were measured with a myoelectricity-evoked potential apparatus (Viking Quest, Nicolet, US). The rats were then used for histology ($n = 12$ per group).

## Surgical Procedure

General anesthesia was achieved by intraperitoneal injection of 30 mg/kg pentobarbital sodium for all surgery. The hair of the left thigh was clipped and the surgical area was scrubbed with 2% iodophor. A 3.0-cm long incision was made in the dorsal skin of the left thigh along the femur. The muscles were split and the sciatic nerve was exposed. The sciatic nerve was transversely cut 15 mm distally from the sciatic notch. In the Control group, the proximal and distal nerve stumps were reconnected using 10–0 Prolene epineurial sutures. In the PCL-amnion nanofibrous membrane group, the repaired site was wrapped with the PCL-amnion nanofibrous membrane after the epineurial sutures. The PCL-amnion nanofibrous membrane overlapped each nerve stump by 5 mm. The diameter of the wraps was larger than that of the sciatic nerve to avoid compressing the nerve. After the operation, rats were kept warm and protected from moisture. The rats were fed separately in a single cage to ensure adequate food and water. The incision was disinfected with iodophor twice a day for three consecutive days. The rats were monitored for plantar ulcers or autophagy.

## Early Measurement of Nerve Regeneration (4 Week Time Point)

At 4 weeks, the rats were euthanized by intraperitoneal injection with sodium pentobarbital (150 mg/kg), and the sciatic nerve was carefully re-exposed and systematically assessed for adhesions scored according to Petersen et al. (31). The inflammation at the repair site was evaluated using histologic, immunofluorescence, and molecular analysis. In addition, the nerve regeneration was assessed using histomorphometric analysis. At 4 weeks, the total RNA from the 15-mm long sciatic nerve centered on the repaired site of eight rats in each group were extracted using TRIzol eagent according to the product description.

### Macrophage Immunofluorescence Assessment

Immunofluorescence staining was performed on 4 μm nerve sections for quantifying the number of macrophages in the nerve repair site and their specific phenotype (M1/M2). After antigen repair, the sections were incubated with diluted normal goat serum for 30 min. The sections were incubated in a humidified chamber at 4°C for 15 h with CD68 monoclonal antibody (Invitrogen, MA5-13324) at a dilution of 1:100, CD206 (Proteintech, 18704-1-AP) at a dilution of 1:100, and iNOS (Bioworld, BS1186) at a dilution of 1:200. The sections were washed in PBS buffer three times and incubated with either a FITC-labeled sheep anti-rabbit IgG (Beyotime, P0186) diluted 1:200 or a Cy3-labeled sheep anti-mouse IgG (Beyotime, A0521) diluted 1:500 in a humidified chamber for 1 h at room temperature. DAPI was dropped on the sections to stain the nuclei for 5 min. Finally, PBS buffer was used to wash the sections four times to remove excessive DAPI. Fluorescence microscopy (Olympus BX53) was used to calculate the number of positive cells per $mm^2$ of tissue area. The average number of positive cells for each nerve was expressed as mean ± SD.

### Masson Staining

To assess collagen deposition at the nerve repair site, Masson staining was performed on the 10 μm nerve sections. The sections were fully dewaxed and soaked in distilled water for 2 min. After washing with tap water for 3–5 min, the sections were stained with Weigert's iron hematoxylin solution for 5–7 min, then washed with running water for 5 min followed by distilled water. The sections were then stained with Biebrich scarlet acid fuchsin solution for 5 min, followed by distilled water. The sections were then stained with phosphotungstic/phosphomolybdic acid for 10 min and aniline blue for 5 min. After washing with distilled water, the sections were washed with 1% acetic acid for 1 min. The sections were then dehydrated with 95% and absolute alcohols. The sections were taken from the nerve at the repair site. An image analysis system (Image J, Nation Institutes of Health) was used to calculate the area of intraneural collagen staining. The intraneural collagen level for each nerve was expressed as mean ± SD.

### Quantitative RT-PCR

The total RNA from nerve tissue (15 mm sciatic nerve centered on the repaired site) was extracted using TRIzol reagent (Tiagen Biochemical Technology, Beijing, China) according to the product description. The concentration of RNA was measured using a Nanodrop 2000 spectrophotometer (NanoDrop Products, Wilmington, DE). cDNA was generated from RNA using Takara PrimeScript RT reagent kit (Bao Biological Engineering, Dalian, China) per the manufacturer's instructions. The PCR reaction was carried out in the PCR amplification instrument by pre-denaturation at 95°C for 30 s, followed by 40 cycles of denaturing at 95°C for 5 s, and annealing/extending at 60°C for 34 s. Relative gene expression data were analyzed using $2^{-\Delta\Delta CT}$. The genes and related specific primers are represented in **Table 1**.

**TABLE 1 |** Primer sequences.

| Primer name | Sequence 5'-3' | Product length |
|---|---|---|
| r-IL6-F | AGAGACTTCCAGCCAGTTGC | 126bp |
| r-IL6-R | CCTCCGACTTGTGAAGTGGT | |
| r-TNF-α-F | AGACCCTCACACTCAGATCATCTTC | 193bp |
| r-TNF-α-R | CTCCGCTTGGTGGTTTGCTA | |
| r-IL10-F | GCAAAGAGAACGCGTGGAAC | 143bp |
| r-IL10-R | GTTCGGTCGGAATAGGTCGG | |
| R-IL13-F | CTCTCGCTTGCCTTGGTGGT | 165bp |
| R-IL13-R | CAGCTGTCAGGTCCACGCTC | |
| r-ACTIN-F | AAGTGCGACGTGGACATCCG | 109bp |
| r-ACTIN-R | GGGCGGTGATCTCCTTCTGC | |

### Assessment of Nerve Histomorphometry

Nerve removed 10 mm distally from the repair site were used to assess the regenerating nerve fibers. The nerve specimens were perfused with 4% paraformaldehyde, then washed with phosphate buffer, fixed in 1% osmium tetroxide, dehydrated with ethyl alcohol, and finally embedded in epon. The nerve was sliced into thin sections (thickness: 4 μm) and stained with toluidine blue. An image analysis system (Image J, Nation Institutes of Health) was used to evaluate the entire nerve cross-section (32). Morphometric measurements of the sciatic nerve included: (1) average axon density (N/mm$^2$), (2) average fiber diameter (μm), and (3) average myelin sheath thickness (μm). All values were expressed as mean ± SD.

## Late Measurement of Nerve Regeneration (16 Week Time Point)

Functional nerve recovery was assessed by SFI at 4, 8, 12, and 16 weeks and electrophysiological analyses at 12 and 16 weeks. At 16 weeks, the rats were euthanized by intraperitoneal injection with sodium pentobarbital (150 mg/kg), and the degree of the gastrocnemius muscle atrophy and nerve regeneration was evaluated.

### Sciatic Functional Index

The gastrocnemius muscle functional recovery was assessed by the SFI at 4, 8, 12, and 16 weeks (33). Prior to operation, rats were trained to walk through a dark 100 × 7 cm closed box. Before walking through the dark box, a paper was placed on the bottom of the box. The rat's rear paws were dipped with ink and measurements of the rear paws print were made when the rats walked on the box. The following formula was used to calculate the SFI:

$$SFI = \frac{-38.3 \times (EPL - NPL)}{NPL} + \frac{109.5 \times (ETS - NTS)}{NTS} + \frac{13.3 \times (EIT - NIT)}{NIT} - 8.8 \quad (1)$$

The paw length (PL) is the length between the top of the third toe to the heel. The toe spread (TS) is the length between the first to the fifth digit. The intermediary toe spread (IT) is the length between the second and fourth digit. The PL, TS, and IT were measured for both the non-operated (N; "normal") and operated (E; "experimental") sides. A SFI value closer to 0 indicates better recovery and a SFI value of −100 indicates complete damage.

### Electrophysiology

To follow the sciatic nerve functional recovery, the CMAP amplitude and latency of the sciatic nerve in each group were measured with a myoelectricity-evoked potential apparatus (Viking Quest, Nicolet, US) at 12 and 16 weeks. The stimulating needle electrode was placed on the proximal end of the sciatic nerve. The recording electrode was placed on the abductor hallucis plantar. The CMAP amplitude and latency were recorded with the stimulating mode (stimulus intensity 40 mV, frequency 1 Hz, duration 0.2 ms). The normal CMAP amplitude and latency were obtained from the contralateral limb. All values were expressed as mean ± SD.

### Muscle Atrophy Assessment

The gastrocnemius muscle was assessed grossly and histologically. After the rats were euthanized, the right (non-operated side) and left (operated side) gastrocnemius muscles were carefully resected. Muscle blood was absorbed and the gastrocnemius muscle weight was recorded. The wet weight recovery ratio was calculated as (gastrocnemius muscle wet weight on the operated side/ gastrocnemius muscle wet weight on the non-operated side) × 100% (34).

Histological analysis was performed for a morphological assessment of gastrocnemius muscle atrophy. The sections taken from the mid-portion of the gastrocnemius muscles were used for morphological assessment. After antigen repair, endogenous peroxidase was blocked by 3% hydrogen peroxide for 20 min. The sections were incubated with normal goat serum for 30 min. The sections incubated in a humidified chamber at 4°C for 15 h with laminin antibody (Bioss, bs-0821R) at a dilution of 1:200. The sections were washed in PBS buffer three times and incubated with a FITC-labeled sheep anti-rabbit IgG (Beyotime, P0186) diluted 1:500. DAPI was dropped on the sections and the nuclei was stained for 5 min. Finally, PBS buffer was used to washed the sections four times to remove excess DAPI. Image J software was used to the average single-muscle fiber cross-sectional area (CSA). Morphometric measurements included: (1) the average single-muscle fiber cross-sectional area (μm $^2$) and (2) the single muscle fiber cross-section area distribution. All values were expressed as mean ± SD.

### Assessment of Nerve Histomorphometry

Sixteen weeks post-surgery, the nerve tissues at the repair site were fixed in 3% glutaraldehyde. The nerve tissues was fixed in 1% osmium tetraoxide solution and then embedded in epoxy resin. Transverse ultra-thin sections were cut from the samples. Sections were stained with 3% uranium acetate and 0.1% lead citrate. Finally, sections were examined with a transmission electron microscope (TEM). Morphometric measurements of the sciatic nerve included: (1) average axon density (N/mm$^2$), (2) average fiber diameter (μm), and (3) average myelin sheath thickness (μm). All values were expressed as mean ± SD.

## Statistics

All values were expressed as mean ± SD and analyzed by SPSS Statistics, version 21.0 software. For multiple group comparisons, one-way ANOVA was used. When significant differences were detected, the SNK-q test was used for pairwise comparisons. The *T*-test was used to compared two groups. $P < 0.05$ was considered significant.

# RESULTS

## PCL-Amnion Nanofibrous Membrane vs. Conventional Epineurial Repair, 4 Weeks

### Gross Examination of the Repair Site

At 4 weeks postoperative, loose adhesions and very little tissue were found around the sciatic nerve in the PCL-amnion nanofibrous membrane group. The surface of the nanofibrous membrane remained smooth (**Figure 1A**). In contrast, extensive and dense adhesions that required more blunt separation were found around the sciatic nerve in the Control group (**Figure 1B**). The scores of the PCL-amnion nanofibrous membrane group were less than those of the Control group ($P < 0.05$) (**Figure 1C**).

### Inflammatory Reaction at the Repair Site

To evaluate the inflammatory response at the nerve repaired site, ED-1 macrophages were labeled with the anti-CD68 antibody (**Figures 2A,B**). The number of intraneural macrophages (per $mm^2$) in the PCL-amnion nanofibrous membrane group (mean, 2,189 ± 464) was significantly less than in the Control group (mean, 4,441 ± 1,194), ($P < 0.05$) (**Figure 2C**), indicating that the PCL-amnion nanofibrous membrane inhibited the inflammatory response at the repair site. In addition, macrophages were classified as M1 or M2 by phenotypic characterization. M1 macrophages were labeled with anti-iNOS antibody and M2 macrophages were labeled with anti-CD206 antibody. The number of M1 macrophages (per $mm^2$) (mean, 3,611 ± 1,182) was significantly greater than M2 macrophages (per $mm^2$) (mean, 1,004 ± 204) in the Control group ($P < 0.05$). However, the number of M2 macrophages (per $mm^2$) (mean, 1430 ± 232) was significantly greater than M1 macrophages (per $mm^2$) (mean, 437 ± 53) in the PCL-amnion nanofibrous membrane group ($P < 0.05$) (**Figures 2D–I**).

The amount of collagen at the sciatic nerve repaired site is shown in **Figure 3**. The mean percentage of intraneural collagen staining (mean, 17.3 ± 1.8%) (**Figure 3A**) in the PCL-amnion nanofibrous membrane group was significantly less than in the Control group (mean, 28.3 ± 2.1%, **Figure 3B**) ($P < 0.05$) (**Figure 3C**).

By analyzing the relative content of cytokine gene expression, the expression of the pro-inflammatory cytokine IL-6 and TNF-α significantly decreased at the repair site in the PCL-amnion nanofibrous membrane group ($P < 0.05$ for each cytokine). Moreover, the expression of the anti-inflammatory cytokines IL-10 and IL-13 significantly increased at the repair site in the PCL-amnion nanofibrous membrane group ($P < 0.05$ for both cytokines) (**Figure 3D**).

### Early Evaluation of Nerve Regeneration

In the PCL-amnion nanofibrous membrane group, the average density of myelinated axons was significantly greater than that of the Control group (per $mm^2$) (25,864 ± 4,195 vs. 15,555 ± 2,796, $P < 0.05$). Furthermore, the average fiber diameter was significantly greater in the PCL-amnion nanofibrous membrane group in comparison with that in the Control group (3.0 ± 2.5 vs. 2.4 ± 2.1 μm, $P < 0.05$). The average myelin sheath thickness was significantly greater in the PCL-amnion nanofibrous membrane group than that in the control group (0.50 ± 0.22 vs. 0.33 ± 0.10 μm, $P < 0.05$) (**Figures 4A–E**).

## PCL-Amnion Nanofibrous Membrane vs. Conventional Epineurial Repair, 16 Weeks

### Sciatic Function Index

The SFI was used to evaluate the function recovery of the gastrocnemius muscle, starting at 4 weeks after never repair. The footprint of the two groups was convenient to calculate SFI. The SFI of each group was similar 4 weeks postoperative ($P > 0.05$). However, at 8, 12, and 16 weeks postoperative, the SFI of the PCL-amnion nanofibrous membrane group was significantly greater than that in the Control group (8 weeks: −68.10 ± 4.07 vs. −73.28 ± 4.78, $P < 0.05$; 12 weeks: −56.05 ± 3.62 vs. −60.74 ± 4.38, $P < 0.05$; 16 weeks: −49.68 ± 3.53 vs. −53.36 ± 2.32, $P < 0.05$) (**Figure 5**).

### Electrophysiology

To follow sciatic functional recovery, the motor amplitude and latency of the sciatic nerve were measured at 12 and 16 weeks after never repair. The motor amplitude in the PCL-amnion nanofibrous membrane group was always significantly greater than that in the Control group ($P < 0.05$) (**Figure 6A**). At 12 weeks, the motor latency in the PCL-amnion nanofibrous membrane group was significantly less than that in the Control group ($P < 0.05$). Although no significance difference at 16 weeks ($P > 0.05$), the motor latency in the PCL-amnion nanofibrous membrane group was less than that in the Control group (**Figure 6B**). Therefore, the number of mature nerve fibers re-innervating the target muscle was larger in the PCL-amnion nanofibrous membrane group than in the Control group.

### Muscle Atrophy

The wet weight recovery ratio of gastrocnemius muscle in the PCL-amnion nanofibrous membrane group was 60.52 ± 3.09% and 47.09 ± 4.05% in the Control group ($P < 0.05$) (**Figure 7A**).

The anti-laminin antibody was used to delimit the muscle fibers. The average single-fiber CSA was greater in the PCL-amnion nanofibrous membrane group (3,924 ± 1,243 vs. 3,265 ± 1,110 $\mu m^2$, $P < 0.05$) (**Figures 7B–D**). The frequency distribution of muscle fibers was plotted by single-fiber CSA (**Figure 7E**). The histogram of the single-fiber area shifted to the right in the PCL-amnion nanofibrous membrane group.

### Morphometric Measures of Late Nerve Regeneration

Sixteen weeks after nerve repair, the average density of myelinated axons was significantly greater than that of the Control group (per $mm^2$) (20,627 ± 3,629 vs. 16,334 ± 1,963,

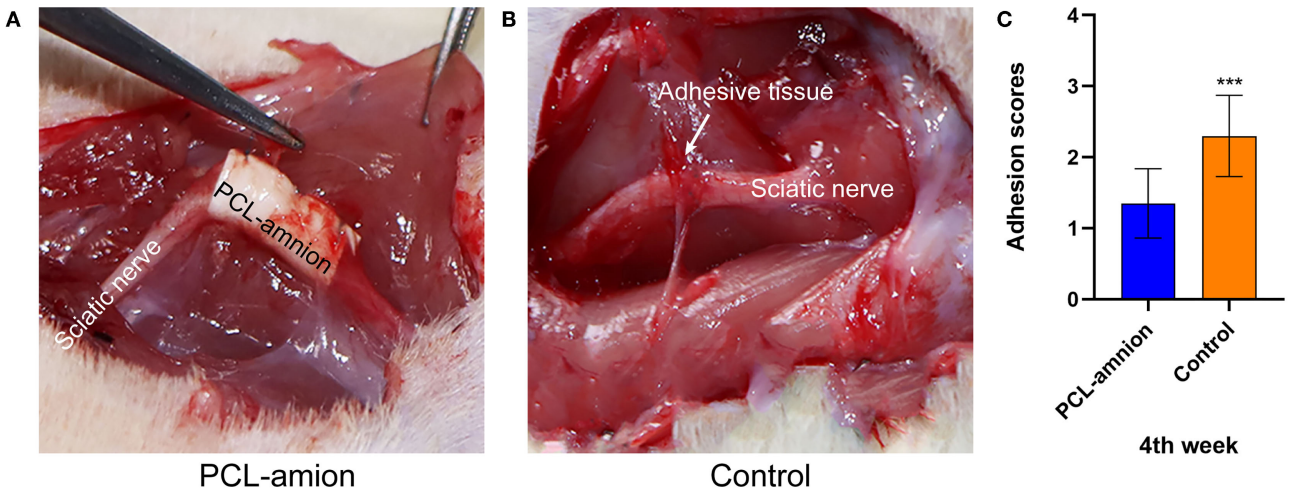


**FIGURE 1 |** Adhesion assessment of the two groups at four weeks. **(A)** The PCL-amnion nanofibrous membrane group. **(B)** The Control group. **(C)** Adhesion scores of each group at each time point ($P < 0.05$). ***$P < 0.001$ compared to the PCL-amnion group.

$P < 0.05$). Furthermore, the average diameter and myelin sheath thickness of the myelinated nerve fibers were significantly greater in the PCL-amnion nanofibrous membrane group than the Control group (3.3 ± 0.7 vs. 2.9 ± 0.7 μm, $P < 0.05$; and 0.78 ± 0.29 vs. 0.50 ± 0.21 μm, $P < 0.05$) (**Figures 8A–E**). In the PCL-amnion nanofibrous membrane group, compared to the control group, nerve fibers were more orderly arranged, the myelin lamina was thicker and denser, and the ultrastructure of microtubules and microfilaments was more regular.

## DISCUSSION

The end-to-end epineurial suture remains the standard surgical approach for neurotmesis. In previous studies, researchers mainly studied how to promote nerve regeneration, ignoring various inhibiting factors in nerve repair, such as scarring formation and adhesion at the nerve repair site (35–37). During peripheral nerve injury repair, nerve scarring obstructs the passage of axon regeneration and compresses nerves by adhesion between nerve and surrounding tissue, which reduces the nerve blood supply and hinders nerve regeneration and functional recovery (38, 39). Although rapidly developing micro-surgical techniques promote the recovery of nerve function, some degree of scarring that still exists in the nerve repair site limits the recovery of nerve function (40). Previous studies found that nerve regeneration required an appropriate micro-environment, which promotes nerve regeneration and reduces scarring formation (41).

The amniotic membrane, as a nerve conduit, can promote nerve regeneration and prevent scarring formation (42, 43). The possible mechanism is as follows: the amniotic membrane acts as a barrier function to prevent scarring proliferation and the invasion of inflammatory cells and fibroblasts (22). A closed regeneration chamber can be formed to avoid the loss of nerve factors secreted by nerve injury sites and provide a favorable micro-environment for nerve regeneration, which directionally directs the regenerated axons to distal target organs. An amniotic membrane contains laminin and fibronectin for promoting axon regeneration (44). An amniotic matrix inhibits tissue fibrosis. The amniotic epithelial cells were inactivated and had low immunogenicity by freeze-drying the amniotic membrane. Hua et al. (45) implanted freeze-dried amniotic membranes with amniotic epithelial cells into animals, and found that freeze-dried amniotic membranes showed low immunogenicity which was similar to immune pardoned tissues. An amniotic membrane and Human Amniotic Epithelial Stem Cells (hAESCs) were implanted into an immunotype-mismatched human subcutaneous pocket, and an amniotic membrane and hAESCs survived in the host for a long time without displaying any infiltration or rejection of host immune cells, indicating that amnion-derived cells, including hAESCs, had low immunogenicity (46). However, an amniotic membrane has weak mechanical strength, rapid degradation, and absorption (22). In order to overcome the shortages of amniotic membrane, we have invented a novel type of biomaterial, PCL-amnion nanofibrous membrane, to reduce prevent scarring proliferation and promote nerve regeneration.

Studies have confirmed that macrophages and cytokines play key roles in peripheral nerve regeneration (47–49). At an early stage of nerve repair (1–10 days), M1 macrophages are mainly activated at the nerve injury site, which secretes pro-inflammatory cytokines and molecules inducing tissue damage and condition aggravation (50). However, at the late stage of nerve repair (15–30 days), M2 macrophages are mainly activated, which inhibit the inflammatory response and promote nerve regeneration (51). In this study, we made a new neural scaffold to create a temporary micro-environment for nerve regeneration, and the ordered nano-scaffold was conducive to the activation of macrophages into M2. At 4 weeks, macrophages in the PCL amniotic membrane group were mainly activated to M2 macrophages. In addition, there were changes in the expression of pro-inflammatory and anti-inflammatory

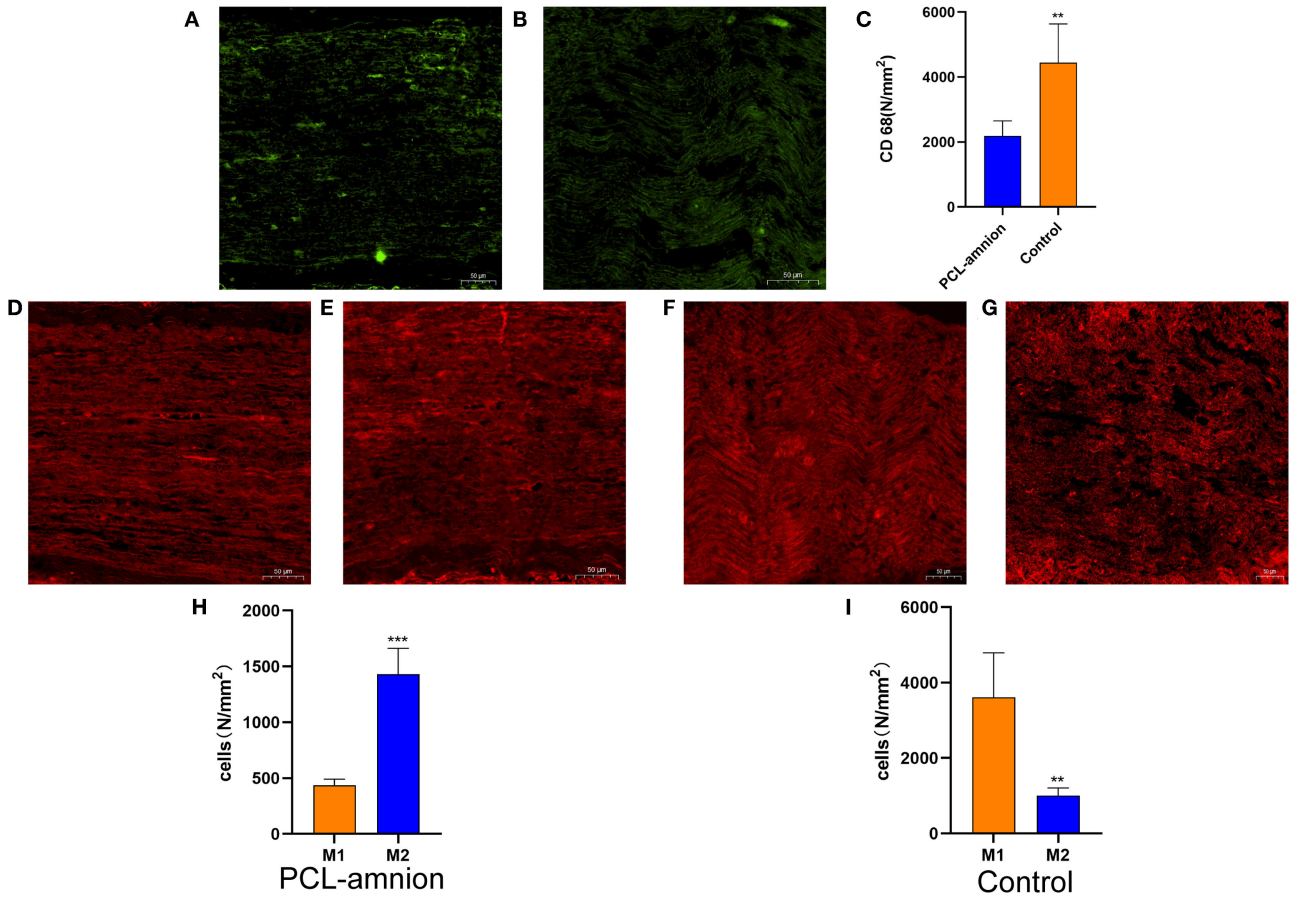


**FIGURE 2 |** Inflammatory reaction assessment at the repair site. **(A–C)** ED1 macrophages of the sciatic nerve at the repair site. **(A)** The PCL-amnion nanofibrous membrane group. **(B)** The Control group. **(C)** The ED1 macrophage count between the two groups were significantly different ($P < 0.05$). $^{**}P < 0.01$ compared to the PCL-amnion group. **(D–I)** M1 and M2 of the sciatic nerve at the repair site. **(D)** M1 of the sciatic nerve in the PCL-amnion nanofibrous membrane group. **(E)** M2 of the sciatic nerve in the PCL-amnion nanofibrous membrane group. **(F)** M1 of the sciatic nerve in the Control group. **(G)** M2 of the sciatic nerve in the Control group. **(H)** The M1:M2 ratio in the PCL-amnion nanofibrous membrane group was 1:3 ($P < 0.05$). $^{***}P < 0.001$ compared to M1. **(I)** The M1:M2 ratio in the Control group was 3:1 ($P < 0.05$). $^{**}P < 0.01$ compared to M1.

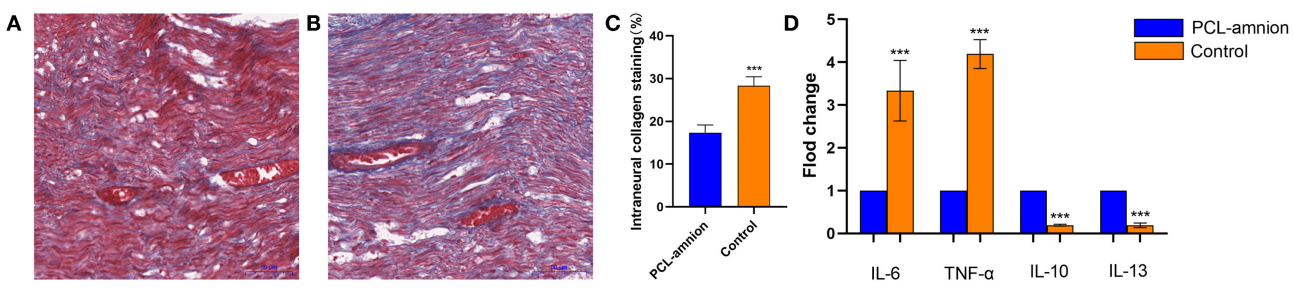


**FIGURE 3 |** Collagen deposition and quantification of cytokine expression at the repair site. **(A)** The PCL-amnion nanofibrous membrane group. **(B)** The Control group. **(C)** Quantification of collagen at the repair site between the two groups were significantly different ($P < 0.05$). **(D)** The gene expression of proinflammatory cytokines IL6 and TNF-a decreased, and the gene expression of anti-inflammatory cytokines IL10 and IL13 increased in the PCL-amnion nanofibrous membrane group ($P < 0.05$ for each cytokine). $^{***}P < 0.001$ compared to the PCL-amnion group.

cytokines. The expression of the anti-inflammatory cytokines IL-10 and IL-13 at the repair site increased significantly more in the PCL-amnion nanofibrous membrane group than in the Control group. These results indicate that the PCL-amnion nanofibrous membrane regulates the proportion of M1/M2 macrophages, inhibiting inflammatory factors and enhancing

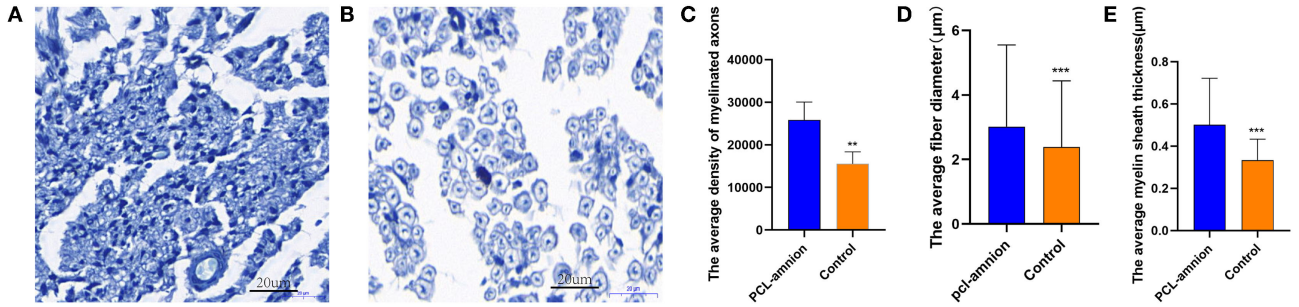


**FIGURE 4 |** Histomorphometry analysis of nerve regeneration. **(A)** The PCL-amnion nanofibrous membrane group. **(B)** The Control group. **(C–E)** More mature fibers were observed in the PCL-amnion nanofibrous membrane group. **(C)** The average density of myelinated axons was significantly different between two groups ($P < 0.05$). **(D)** The average fiber diameter of myelinated nerve fibers was significantly different between two groups ($P < 0.05$). **(E)** The average myelin sheath thickness of the sciatic nerve was significantly different between two groups ($P < 0.05$). ***$P < 0.01$ compared to the PCL-amnion group. **$P < 0.001$ compared to the PCL-amnion group.

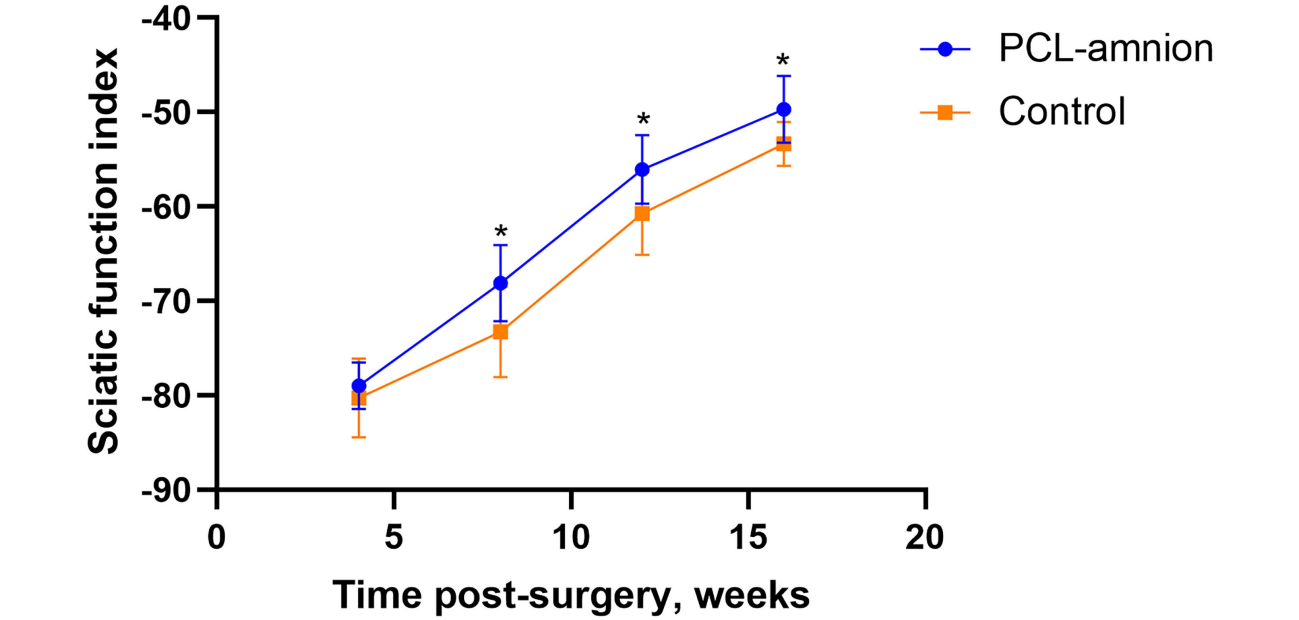


**FIGURE 5 |** Sciatic function index. The SFI between the two groups was significantly different at 8, 12, and 16 weeks ($P < 0.05$). *$P < 0.05$ compared to the control group.

the expression of anti-inflammatory cytokine. The Masson's trichrome staining presented in this study showed that the collagen deposition at the repair site was markedly less in the PCL-amnion nanofibrous membrane group than in the Control group. The differential cytokine expression reduced the undesirable immune responses of scarring and fibrosis.

The PCL-amnion nanofibrous membrane has a similar size as matrix collagen fiber in order to facilitate the tissue repair, and the effective scaffold pore structure created by electrostatic spinning technology is to facilitates the transfer of bioactive molecules, nutrients, and metabolic wastes. In this study, the PCL-amnion nanofibrous membrane consists of PCL in the outer layer and amniotic membrane in the inner layer. The amniotic membrane can slowly release TGF-β1, bFGF, PDGF, and NGF cytokines (52). These cytokines continuously diffuses through the pore structure of PCL to the nerve repair site and promotes the proliferation of Schwann cells and axon regeneration. In a previous study, PCL with adipose stem cells was used as a scaffold for nerve regeneration (53). In a rat sciatic nerve transection model, a PCL nanofibrous scaffold loaded by a mesenchymal stem cell condition medium was shown to promote nerve regeneration (54). For repair of sciatic nerve injury, bFGF-chitosan scaffolds were prepared and used to facilitate nerve regeneration (55). At 16 weeks, the axon image showed that there was more mature fibers crossing the suture site in the PCL-amnion nanofibrous membrane group than in the Control group. The nerve fibers were arranged regularly in the PCL-amnion nanofibrous membrane group. Compared with those in

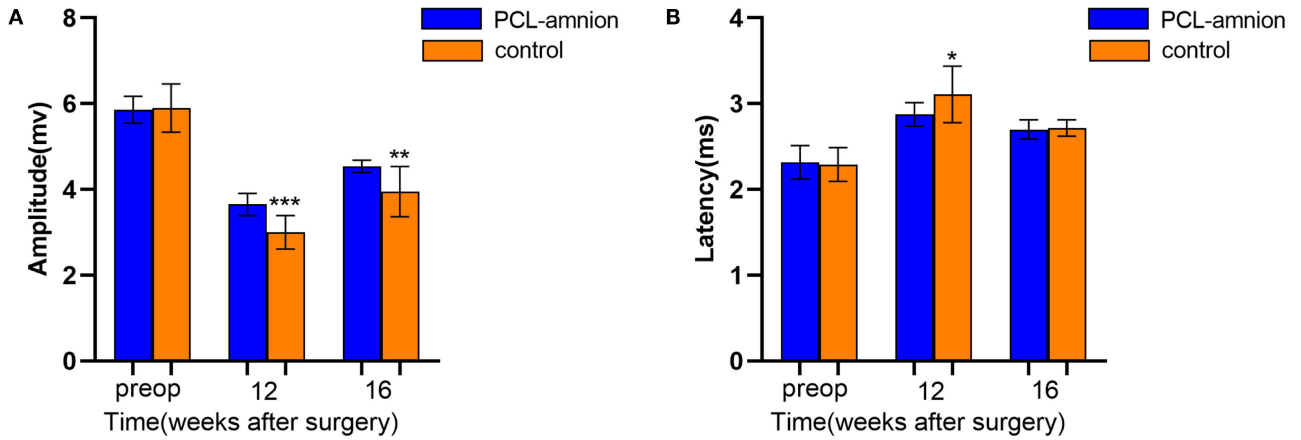


**FIGURE 6 |** Electrophysiology testing. The motor amplitude and latency were analyzed to assess the functional recovery of the sciatic nerve. **(A)** The PCL-amnion nanofibrous membrane group shows significantly greater amplitude at 12 and 16 weeks ($P < 0.05$). ***$P < 0.001$ compared to the PCL-amnion group at 12 weeks. ***$P < 0.01$ compared to the PCL-amnion group at 16 weeks. **(B)** The PCL-amnion nanofibrous membrane group shows significantly greater latency at 12 weeks ($P < 0.05$); however, the latencies between two groups were not significantly different at 16 weeks ($P > 0.05$). *$P < 0.05$ compared to the PCL-amnion group at 12 weeks.

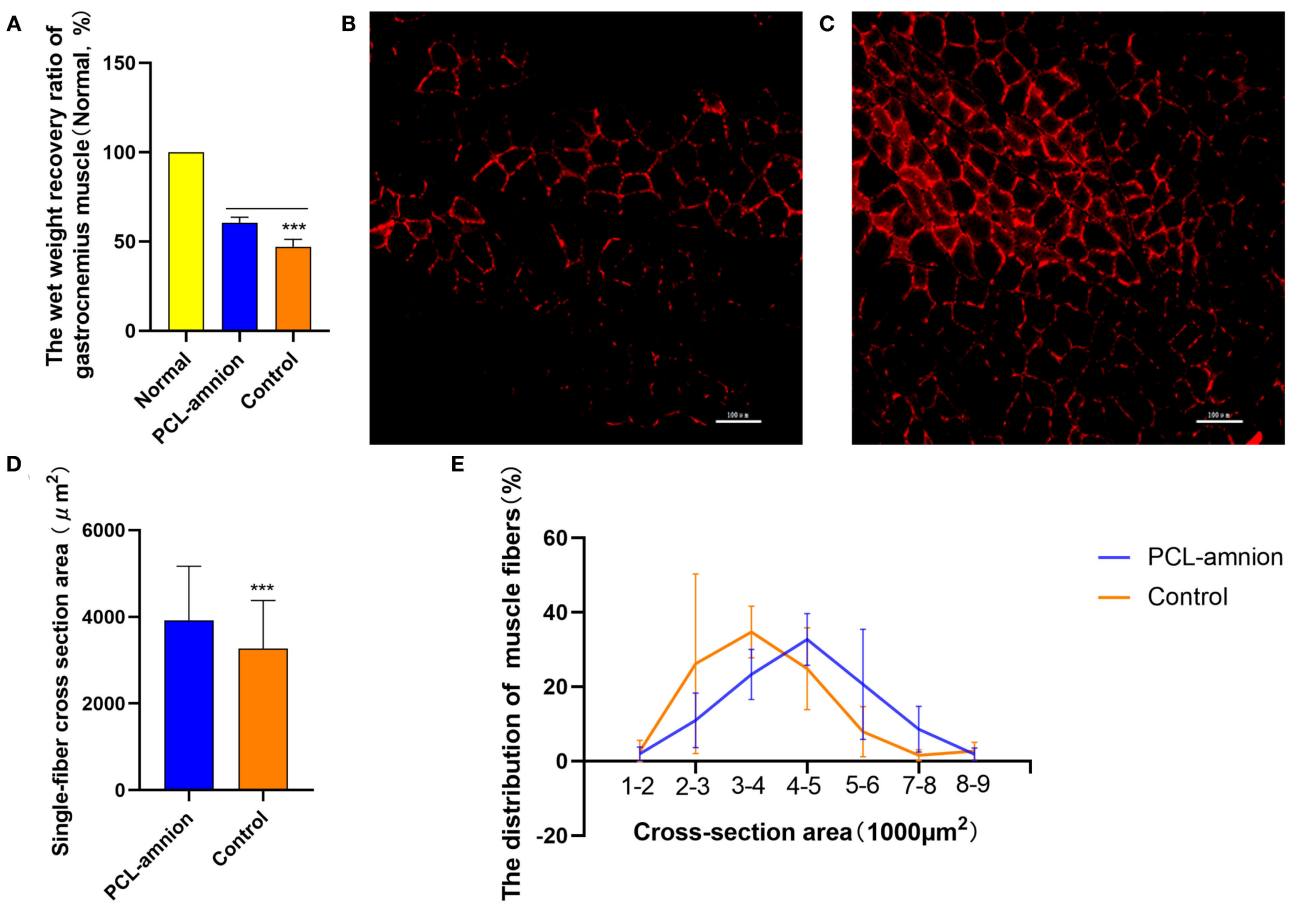


**FIGURE 7 |** Gastrocnemius muscle analysis. **(A)** Wet weight recovery ratio of gastrocnemius muscle in the PCL-amnion nanofibrous membrane group is significantly higher in comparison with the control group ($P < 0.05$). **(B)** The PCL-amnion nanofibrous membrane group. **(C)** The Control group. **(D)** Average single-fiber CSA. The PCL-amnion nanofibrous membrane group shows significantly greater single-fiber CSA in comparison with the control group ($P < 0.05$). **(E)** Distribution of single-fiber CSA. ***$P < 0.001$ compared to the PCL-amnion group.

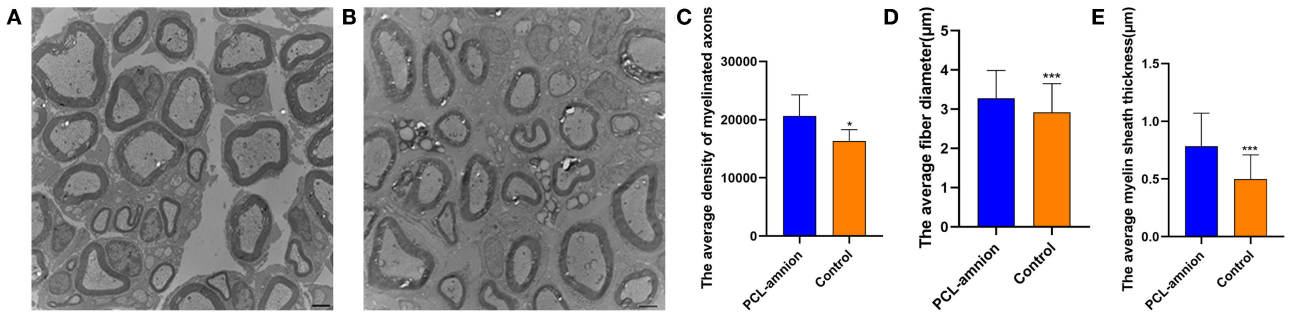


**FIGURE 8 |** Transmission electron microscopy images of the nerve tissues at 16 weeks. **(A)** The PCL-amnion nanofibrous membrane group. **(B)** The Control group. **(C)** The average density of myelinated axons was significantly different between two groups ($P < 0.05$). **(D)** The average fiber diameter of myelinated nerve fibers was significantly different between two groups ($P < 0.05$). **(E)** The average myelin sheath thickness of the sciatic nerve was significantly different between two groups ($P < 0.05$). *$P < 0.05$ compared to the PCL-amnion group. ***$P < 0.001$ compared to the PCL-amnion group.

the Control group, the axonal diameter and the myelin sheath in the PCL-amnion nanofibrous membrane group were greater and thicker, respectively. In addition, a shorter motor latency time, greater CMAP amplitudes, higher wet weight recovery ratio, and higher single-fiber muscle cross-sectional area were demonstrated in the PCL-amnion nanofibrous membrane group in comparison with control group.

## CONCLUSIONS

The present study demonstrated that the PCL-amnion nanofibrous membrane with specific fibers and large pores markedly enhanced Schwann cell proliferation, axonal regeneration, and prevented scar formation at the nerve repair site with the end-to-end epineurial suture. Importantly, the PCL-amnion nanofibrous membrane drove macrophage polarization into the M2 phenotype, which promoted nerve regeneration and functional recovery. Our findings provided a new treatment approach to prevent scar formation at the nerve repair site and promote nerve regeneration, which should aid the design of next generation nerve wraps.

The limitation of the present study is lacking of the tests for immunological safety, cytocompatibility and cytotoxicity of PCL amniotic nanofiber membrane. In the further research work, we conduct the immunological safety, cytocompatibility and cytotoxicity test for our PCL amniotic nanofiber membrane. Moreover, we will continue to explore how macrophages are regulated and activated by the PCL-amnion nanofibrous membrane, how M1 macrophages affected Wallerian degeneration, and M2 macrophages affected Schwann cell proliferation and axonal regeneration. Furthermore, the disadvantage of PCL is its slow rate of degradation. An ideal wrapping material begins degradation after epineurium healing, and nerve fibers cross the repair site. Therefore, PCL and other natural polymer materials can be combined to form a nerve wrap to improve the biodegradation rate.

In summary, our findings provided a new treatment approach to prevent scar formation at the nerve repair site and promote nerve regeneration, which should aid the design of next generation nerve wraps.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by according to the Declaration of Helsinki and approval from the Medical Ethics Committee of The Third Hospital of Hebei Medical University, an amniotic membrane was provided by the Department of Obstetrics and Gynecology from June 1st of 2021 to June 30th of 2021. The patients/participants provided their written informed consent to participate in this study. The study was conducted under all protocols approved by the Animal Research Ethical Committee of The Third Hospital of Hebei Medical University and in line with the guidelines for animal care and use (Approval number: Z2021 - 008 - 1).

## AUTHOR CONTRIBUTIONS

DT: conceptualization, funding acquisition, project administration, and writing—review and editing. JB, CL, and LK: data curation. JB, CL, ST, and KY: formal analysis. CL: resources. JB, CL, and KY: software. JB: writing—original draft. All authors contributed to the article and approved the submitted version.

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# MRI-Based Optimization Design of the Pre-Spinal Route of Contralateral C7 Nerve Transfer for Spastic Arm Paralysis

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**Purpose:** The prespinal route of contralateral cervical 7 nerve transfer developed by Prof. Wendong Xu helps realize the direct anastomosis of the bilateral cervical 7 nerves. However, 20% of operations still require a nerve graft, which leads to an unfavorable prognosis. This study aims to explore the optimized prespinal route with MRI to further improve the prognosis.

**Methods:** The current study enrolled 30 patients who suffered from central spastic paralysis of an upper limb and who underwent contralateral cervical 7 nerve transfer via Prof. Xu's prespinal route through the anterior edge of the contralateral longus colli. MRI images were used to analyze the route length, vertebral artery exposure, and contralateral cervical 7 nerve included angle. Three prespinal routes were virtually designed and analyzed. The selected optimal route was applied to another 50 patients with central spastic paralysis of an upper limb for contralateral cervical 7 nerve transfer.

**Results:** By the interventions on the 30 patients, the middle and posterior routes were shorter than the anterior route in length, but with no statistical difference between the two routes. Of 30 contralateral vertebral arteries, 26 were located at the posterior medial edge of the longus colli. The average included angles of the anterior, middle, and posterior routes were $108.02 \pm 7.89°$, $95.51 \pm 6.52°$, and $72.48 \pm 4.65°$, respectively. According to these data, the middle route was optimally applied to 50 patients, in whom the rate of nerve transplantation was only 4%, and no serious complications such as vertebral artery or brachial plexus injury occurred.

**Abbreviations:** C7, cervical 7; CC7, contralateral cervical 7; CNS, central nervous system; CUBE, 3D fast spin echo with an extended echo train acquisition; FIESTA-C, fast imaging employing steady-state acquisition-constructive interference steady state; FOV, field of view; MRI, magnetic resonance imaging; STIR, short tau inversion recovery; TE, echo time, TR, repetition time; T1WI, T1 weighted imaging; T2WI, T2 weighted imaging.

**Conclusion:** The low rate of nerve transplantation in 50 patients and the absence of any serious complications in these cases suggests that the middle route is the optimal one.

**Keywords: magnetic resonance imaging (MRI), spastic arm paralysis, contralateral C7 transfer, pre-spinal routes, optimization design**

## INTRODUCTION

Brachial plexus injury caused by trauma, birth injury, and other things can seriously impact the function of the paralyzed upper limb, and spastic hemiplegia of the upper extremity due to central paralysis is a common sequela that significantly affects the quality of life of patients (1–3). The number of such patients is very high, which can have practical consequences for those caring for them and for society as a whole. The effectiveness of common rehabilitation therapy is extremely limited, which makes it difficult to improve the function of the paralysed limb. The prespinal route of "contralateral cervical 7 (C7) nerve transfer," which is the route through the anterior edge of the contralateral longus colli, the anterior edge of the vertebral body, the posterior edge of the esophagus, and the anterior edge of the longus colli on the paralyzed side, hence known as the anterior route, was developed by Prof. Wendong Xu's team and has become a new and effective approach to the treatment of this condition (4, 5).

During the operation, if the bilateral C7 nerves cannot be directly anastomosed, other nerves need to be grafted, which will reduce the surgical effect. Compared with the early anterior cervical subcutaneous path, Prof. Xu's prespinal route significantly shortens the distance of transposition and achieves direct anastomosis of bilateral C7 nerves, thus reducing trauma, the length of the operation, recovery time, and time for nerve regeneration, thus improving the therapeutic effect. However, 20% of procedures still necessitate the use of a graft regardless of the shortened pathway (6). In addition, the regional anatomy of the transposition path is relatively complex, mainly involving the vertebral artery and brachial plexus, which is closely related to the prespinal route (7). All these factors can restrict the wide application of the operation.

At present, imaging examination has become an important technique in preoperative evaluation, since it is capable of clearly showing the location relationship where the anatomical structures are located in the surgical area, performing accurate data measurement and precise positioning, and providing a data basis for a particular surgical intervention. For the prespinal route of contralateral C7 nerve transposition, however, a standard preoperative evaluation system is not available. Therefore, the objective of our study was to explore the optimized prespinal route by MRI simulation and evaluate the position and relationship between the locations of important anatomical structures, so that we could shorten the route distance, evaluate the safety of the surrounding structures, improve the curative effect, and avoid serious complications. This could thus provide a scientific basis for clinically designing a shorter and safer transfer route for direct and tension-free anastomosis of the bilateral C7 nerve.

## MATERIALS AND METHODS

### Patients

A total of 80 CNS injury patients were enrolled in this study, 30 of whom underwent contralateral C7 nerve transfer surgery via the prespinal route through the anterior edge of the contralateral longus colli from June 2018 to December 2019, and the remaining 50 of whom had received the same surgery via the prespinal route through the middle of the contralateral longus colli from January 2020 to December 2020. There were no statistically significant differences in age and gender at scanning within the groups that were to receive brachial plexus MRI examination. Prior to the examination, all patients signed the informed consent form for the current study, the protocol of which was approved by the Research Ethics Committee of Shanghai Jingan District Central Hospital.

### Surgical Procedure

As previously reported (6), the surgical procedure was as follows: A 15 cm transverse incision was made approximately 2 cm superior to the clavicle at the bottom of the neck so that the brachial plexus nerves were bilaterally exposed, superior to the clavicle. On the paralyzed side, the C7 nerve was severed near the intervertebral foramen, and on the nonparalyzed side, the C7 nerve was severed, as distally as possible, proximal to the point at which it combined with the fibers of other brachial plexus nerves. The anterolateral aspect of the C7 vertebral body was bluntly dissected so that the esophagus was exposed anterior to the vertebral body, thus creating a conduit between the spinal column and the esophagus. Afterward, the cut end of the C7 nerve on the nonparalyzed side was drawn through the prespinal route (the anterior, middle, or posterior route) to the paralyzed side to be anastomosed, directly without a graft or indirectly with a graft, to the cut end of the C7 nerve using microsurgical epineurium suturing (**Figure 1**). The whole procedure was performed by a senior surgeon.

### MRI Scanning

MRI scanning was performed using a 3.0 T scanner on the patient, who lay supine, with their arm positioned by their side. The standard sequence protocol involved axial T1WI, axial T2WI, 3D-fast imaging employing steady-state acquisition-constructive interference steady state, and 3D fast spin echo with an extended echo train acquisition (CUBE)-short tau

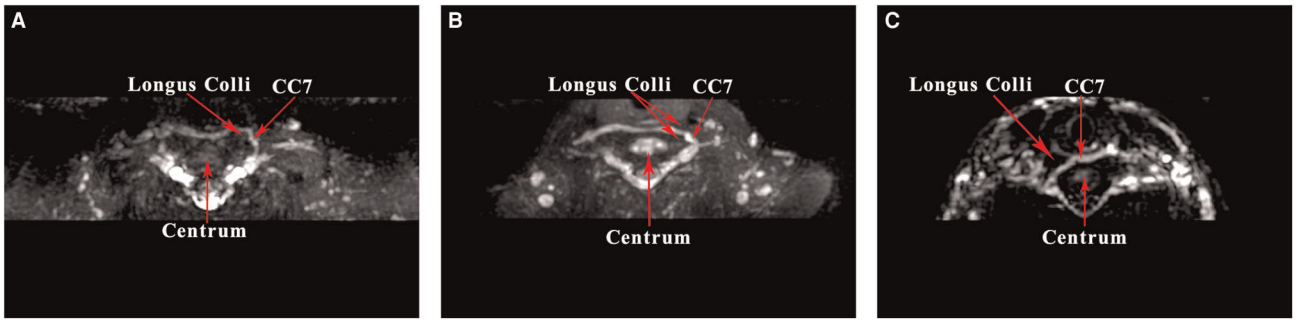


**FIGURE 1** | contralateral cervical 7 (CC7) nerve transfer operation on MRI by 3D CUBE-STIR sequences: (**A**) anterior route, (**B**) middle route, (**C**) posterior route.

inversion recovery (STIR). The scanning parameters of the T1WI sequence were as follows: FOV 240 mm, thickness/interval 3/0 mm, resolution 288 × 256, TR 582 ms, and TE 6.7 ms and those of the 3D CUBE-STIR sequence were as follows: FOV 280 mm, thickness/interval 1.6/0 mm, resolution 320 × 224, TR 4,750 ms, and TE 179 ms. The brachial plexus MRI images of each patient were carefully evaluated.

The MRI datasets were imported into the post-processing software package of Image J 1.52p (Wayne Rasband National Institute of Health, USA). On T1WI images at the level of the C7 nerve outlet of the intervertebral foramen, the following were visible: the trace lines from the contralateral to the paralyzed side along the anterior edge of longus colli on the contralateral side, the anterior edge of the vertebral body, the posterior edge of the esophagus, the anterior edge of the longus colli on the paralyzed side (the anterior route); through the middle of longus colli on the contralateral side, the anterior edge of the vertebral body, the posterior edge of the esophagus, the anterior edge of the longus colli on the paralyzed side (the middle route); and the posterior edge of longus colli on the contralateral side, the anterior edge of the vertebral body, the posterior edge of the esophagus, and the anterior edge of the longus colli on the paralyzed side (the posterior route). We usedthe segmented line tool to draw different surgical paths manually before employing the fit spline to smooth the path curve and the trajectory measurement tool to automatically calculate the lengths of different surgical paths.

In all the patients, the lengths of the anterior, middle, and posterior routes were automatically measured using Image J software (**Table 1**; **Figures 2**, **3**), as were the contralateral C7 nerve included angles of the three prespinal routes (anterior, middle, or posterior) simulated at the level of the superior margin of the C7 vertebra on the preoperative T1WI sequence (**Table 2**; **Figures 4**, **5**).

## Statistical Analysis

The Kolmogorov–Smirnov test was applied to each continuous variable to examine whether a normal distribution could be assumed, after which the variable was summarized as mean ± SD as appropriate. The group means were compared using one-way ANOVA or independent sample *t*-tests, and the results were presented as odds ratios with 95% confidence intervals. A value of $p < 0.05$ was considered statistically significant. The statistical analysis was performed on SPSS, version 17.0 (SPSS, Chicago, IL, USA).

**TABLE 1** | The lengths of three prespinal routes on T1WI.

| Group | *N* | Mean (cm) | Std. deviation | Std. error | 95% Confidence interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower bound | Upper bound |
| Anterior route | 30 | 5.79 | .59 | .06 | 5.66 | 5.91 |
| Middle route | 30 | 4.70* | .51 | .05 | 4.60 | 4.81 |
| Posterior route | 30 | 4.59* | .57 | .06 | 4.47 | 4.71 |

*T1WI, T1 weighted imaging;* $^{*}p < 0.001$; $^{\&}p > 0.05$.

## RESULTS

In the contralateral C7 nerve transfer operation, the three prespinal routes were presented on MRI by using the 3D CUBE-STIR sequence (**Figure 1**). According to the measured lengths of the three prespinal routes simulated at the level of the superior margin of the C7 vertebra on the preoperative T1WI sequence, the average lengths of the anterior, middle, and posterior routes were 5.79 ± 0.59 cm, 4.70 ± 0.51 cm, and 4.59 ± 0.57 cm, respectively. Statistical analysis showed no significant difference between the middle and the posterior route ($p = 0.57$), while the anterior route was significantly longer than the other two (all $p < 0.001$; **Table 1**; **Figures 2**, **3**).

From the measurement of the contralateral C7 nerve included angles of the three prespinal routes (the anterior, middle, or posterior route) simulated at the level of the superior margin of the C7 vertebra on the preoperative T1WI sequence (represented by a schematic map), the average included angles of the anterior, middle, and posterior routes were 108.02 ± 7.89°, 95.51 ± 6.52°, and 72.48 ± 4.65°, respectively. Statistical analysis showed significant differences between the anterior, middle, and posterior routes (all $p < 0.001$; **Table 2**; **Figures 4**, **5**).

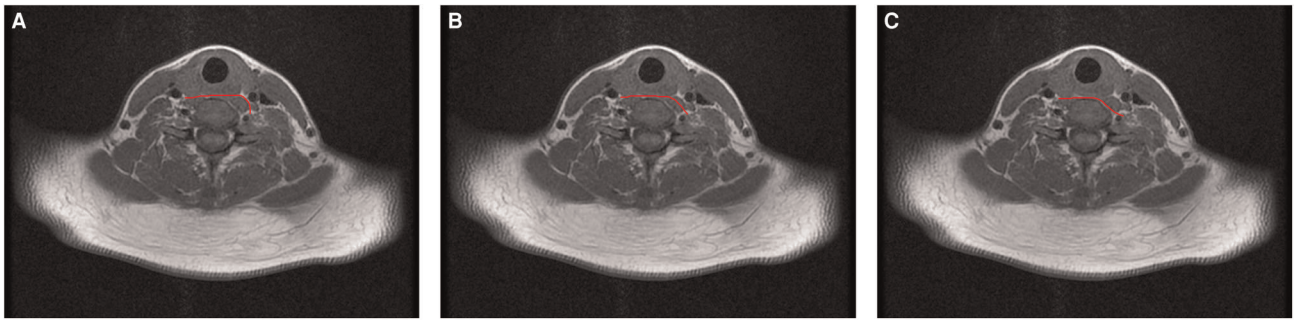


**FIGURE 2** | Prespinal routes of CC7 transfer surgery on T1-weighted images (T1WI): (**A**) anterior route, (**B**) middle route, (**C**) posterior route.

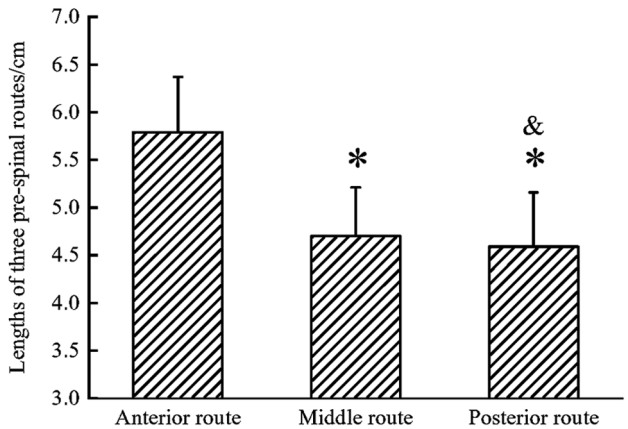


**FIGURE 3** | Statistical chart of lengths of three prespinal routes on T1WI. The lengths of the two were statistically shorter than that of the anterior route (the middle, $p < 0.001$; the posterior, $p < 0.001$). No significant difference was observed between the middle and the posterior route ($p = 0.57$).

**TABLE 2** | Included angle of the CC7 nerve via three prespinal routes.

| Group | *N* | Mean (∠) | Std. deviation | Std. error | 95% Confidence interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower bound | Upper bound |
| Anterior route | 30 | 108.02 | 7.89 | 1.76 | 104.33 | 111.72 |
| Middle route | 30 | 95.51* | 6.52 | 1.46 | 92.46 | 98.56 |
| Posterior route | 30 | 72.48*& | 4.65 | 1.04 | 70.30 | 74.65 |

*CC7, contralateral cervical 7*; *$p < 0.001$; &$p > 0.05$.

An MRI showed that the contralateral vertebral artery was located at the posterior medial edge of the longus colli in 26 cases (26/30), at the lateral edge of the longus colli in 3 cases (3/30), and at the anterior margin of the longus colli in 1 case (1/30; **Figure 6**).

According to the data of the first 30 patients, who underwent contralateral C7 nerve transfer surgery through the anterior route, and of another 50 patients who underwent contralateral C7 nerve transfer surgery through the middle route, 2 were found to have nerve transplantation, the rate of which was 4%, with no occurrence of serious complications such as vertebral artery and brachial plexus injury.

## DISCUSSION

Peripheral nerve transfer, first carried out by Prof. Yudong Gu in 1970, was first applied to patients with brachial plexus avulsion through phrenic nerve transplantation (8, 9). In 1986, academician Yudong Gu pioneered the contralateral C7 nerve transfer in the world to treat patients with total brachial plexus injury by creating the classic anterior cervical subcutaneous route contralateral C7 nerve transfer, which has been widely used (10–12). Since then, Prof. Wendong Xu has improved the surgery by developing the anterior route, for the contralateral C7 nerve transfer in treating patients with brachial plexus injury and central hemiplegia (13, 14). This approach significantly shortens the route, actualizing the direct anastomosis of the bilateral C7 nerve, reducing the rate of nerve transplantation, and achieving remarkable efficacy (4).

In our previously reported clinical study, however, we had found that the nerve transplantation rate of the anterior route in the treatment of unilateral arm paralysis due to central paralysis was 20% (6). According to the clinical follow-ups, the curative effect on the patients requiring nerve transplantation was found to be worse than that of bilateral C7 nerve direct anastomosis, increasing the difficulty of the surgery and the uncertainty of the prognosis (15). As for surgical complications, so far no serious adverse events related to the surgery have been reported in the clinical research by Prof. Xu's team (16). However, it was reported that the total incidence of complications was 5.4% (23 of 425), which was mainly related to the prespinal route (the anterior route), exploration and transection of contralateral C7 nerve, and that two cases of more serious vertebral artery injury accounted for 0.47% and four cases of contralateral brachial plexus injury, 0.94% (7).

Therefore, we tried to use MRI images to simulate the prespinal routes to explore a better and shorter surgical path

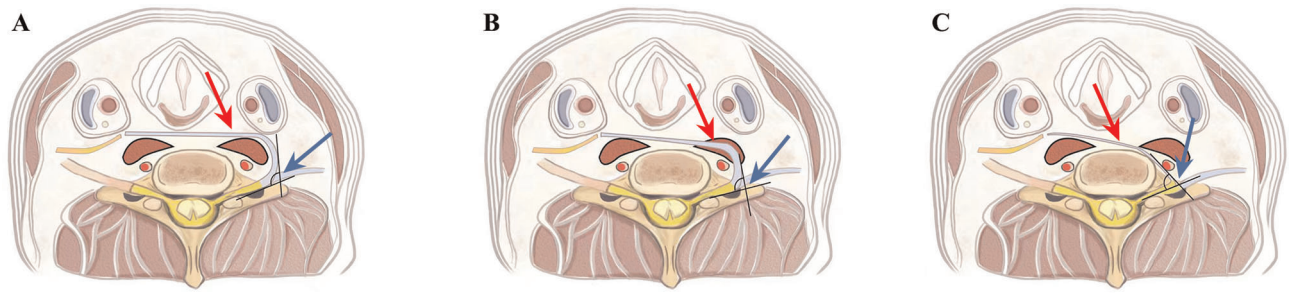


**FIGURE 4** | The included angle of CC7 nerve transfer via three prespinal routes: (**A**) anterior route, (**B**) middle route, (**C**) posterior route.

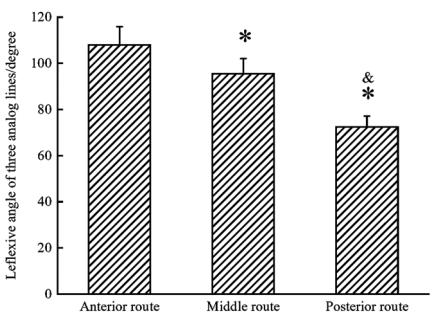


**FIGURE 5** | Statistical chart of the CC7 nerve included angles of three prespinal routes. *The included angles of the two were significantly smaller than that of the anterior route (the middle, $p < 0.001$; the posterior, $p < 0.001$). &The included angle of the middle route was larger than that of the posterior route ($p<0.001$).

through which to reduce the probability of nerve transplantation and the risk of surgery.

On the basis of Prof. Xu's modified prespinal route (the anterior route) (4, 5), we simulated three prespinal routes at the upper edge of the C7 vertebral body on MRI images: the anterior, middle, and posterior routes. Of the three routes, in the 30 patients who underwent contralateral C7 nerve transfer surgery through the anterior route, the anterior route was the longest, with an average length of 5.79 ± 0.59 cm. In our previously reported clinical study (6), six patients who underwent contralateral C7 nerve transfer surgery through the anterior route received nerve transplantation, and the mean length of the contralateral C7 nerve measured during the surgery (5.7 ± 0.6 cm in the anterior division and 5.2 ± 0.6 cm in the posterior division) was shorter than the anterior route (5.79 ± 0.59 cm) but longer than the middle route (4.70 ± 0.51 cm) and the posterior route (4.59 ± 0.57 cm). If the six patients received the middle route or the posterior route, therefore, a direct and tension-free anastomosis of the bilateral C7 nerve could be realized. According to the statistics of the anatomic position of the vertebral artery in the surgical access area, it was found that at the level of the superior edge of the C7 vertebral body, the vertebral artery was closely related to the longus colli, and most of the vertebral arteries located in the posterior or posterolateral part of the longus colli (17, 18). In our study, the contralateral vertebral arteries were located at the posterior medial margin of the longus colli in 26 of 30 patients, at the lateral margin of the longus colli in 3 of 30 cases, and at the anterior margin of the longus colli in 1 of 30 cases. This suggests that the vertebral artery can be more successfully avoided by taking the anterior and middle routes than by taking the posterior route.

There exist cervical thoracic ganglia in the space between the anterior part of the vertebral body and the medial margin of the longus colli at the upper edge of the C7 vertebral body (19). If the posterior route is selected, it is likely to cause sympathetic nerve and vertebral artery damage (20, 21). As realized by surgeons, when the incision of the posterior route is deeper, surgery becomes more difficult, and when the included angle of the contralateral C7 nerve is smaller in the posterior route, the C7 nerve conduction is more likely to be affected. Based on the analytical data on the three routes and where the important anatomical structures are located along the route, it could be concluded that the middle route was surgically the best, which was characterized by a short distance, high safety, and less impact on the function of the contralateral C7 nerve.

Of the 50 patients with spastic upper limb paralysis caused by central palsy who were selected to undergo the contralateral C7 nerve transfer through the middle route, only 2 patients received nerve transplantation at a rate of 4%; the rate was significantly lower when compared with that in the case of the anterior route (6/30, 20% vs. 2/50, 4%; $p = 0.021$) (6), even without structural injuries of the vertebral artery, brachial plexus, and other important tissue.

Technically mature MRI on brachial plexus is both noninvasive and safe. The simulation and optimization of the prespinal route through the anterior route and the presentation of where the anatomical structures are located in the area to be operated on facilitate the obtainment of data regarding the displacement route, the location of the adjacent important anatomical structures, the adjacent relationship, and the anatomical variation prior to the surgery. Thus, the technique provides a scientific basis for designing a better transfer route for a direct and tension-free anastomosis of the bilateral C7 nerve, avoiding the occurrence of serious complications, reducing surgical trauma, improving surgical safety and efficacy, and realizing

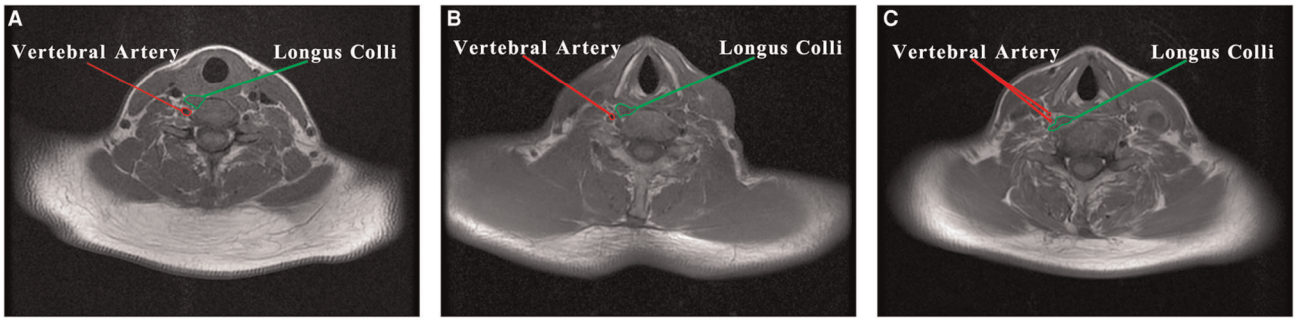


**FIGURE 6** | Anatomic relationship between vertebral artery and longus colli: (**A**) the vertebral artery located at the posterior medial margin of the longus colli (26/30), (**B**) the vertebral artery located at the lateral margin of the longus colli (3/30), (**C**) the vertebral artery located at the anterior margin of the longus colli (1/30).

precise and personalized treatment. As a result of this study, we can conclude that MRI can provide technical support for the popularization and application of contralateral C7 nerve transfer.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Research Ethics Committee of Shanghai Jing'an District Central Hospital. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

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# Comparison of characteristics between neuropathic pain and non-neuropathic pain in patients with diabetic carpal tunnel syndrome: A cross-sectional study

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**Background:** The aim of the study was to compare the clinical characteristics of diabetic carpal tunnel syndrome between patients with neuropathic pain (NeuP) and non-NeuP.

**Methods:** We enrolled 276 patients with diabetic carpal tunnel syndrome. Pain symptoms were evaluated using a visual analog scale. Douleur Neuropathique 4, the Neuropathic Pain Symptoms Inventory questionnaire, and the body map were used to assess neuropathic symptoms. Baseline information, clinical manifestations, electrophysiological test results, and psychological status were compared between the neuropathic pain (NeuP) and non-NeuP to identify the risk factor for NeuP occurrence.

**Results:** Results showed that the degree of pain was more severe in NeuP patients than in nociceptive pain patients ($p = 0.025$). The frequencies of light touch and pinprick were more pronounced in the NeuP group than in the non-NeuP group (light touch: $p = 0.001$; pinprick: $p = 0.004$). There were 48 and 27 NeuP patients with extramedian and proximal spread, respectively, whereas in the non-NeuP group, there were 11 and 9 patients, respectively ($p = 0.03$). Electrophysiological results showed that patients in the NeuP group exhibited greater sensory nerve conduction velocity impairment compared with the non-NeuP group ($p = 0.033$). Pain Catastrophizing Scale total scores of the NeuP group were significantly higher than those of the non-NeuP group ($p = 0.006$).

**Conclusion:** Of the 276 diabetic carpal tunnel syndrome patients studied, the majority had NeuP. Furthermore, light touch, electrophysiological test results, and psychological factors were found to be related to NeuP occurrence in patients with diabetic carpal tunnel syndrome.

KEYWORDS

carpal tunnel syndrome, neuropathic pain, diabetic mellitus, cross-sectional study, psychologcal assessment

## Introduction

Carpal tunnel syndrome is the most common peripheral nerve entrapment disease with an incidence as high as 10% in the general population (1). The primary symptoms of carpal tunnel syndrome include paresthesia and neuropathic pain (NeuP) in the median territory, weakness of hand grasp, and thenar wasting. NeuP, especially nocturnal pain, is the primary complaint of patients with carpal tunnel syndrome. Pain symptoms directly and negatively impact the sleep quality and hand function of patients, which results in psychological states of anxiety and depression (2).

The International Association for the Study of Pain (IASP) defines NeuP as "pain caused by a lesion or disease of the somatosensory nervous system." The prevalence of NeuP in carpal tunnel syndrome patients varies from 31% to 80% across studies (3–5). Researchers have dedicated significant effort to determining the characteristics of NeuP in patients with carpal tunnel syndrome. Matesanz et al. reported that the severity of NeuP is associated with more pronounced deficits in emotional well-being and sleep quality (3). Oteo-Alvaro and Marin revealed that numbness/tingling, pain intensity, and neurologic affectation are risk factors for NeuP (4). Moreover, Sonohata et al. found that carpal tunnel release can alleviate NeuP (5).

Diabetes mellitus is a shared risk factor for NeuP and carpal tunnel syndrome (6), and NeuP is often the most pronounced manifestation of carpal tunnel syndrome. Carpal tunnel syndrome and diabetes mellitus have synergistic effects on median nerve injury (7–9). Thus, carpal tunnel syndrome, NeuP, and diabetes mellitus may interact to form a mutual response to the progress of the diseases. Therefore, patients with NeuP who have diabetic carpal tunnel syndrome are likely to present with characteristics that are distinct from those with nondiabetic carpal tunnel syndrome. Understanding the specific symptoms of this subgroup of carpal tunnel syndrome patients could enable more accurate diagnosis and the development of more focused therapies. However, analyses of the characteristics of NeuP in patients with diabetic carpal tunnel syndrome are scarce.

In our clinical practice, we have observed differences between NeuP patients and non-NeuP patients of carpal tunnel syndrome in terms of demographic information, clinical manifestation, and psychological state. Therefore, in the current study, we enrolled 276 diabetic carpal tunnel syndrome patients and divided them into two groups according to the pain symptoms .Then, we compared the clinical characteristics between these two groups to identify possible risk factors for the occurrence of NeuP in patients with diabetic carpal tunnel syndrome. The characteristics of NeuP in diabetic carpal tunnel syndrome patients would provide the hints for the hand surgeons to take some interventions earlier.

## Patients and methods

This study was approved by the Ethical Committee of Shenzhen People's Hospital.

### Study population

All participants diagnosed with both carpal tunnel syndrome and diabetes mellitus in the department of hand and microvascular surgery which focused on the peripheral nerve surgery in our hospital, between June 1, 2020 and June 2, 2021, who provided written informed consent and were willing to participate, were recruited into this cross-sectional study. We recruited 276 unilateral carpal tunnel syndrome patients (216 women and 60 men) who were screened according to the inclusion and exclusion criteria. Eligible participants included patients who were aged over 18 years, and who were diagnosed with both carpal tunnel syndrome and diabetes mellitus (Type 1 or Type 2) based on symptoms, physical examinations, and electrophysical tests. The exclusion criteria were as follows: bilateral carpal tunnel syndrome, acute complications of diabetes mellitus (e.g., renal failure, foot ulcers, and severe infection), other NeuP diseases (e.g., peripheral nerve lesions, brain and spinal cord lesions, thyroid dysfunction, and multiple sclerosis), and inability to read or write Chinese (illiterate or an ethnic minority). Patients diagnosed with bilateral carpal tunnel syndrome were excluded because they always presented with different clinical manifestations between the right and left hands, creating difficulties in the data analysis.

### Definite diagnosis of neuropathic pain and carpal tunnel syndrome

Firstly, the visual analog scale (VAS) was used to evaluate whether patients with diabetic carpal tunnel syndrome presented with pain symptoms. Patients after the VAS scale evaluation were diagnosed using the Douleur Neuropathique 4 (DN-4) scale, which is the common diagnosis standard for NeuP (10). The DN-4 scale was used for the definite diagnosis of NeuP in patients with diabetic carpal tunnel syndrome. The questionnaire consists of ten questions evaluating sensory descriptors, and a sensory examination assessing tactile sensation, pinprick, and allodynia. Patients with a DN-4 score of $\geq$4 were diagnosed with NeuP (11).

Diagnosis of carpal tunnel syndrome was based on clinical manifestations, physical examinations, and electrophysiological tests. The symptoms of carpal tunnel syndrome were numbness or tingling in the median nerve distribution for at least one month. The physical examination for carpal tunnel

syndrome detected paresthesia with or without thenar atrophy. The electrophysiology test for carpal tunnel syndrome involved detection of delayed median nerve terminal latency (>3.6 ms). Patients with symptoms but the normal electrophysiology were also diagnosed with carpal tunnel syndrome (12). Patients with extramedian and proximal spread symptoms were also evaluated using spinal MRI or computed tomography to exclude cervical spine-related diseases.

## Baseline characteristics

We reviewed the medical records from the hospital database of all participants to collect basic information, including sex, age, height, weight, educational status, and living habits. We also reviewed the duration of clinical manifestations, including median symptom duration and diabetes symptom duration. Levels of glycosylated hemoglobin (HbA1c) were recorded to determine the severity of diabetes during the past 3 months.

## Clinical manifestations

To evaluate the clinical symptoms of diabetic carpal tunnel syndrome, we used the Boston Carpal Tunnel Syndrome Questionnaire (BCTQ). The BCTQ comprises a symptom severity score (BCTQ-S) and a functional status score (BCTQ-F), which represent symptom severity and functional deficit, respectively, after median nerve compression (13). The BCTQ-S consists of 11 questions related to symptom severity, whereas the BCTQ-F consists of eight questions on hand function during daily activities. Each question is rated on a five-point scale from 1 (none) to 5 (most severe), and the average score of each item is calculated. The validated translated Chinese version of the BCTQ was used for the current study (12).

In addition to the BCTQ, each participant underwent a physical examination of the sensory and motor function of the median nerve. Light touch and pinprick were evaluated using cotton wool and a neurotip on the palm side of the index finger. The sensation was recorded as normal or reduced compared with the same finger on the other hand. The Tinel sign test involves tapping over the median nerve at the entrance of the carpal tunnel, and is considered positive if the patient senses numbness, tingling, and shooting pains in the thumb, index finger, middle finger, the radial half of the ring finger, and the palm. The Phalen test involves flexion of the wrist to the unforced extreme angle for 60 s, and a positive test is recorded if numbness, tingling, and shooting pains are experienced or exaggerated at the distribution of the median nerve. We also evaluated the muscle strength of the abductor pollicis brevis muscle according to the Medical Research Council scale.

## Electrophysiological test

The electrophysiological test was performed using the KEY POINT (Alpine Biomed, Denmark) system. The hand temperature was maintained above 31°C. Median nerve motor function was evaluated by median nerve terminal latency and the compound muscle action potential (CMAP), which were recorded from the abductor pollicis brevis muscle while applying stimulation from the wrist to antecubital fossa. Median nerve sensory function was evaluated by sensory nerve conduction velocity (SNCV), which was recorded from the wrist while stimulating the index finger.

## Pain evaluation

The VAS was used to evaluate the degree of general pain of participants. Patients selected a number that corresponded to their recently experienced pain, on a scale from 0 (no pain) to 10 (the worst pain). We then applied the Neuropathic Pain Symptom Inventory (NPSI), which is a widely used tool for characterizing NeuP symptoms (14, 15). NPSI is a self-reported questionnaire that is specifically designed to evaluate NeuP symptoms and has been validated in more than 50 different languages, including Chinese. This questionnaire comprises five subgroups that represent four aspects of NeuP: burning spontaneous pain, pressing spontaneous pain, paroxysmal pain, evoked pain, and paresthesia/dysesthesia. We also used a combination of the VAS and the body map to indicate pain distributions involved in diabetic carpal tunnel syndrome. We determined and analyzed the involved nerve distribution areas, which included fingers, palm, extramedian distributions, and proximal areas. After comparing the pain distributions between the NeuP group and the non-NeuP group, we investigated the characterstics of neuropathic pain distribution.

## Psychological status evaluation

The pain catastrophizing scale (PCS) consists of 13 self-reported items that measure pain-related catastrophizing phenomena in the context of actual or anticipated pain (16). The PCS questionnaire was used to evaluate patients' psychological status. The PCS measures catastrophizing phenomena along three dimensions: rumination, magnification, and helplessness (17). It has been widely applied to evaluate various pain conditions, such as low back pain, diabetic pain, NeuP, and stroke pain, and has been shown to be related to pain outcomes. We compared PCS scores between NeuP and non-NeuP groups to determine the

pain catastrophizing effect of NeuP in patients with diabetic carpal tunnel syndrome.

## Statistical analysis

Student's t-tests were used to compare the continuous variables between groups, chi-square tests were used to analyze the rate and constituent ratio index, the Shapiro-Wilk test was used to test data normality, and non-normal data were analyzed using the Wilcoxon test. We calculated means and standard deviations for continuous data and frequencies for categorical data. The SPSS software (version 23.0, IBM, New York) was used for all data analyses. We used a $p < 0.05$ to signify significance.

# Results

## Demographic factors between the two groups

The demographic information is listed in **Tables 1**. A total of 198 (71.7%) participants with a DN-4 score of ≥4 were classified into the NeuP group, and the remaining 78 (28.3%) participants with a DN-4 score of <4 were classified into the non-NeuP group. Among the non-NeuP group, half ($n = 39$) of the patients were classified as having no pain, and the other half were classified as having nociceptive pain. We also tested the VAS scores of the NeuP and non-NeuP groups. The VAS score of the NeuP group was 5.5 ± 3.2, which was significantly higher than that of the non-NeuP group ($p = 0.021$). We also compared VAS scores between the NeuP and nociceptive pain patients, and found that the degree of pain of NeuP patients was greater than that of nociceptive pain patients ($p = 0.025$). Notably, we also found that the median symptom duration of the NeuP group was 4.12 ± 2.11 months, whereas the median symptom duration of the non- NeuP group was 8.4 ± 3.22 months ($p = 0.014$). This indicated that diabetic carpal tunnel syndrome patients with NeuP experienced pain symptoms over a shorter period. Finally, we compared the neuropathic pain proportion between the Type 1 or Type 2 diabetic mellitus and the results showed no significant difference ($p = 0.423$).

TABLE 1 Demographic information of the two groups.

| | All | No NeuP | NeuP | *P*-value |
|---|---|---|---|---|
| No of participants, *n* (%) | 276 | 78 (28.3%) | 198 (71.7%) | |
| VAS | 4.5 ± 3.6 | 1.8 ± 1.2 | 5.5 ± 3.2 | **0.021** |
| Gender, *n* (%) | | | | 0.760[a] |
| Female | 216(78.26%) | 59(75.6%) | 157(79.3%) | |
| Male | 60(21.84) | 19(24.4%) | 41(20.7%) | |
| Age, years | 58.0 ± 19.2 | 52.2 ± 10.2 | 60.3 ± 14.9 | 0.133 |
| Mean height, cm | 163.6 ± 21.3 | 159.3 ± 12.2 | 165.4 ± 21.0 | 0.159[b] |
| Mean weight, kg | 74.3 ± 26.3 | 69.3 ± 21.5 | 76.4 ± 23.9 | 0.062 |
| Educational degree, years | 9.0 ± 3.4 | 8.1 ± 2.2 | 9.3 ± 3.11 | 0.681 |
| Living habits | | | | |
| Smoking, *n* (%) | 190(68.8%) | 51(65.4%) | 139(70.0%) | 0.512[a] |
| Cigarettes per day | 8.2 ± 7.9 | 7.4 ± 6.3 | 8.5 ± 7.6 | 0.534[b] |
| Drinking, *n* (%) | 131(47.5%) | 30(38.5%) | 101(51.0%) | 0.214[a] |
| Alcohol per day, gram | 35.3 ± 32.6 | 23.5 ± 11.4 | 40.0 ± 34.1 | 0.256 |
| Median symptom duration, mo | 5.3 ± 2.8 | 8.4 ± 3.2 | 4.1 ± 2.1 | **0.014** |
| Diabetes symptom duration, mo | 32.3 ± 13.5 | 30.1 ± 7.1 | 33.1 ± 12.3 | 0.331[b] |
| HbA1c, % | 7.0 ± 1.1 | 6.9 ± 1.1 | 7.1 ± 0.9 | 0.546 |
| Types of DM, *n* (%) | | | | |
| Type 1 DM | 11(3.99%) | 4(36.36%) | 7(63.64%) | 0.423[a] |
| Type 2 DM | 265(96.01%) | 74(27.92%) | 191(72.08%) | |

Figures of vas score, age, educational degree, mean height, mean weight, cigarettes/alcohol per day, symptom duration and HbA1c were presented with Mean ± SD. Other figures were presented with numbers (percentage).
VAS: Visual Analogue Scale, HbA1c: glycosylated hemoglobin, DM: diabetic mellitus.
Bold values meant *P*-value <0.05 and the figures are statistically different.
[a]Chi-square test.
[b]Mann-Whitney test.

## Paresthesia is related to the occurrence of neuropathic pain

Comparisons of clinical manifestations between the two groups are shown in **Table 2**. We used the BCTQ to assess symptom severity and limb function. Results showed that there was no significant difference between the two groups in terms of functional status ($p = 0.391$). However, symptom severity was greater in the NeuP group than in the non-NeuP group ($p = 0.037$).

Furthermore, the frequencies of light touch and pinprick were more pronounced in the NeuP group than in the non-NeuP group (light touch: $p = 0.001$; pinprick: $p = 0.004$). There were no significant differences in Phalen and Tinel signs, which are considered two important physical examination components in the diagnosis of carpal tunnel syndrome, between the two groups.

## Electrophysiological tests provide clues for neuropathic pain

Electrophysiological results showed that patients in the NeuP group exhibited more SNCV impairment than the non-NeuP group ($p = 0.033$), which provides evidence that nerve injury is related to the occurrence of NeuP (**Table 3**). Although the CMAP did not significantly differ between the two groups, the

TABLE 2 Clinical presentations of the two groups.

| | No NeuP | NeuP | *P*-value |
|---|---|---|---|
| Boston Carpal Tunnel Questionnaire | | | |
| Symptom Severe Score | 2.1 ± 0.7 | 2.9 ± 1.1 | 0.037 |
| Functional Status Score | 2.4 ± 0.6 | 2.8 ± 0.5 | 0.391 |
| Clinical examination *n* abnormal (%) | | | |
| Light tough | 10(12.8%) | 89(44.9%) | **0.001**[a] |
| Pinprick | 15 (19.2%) | 103(52.0%) | 0.004 |
| Phalen test | 53 (67.9%) | 135 (68.2%) | 0.771 |
| Tinel sign | 32 (41.0%) | 98(49.5%) | 0.514 |
| Compression sign | 29(37.2%) | 85(43.0%) | 0.122 |
| Muscle strength | | | |
| MRC3 | 0(0.00%) | 7(3.5%) | 0.486[a] |
| MRC4 | 15(19.2%) | 14(7.1%) | 0.542 |
| MRC5 | 63(80.8%) | 177(89.4%) | 0.061 |
| Thenar atrophy | 15 (19.2%) | 35 (19.7%) | 0.451[a] |

The data sets of symptom severe score and functional status score were expressed as Mean ± SD.
The data sets of other clinical symptoms were expressed as *n* (%).
Bold values meant *P*-value <0.05 and the figures are statistically different.
MRC, Medical Research Council Muscle Strength Scale.
[a]Chi-square test.

median nerve terminal latency of the NeuP group was more prolonged than that of the non-NeuP group (CMAP: $p = 0.341$; median nerve terminal latency: $p = 0.043$; Table 3).

## Neuropathic pain patients tended to experience more extramedian and proximal spread symptoms

After determining the possible risk factors for NeuP in diabetic carpal tunnel syndrome patients, we analyzed the symptom characteristics of NeuP in both groups (Tables 4, 5).

Firstly, we found that the symptom distribution of both groups differed significantly. There were 48 and 27 patients in the NeuP group who had extramedian and proximal spread, respectively, whereas 11 and 9 patients in the non-NeuP group had extramedian and proximal spread, respectively ($p = 0.03$). This suggests that NeuP was not strictly confined to the median nerve distribution. In the non-NeuP group, the majority of patients (44.9%) had symptoms involving two fingers. However, in the NeuP group, most patients (37.8%) had symptoms around the area outside of the median nerve. The analysis of the pain symptoms of diabetic carpal tunnel syndrome patients using the NPSI showed that the most predominant symptoms were paresthesia/dysesthesia, which was observed in 83.33% of patients in the NeuP group and 71.80% of patients in the non-NeuP group. However, we also found that the severity of paresthesia/dysesthesia in the NeuP group was higher than in the non-NeuP group ($p = 0.001$). In addition, the NeuP group had more deep and superficial spontaneous pain than the non-NeuP group (deep spontaneous pain: $p = 0.004$; superficial spontaneous pain: $p = 0.041$).

TABLE 3 Electrophysiologic tests of the two groups.

| | No NeuP | NeuP | *P*-value |
|---|---|---|---|
| Sensory nerve conduction velocity(m/s) | 34.7 ± 8.9 | 30.5 ± 7.9 | 0.033[a] |
| Median nerve terminal latency (ms) | 5.1 ± 1.5 | 6.1 ± 1.9 | 0.043 |
| CMAP (mv) | 5.6 ± 3.4 | 5.8 ± 3.2 | 0.341 |

Data sets of the electrophysiologic tests were presented with Mean ± SD.
CMAP, compound muscle action potential.
[a]Mann-Whitney test.

TABLE 4 Symptomatic comparison of neuropathic pain symptoms between two groups.

| Type | Subgroup | No NeuP | | NeuP | | |
|---|---|---|---|---|---|---|
| | | N | Score | N | Score | P |
| Burning (Superficial) | Spontaneous Pain | 4 (5.1%) | 1.8 ± 0.4 | 56 (28.3%) | 4.8 ± 2.1 | 0.041[a] |
| Pressing (Deep) | Spontaneous Pain | 4 (5.1%) | 1.8 ± 0.4 | 20 (10.1%) | 4.1 ± 2.3 | 0.004 |
| | Paroxysmal Pain | 3 (3.8%) | 2.4 ± 1.0 | 64 (32.3%) | 5.3 ± 2.9 | 0.241 |
| | Evoked Pain | 3 (3.8%) | 3.1 ± 2.0 | 43 (21.7%) | 3.3 ± 2.6 | 0.835 |
| | Paresthesia/ Dysesthesia | 56 (71.8%) | 3.1 ± 2.5 | 165 (83.33%) | 6.7 ± 2.0 | 0.001 |

Figures of pain scores of different subgroups were presented with Mean ± S.
[a]Mann-Whitney test.

TABLE 5 Comparison of symptoms distributions between different groups.

| Fingers affected, *n* (%) | No NeuP | NeuP | *P*-value |
|---|---|---|---|
| 1 | 6 (7.7%) | 10(5.1%) | 0.03[a] |
| 2 | 35 (44.9%) | 40(20.2%) | |
| 3 | 8(10.3%) | 14(7.1%) | |
| 4 | 9(11.5) | 59(29.8%) | |
| Extra median spread | 11(14.1%) | 48(24.2%) | |
| Proximal spread | 9(11.5%) | 27(13.6%) | |

[a]Chi-square test.

## Pain catastrophization always accompanies neuropathic pain patients

Given that NeuP affects the psychological status of carpal tunnel syndrome patients, we also evaluated PCS scores between the two groups (Table 6). The PCS total score of the NeuP group was significantly higher than that of the non-NeuP group ($p = 0.006$). The rumination score of the NeuP

TABLE 6 Comparison of psychologic factors in two groups in diabetic CTS patients.

| Emotional well being | No NeuP | NeuP | *P*-value |
|---|---|---|---|
| PCS total | 8 ± 2.3 | 14 ± 4.2 | 0.006[a] |
| Rumination | 2.6 ± 1.2 | 4.7 ± 3.4 | 0.03 |
| Magnification | 3.0 ± 1.0 | 5.5 ± 2.3 | 0.04 |
| Helplessness | 2.8 ± 1.5 | 4.0 ± 2.3 | 0.062 |

PCS, Pain Catastrophizing Scale.
[a]Mann-Whitney test.

group was 4.7 ± 3.4, whereas that of the non-NeuP group was 2.6 ± 1.2 ($p = 0.03$). The magnification score of the NeuP group was also significantly higher than that of the non-NeuP group ($p = 0.04$). The helplessness score of the NeuP group was markedly higher than the non-NeuP group, although the difference was not significant ($p = 0.062$).

## Discussion

Diabetes mellitus is considered a putative risk factor for carpal tunnel syndrome and is also a shared risk factor for NeuP (18, 19). There has been extensive research investigating the characteristics of NeuP in patients with carpal tunnel syndrome and diabetic carpal tunnel syndrome (3, 4, 20). However, few studies have elucidated the risk factors, symptom characteristics, and related psychological factors of patients with both diabetic carpal tunnel syndrome and NeuP. Therefore, we studied 276 diabetic carpal tunnel syndrome patients and divided them into a NeuP and non-NeuP groups according to DN-4 scale scores. We compared clinical data between the two groups to obtain a better understanding of this subgroup of patients with carpal tunnel syndrome.

The prevalence of NeuP in this cohort of patients was 71.7%, which indicated that the majority of the diabetic carpal tunnel syndrome patients experienced NeuP. Previous studies have also reported similar results. Oteo-Alvaro and Marin reported that 76.7% of patients with carpal tunnel syndrome have NeuP (4). Matesanz et al. also reported a prevalence as high as 80% (3). Moreover, Esma reported that 72.7% of carpal tunnel syndrome patients develop NeuP symptoms. Taken together, we found that the occurrence rate of NeuP in diabetic carpal tunnel syndrome patients is comparable to that in all carpal tunnel syndrome patients.

We then compared demographic data between the two groups, which included sex, age, height, weight, educational status, living habits, symptom duration, and HbA1c level. Of these risk factors, median symptom duration showed a significant difference between the non-NeuP and NeuP groups. The median symptom duration of the NeuP group was shorter than that of the non-NeuP group, which indicated that the NeuP patients developed NeuP at an earlier period. This contradicts the common notion that severity of pain symptoms is correlated with disease duration. A possible explanation is that the median nerve injury of the NeuP group was more severe than that of the non-NeuP group; therefore, patients in the NeuP group developed pain symptoms earlier (21). Although differences in alcohol use and the amount of alcohol consumed did not reach significance, the difference between the NeuP and non-NeuP groups is nevertheless notable because alcoholic neuropathy may contribute to NeuP.

Light touch is a prominent symptom of diabetic carpal tunnel syndrome (1). We found that the incidence of abnormal light touch in the NeuP group was significantly higher than that in the non-NeuP group. Previous studies have shown that sensory function is related to the pain phenomenon (22, 23). Different sensory functional deficits may have different underlying mechanisms. In addition, we found that the SNCV of the NeuP group was significantly slower than that of the non-NeuP group. However, the CMAP did not differ significantly between the two groups. The electrophysiological results showed that the sensory nerve is predisposed to be affected in the NeuP group, whereas motor nerve function does not play a central role in the occurrence of NeuP. The electrophysiological results also suggested that the slower SNCV of the NeuP group accounts for the prolonged median nerve terminal latency.

Comparison of the degree of pain showed that spontaneous pain, both superficial and deep, was more severe in the NeuP group compared with the non-NeuP group. Paresthesia and dysesthesia were also significantly different between the two groups. These results were consistent with the clinical manifestations and electrophysiological results.

In addition to pain symptoms, we investigated pain distributions in the two groups. Results showed that the NeuP group had more extramedian and proximal distributions than the non-NeuP group. It has been reported that central mechanisms are also involved in NeuP in patients with carpal tunnel syndrome (24–26). Our results confirmed that central mechanisms play a vital role in diabetic NeuP. The central mechanisms of carpal tunnel syndrome include sensitization and descending facilitation. Previous studies have reported that hyperalgesia, allodynia, and wind-up in extramedian territories are the main sensitization presentations (27–29). In our study, we found that sensitization also correlated with the occurrence of NeuP in diabetic carpal tunnel syndrome patients.

Previous studies have reported that carpal tunnel syndrome patients' mindset and pain catastrophization are related to outcomes (2). We revealed that the NeuP symptoms of diabetic carpal tunnel syndrome patients involved pain

catastrophization (30). The underlying mechanism may be that nocturnal pain exerts a negative effect on sleep quality. Moreover, declining grip strength may hinder the work and daily life abilities of patients (31). Insomnia and other life disturbances may also predispose patients to catastrophize, which subsequently creates a vicious cycle (32). Furthermore, pain catastrophization is considered to be related to central mechanisms. The pain catastrophization process is involved in mediating the association between central sensitization and pain expectancy (33). Pain catastrophization also exerts harmful and maladaptive effects on the social environment, and amplifies the central processing of pain (34). Therefore, sociopsychological interventions should be developed to disrupt the pain catastrophization process.

Several limitations of the current research warrant discussion. This was a croos-sectional study; thus,we could only provide potentially related factors for the occurrence of NeuP in diabetic carpal tunnel syndrome patients. Determining the exact cause-effect relationship requires further case-control and cohort studies. In addition, we excluded bilateral carpal tunnel syndrome patients because bilateral clinical manifestations present difficulties in making comparisons. However, bilateral symptoms may also be a factor relevant to NeuP. Therefore, in future research, we encourage case-control and cohort studies that include bilateral carpal tunnel syndrome patients.

## Conclusion

Our cross-sectional study of 276 diabetic carpal tunnel syndrome patients revealed that NeuP accounted for the majority of those patients. A total of 198 (71.7%) participants were diagnosed as NeuP. Light touch and electrophysiological test results were related to the occurrence of NeuP. Patients in the NeuP group tended to experience more extramedian and proximal symptoms. Moreover, pain catastrophization was associated with the occurrence of NeuP in patients with diabetic carpal tunnel syndrome.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The studies involving human participants were reviewed and approved by the Ethical Committee of Shenzhen First People's Hospital. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

YL was the primary contributor to the study designation and implementation. YZ supervised all study procedures and revised the manuscript. RHW completed part of the manuscript writing. ZT performed all statistical analyses and checked the data. CC participated in the design of the pain scale. DY supervised and developed the clinical follow-up process. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Etiological and epidemiological characteristics of surgically treated radial nerve lesions: A 20-year single-center experience

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**Introduction:** Radial nerve lesions present a clinical entity that may lead to disability, psychological distress, and job loss, and thus requires great attention. Knowledge of the etiology and exact mechanism of the nerve impairment is of great importance for appropriate management of these patients, and there are only a few papers that focused on these features in patients with surgically treated radial nerve lesions. The lack of studies presenting the etiology and injury mechanisms of surgically treated radial nerve lesions may be due to a relatively small number of specialized referral centers, dispersion to low-flow centers, and a greater focus on the surgical treatment outcomes.

**Aim:** The aim of this study was to describe the etiological and epidemiological characteristics of patients with surgically treated radial nerve lesions of various origins.

**Methods:** This retrospective study evaluated 147 consecutive patients with radial nerve lesion, treated in the department during the last 20 years, from January 1, 2001, until December 31, 2020.

**Results:** The majority of patients belonged to the working population, and 70.1% of them were male. Most commonly, the etiology of nerve lesion was trauma (63.3%) or iatrogenic injury (28.6%), while the less common origin was idiopathic (4.1%) or neoplastic (4.1%). The most frequent location of the lesion was in the upper arm, followed by the elbow and forearm. Fracture-related contusion was the most common mechanism (29.9%), followed by postoperative fibrosis (17.7%), lacerations (17.7%), and compression (15.6%).

**Conclusion:** Based on the fact that traumatic or iatrogenic injuries constitute the majority of cases, with their relevant mechanisms and upper arm predomination, it is crucial to raise awareness and understanding of the radial nerve injuries among orthopedic surgeons to decrease the numbers of these patients and properly preserve or treat them within the initial surgery.

KEYWORDS

radial nerve, etiology, epidemiology, mechanism of injury, surgery

# Introduction

Radial nerve lesions present a clinical entity that may lead to functional loss (1), disability (2), psychological distress (3), and job loss (4) and should be, therefore, recognized as a significant socioeconomic problem (5, 6). Knowledge of the etiology and exact mechanism of the nerve impairment is of great importance for appropriate management of these patients (7), and there are only a few papers that focused on these features in patients with surgically treated radial nerve lesions (8, 9).

While many of the posture or compression-related radial nerve palsies may recover spontaneously, as do some of the contusion lesions associated with bone fractures (10, 11), radial nerve lesions demanding surgery are most commonly caused by trauma (8, 12), unlike lesions of the median and ulnar nerve, whose origin is most often idiopathic entrapment (13). While the frequency of iatrogenic radial nerve lesions referred for surgery is similar to that of other major nerves of the arm (14), neoplastic lesions are rare and account only for a small portion of all peripheral nerve tumors (15).

The radial is the deep seated nerve, adjacent to the bones and frequently subjected to fracture-related contusion (8, 16) or laceration (16–18), by the rule a consequence of humeral shaft fracture in the upper arm (8, 19), while the lesions of the trunk or its main branches in the distal parts of the arm are mostly associated with the elbow, radius, and ulna fractures (8). Less frequently, the nerve may be compressed, contused, lacerated, or cut without an associated bone fracture (8, 9, 20–23).

Because of its frequent association with humeral shaft fracture (24), the majority of studies concerning radial nerve lesions have focused on patients with this associated fracture (10, 16–18, 20, 25). The lack of studies presenting the etiology and injury mechanisms of surgically treated radial nerve lesions may also be due to a relatively small number of specialized referral centers, dispersion to low-flow centers, and a greater focus on the surgical treatment (26).

The aim of this study was to describe the etiological and epidemiological characteristics of patients with surgically treated radial nerve lesions of various origins in a single-center during a 20-year period.

# Materials and methods

## Patients

This is a retrospective study that included 147 consecutive patients with radial nerve lesion treated at the Department for Peripheral Nerve Surgery, Functional Neurosurgery and Pain Management Surgery, Clinic for Neurosurgery, University Clinical Center of Serbia, in Belgrade, Serbia, in a 20-year period from January 1, 2001, to December 31, 2020.

The patients with radial nerve lesions were included in the study according to the following criteria:

### Inclusion criteria

- Patients with ultrasonography and electromyoneurography verified radial nerve lesion referred for surgery and treated during the study period.
- Radial nerve lesion located in the upper arm, elbow, or forearm region.
- Lesion of the radial nerve main branches (deep-motor and superficial-sensitive).
- Posterior interosseous nerve (PIN) lesion.
- Superficial sensory radial nerve (SSRN) lesion.

### Exclusion criteria

- Patients with radial nerve lesion undergoing conservative treatment.
- Radial nerve lesion in the infraclavicular region, as the part of brachial plexus injury.

## Data retrieval

All data in the study were obtained by reviewing patients' hospital records and follow-up examinations. We collected data on age (<25, 26–50, 51–75), gender (male/female), whether belonging to the working-age population (27), area of residence (urban/rural), tobacco smoking (yes/no), associated diseases, etiology of nerve lesion (traumatic/iatrogenic/neoplastic/idiopathic), and mechanism of nerve injury. In addition, for patients with traumatic injuries, we noted the

energy of the trauma (high-energy/low-energy), associated injuries, and nerve continuity (preserved/disrupted).

## Statistical analysis

All statistical procedures were performed with SPSS v26.0 software package (IBM Corporation, Armonk, NY, USA). For descriptions of the parameters of interest, we used the methods of descriptive statistics: mean, median, range, absolute (N), and relative (%) frequencies. The normality of data was assessed using the Shapiro–Wilk test. The association between patients' groups was analyzed using the Chi-square test with a 95% confidence interval, and statistical significance set at $p < 0.05$.

# Results

Out of all studied patients, 104 (70.7%) were male and 43 (29.3%) were female. The patients' age ranged from 12 to 75 years, and the mean age of the population was 38.2 ± 15.3. Two-thirds of the male patients −69 (66.3%) were younger than 40 years (mean age = 35.4), while female patients had more even distribution, counting 24 (55.8%) older than 40 (mean age = 45.1). All patients aged under 18 years were males (12, 14, and 16 years old). The youngest female patient was aged 18, while the oldest male and female patients were aged 72 and 75, respectively.

The majority of studied patients—137 (93.20%)—belonged to the working-age population, and there was a statistically significant difference in the male to female ratio regarding the analyzed age groups: the majority of male patients belonged to the group aged 0–25 years (87.1%), while the most of the women were aged 26–50 years (75.6%). Slightly more than a half of the patients—79 (53.7%)—lived in urban places, while 68 (46.3%) lived in rural places. Comorbidities were present in 43 (29.2%), and 61 (41.5%) patients were tobacco smokers before and at the time of surgery (Table 1).

The most common location of the nerve lesion was in the upper arm −110 (74.8%), followed by the elbow −24 (16.3%) and forearm −13 (8.8%). Almost all elbow injuries—21 (87.5%)—involved the radial nerve trunk, while only 2 involved both radial nerve main branches. The majority of forearm nerve lesions—11 (84.6%)—involved PIN, while only 2 (15.4%) involved SSRN. The mechanisms of nerve injury at different locations in the upper extremity are presented in Figure 1.

Out of all studied patients, 100 (68.0%) had preserved, while 47 (32.0%) had disrupted nerve continuity (complete vs. partial disruption = 46:1).

Out of the total 147 patients, the majority (129) were trauma patients. Nerve injury in these occurred due to the trauma in 93 (72.1%) patients, while 36 (24.5%) developed iatrogenic nerve injury. The remaining six iatrogenic injuries occurred in nontraumatized patients. Neoplastic and idiopathic nerve lesions involved six patients each (Table 2).

TABLE 1 Patient distribution with reference to comorbidities and tobacco smoking within gender and age groups.

| Comorbidities | Gender | | Age groups | | | Total |
|---|---|---|---|---|---|---|
| | Male | Female | 0–25 | 26–50 | 51–75 | |
| Chronic hypertension | 10 | 9 | — | 8 | 11 | 19 |
| Diabetes mellitus | 4 | 4 | 1 | 4 | 3 | 8 |
| Hypothyroidism | — | 5 | — | 4 | 1 | 5 |
| Ischemic heart disease | 2 | — | — | — | 2 | 2 |
| Chronic hypertension and diabetes mellitus | 7 | 2 | — | 2 | 7 | 9 |
| Total | 23 | 20 | 1 | 18 | 24 | 43 |
| Tobacco smoking | 39 | 22 | 15 | 26 | 20 | 61 |

Most of the studied patients—129 (87.7%)—developed nerve lesion due to trauma (high-energy vs. low-energy trauma = 71:58). Males were more commonly injured during road traffic accidents [31 (77.5%)], occupational accidents [27 (87.1%)], and physical confrontation [8 (100%)], while more than a half of the females [20 (54.0%)] were injured during fall from the standing position. Table 3 presents further details on the cause of trauma.

Excluding the radial nerve injury, most of the traumatized patients [110 (85.3%)] had other associated injuries (Table 4), the majority of which [79 (71.9%)] had a humeral shaft fracture.

Table 5 reviews the causes and mechanisms of traumatic nerve injuries. The most common cause were road traffic accidents −27 (29.0%), occupational accidents −26 (28.0%) and falls from the standing position −20 (21.5%). The most common mechanisms of nerve injury were fracture related contusion −44 (47.3%) and laceration −18 (19.3%). The majority of fracture related contusions −30 (68.2%) were a consequence of humeral shaft fracture, as well as 13 (72.2%) lacerations, and 2 (28.6%) cuts. The elbow fractures resulted in 7 (15.9%) contusions and 1 laceration, while radius and/or ulna fractures resulted in 7 (15.9%) contusions and 2 lacerations. The 14 contusions, 5 cuts, and 2 lacerations, without an associated fracture, were a consequence blunt trauma or injury by a sharp object. Two injuries by a sharp object resulted in posttraumatic fibrosis, while all compression injuries occurred due to bad posture during sleep (Saturday night palsy).

Table 6 reviews the causes and mechanisms of iatrogenic nerve injuries. The most common cause was open reduction and internal fixation (ORIF) of the humeral shaft [29 (69.0%)]. The most common mechanism of nerve injury associated with ORIF was postoperative fibrosis [20 (69.0%)], while the less common were nerve entrapment between the

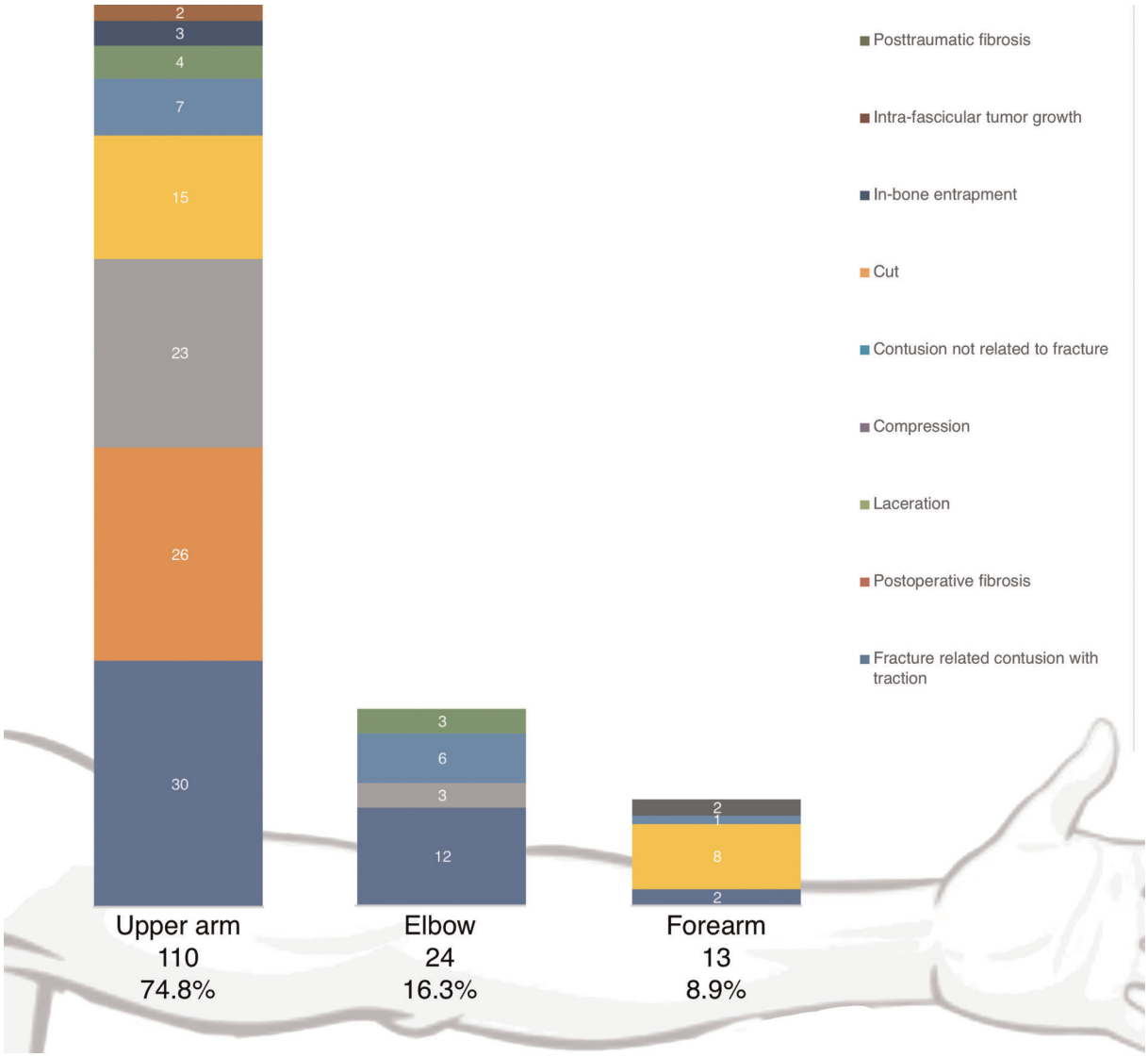


FIGURE 1
Distribution of the patients with reference to location and mechanism of nerve injury.

TABLE 2 Distribution of the patients with reference to etiology, among gender, age groups, and location of the nerve lesion.

| Etiopathogenesis of nerve lesion | Gender | Age groups | | | Location of nerve lesion | | | Total ($n = 147$) |
|---|---|---|---|---|---|---|---|---|
| | | 0–25 | 26–50 | 51–75 | Upper arm | Elbow | Forearm | |
| Traumatic | M | 24 | 39 | 11 | 51 | 20 | 3 | 93 |
| | f | 4 | 9 | 6 | 13 | 4 | 2 | |
| Iatrogenic | m | 3 | 11 | 7 | 21 | — | — | 42 |
| | f | 2 | 7 | 12 | 21 | — | — | |
| Neoplastic | M | — | 4 | — | 2 | — | 2 | 6 |
| | F | — | 2 | — | 2 | — | — | |
| Idiopathic | M | — | 4 | — | — | — | 4 | 6 |
| | F | — | 2 | — | — | — | 2 | |

TABLE 3 Cause of trauma, age, and gender distribution in 129 traumatized patients.

| Cause of trauma (*n* of patients = 129) | Gender | | Age groups | | | Total |
|---|---|---|---|---|---|---|
| | Male | Female | 0–25 | 26–50 | 51–75 | |
| Road traffic accident | 31 | 9 | 13 | 26 | 1 | 40 |
| Fall from the standing position | 18 | 20 | 8 | 9 | 21 | 38 |
| Occupational accident[a] | 27 | 4 | 5 | 13 | 13 | 31 |
| Bad posture during sleep | 5 | 3 | — | 8 | — | 8 |
| Physical confrontation | 8 | — | 5 | 3 | — | 8 |
| Heavy object crushing | 2 | — | 2 | — | — | 2 |
| Shooting with firearms | 1 | — | 1 | — | — | 1 |
| Traction by a dog leash | — | 1 | — | — | 1 | 1 |
| Total | 92 | 37 | 33 | 60 | 36 | 129 |

[a]Occupational accidents included crushing and/or traction by a heavy machine, falls from the height, heavy object crushing, and injuries by a sharp object.

bone fragments [3 (10.3%)], nerve compression by a plate [3 (10.3%)], and nerve laceration [3 (10.3%)]. The osteosynthesis material removal led to nerve laceration in five patients. All cases of tumor resection (six) resulted in postoperative fibrosis. In two cases, repositioning under general anesthesia led to the compression injury.

All patients with neoplastic etiology of the nerve lesion (male vs. female = 4:2) had benign peripheral nerve sheath tumor (PNST), out of whom three had schwannoma (arising from the sensory fibers), two had neurofibroma (arising from the motor fibers), and one had a hybrid tumor with neurofibroma capsule and schwannoma tissue (arising from the sensory fibers). All but two schwannomas in the forearm region originating from the SSRN were located in the upper arm region.

All patients with idiopathic etiology of the nerve lesion (male vs. female −4:2) had PIN entrapment syndrome due to the nerve compression at the supinator muscle arch—the arcade of Frohse.

## Discussion

For more than 40 years, our department is dedicated to the surgery of peripheral nerves. In the last 20 years, we surgically treated 147 patients with radial nerve lesion, which is a remarkable number of cases. The Department for Peripheral Nerve Surgery, Functional Neurosurgery, and Pain Management Surgery at the Clinic for Neurosurgery, Clinical Center of Serbia in Belgrade, Serbia, is a referral center for peripheral nerve injuries and diseases, serving the approximate population of 7 million people of Serbia (28), where every patient in the need for nerve surgery should be

TABLE 4 Location of nerve lesion and other associated injuries.

| Number of other associated injuries (*n* of patients = 110) | Location of nerve lesion | | | Total |
|---|---|---|---|---|
| | Upper arm | Elbow | Forearm | |
| One other associated injury | 52 | 11 | 1 | 64 |
| HSF | 51 | — | — | 51 |
| Lateral epicondyle fracture | — | 2 | — | 2 |
| Elbow fracture | — | 8 | — | 8 |
| Ulna fracture | — | — | 1 | 1 |
| Biceps muscle | 1 | — | — | 1 |
| Brachioradial muscle | — | 1 | — | 1 |
| Two other associated injuries | 14 | 9 | 3 | 26 |
| Radius and ulna fracture | — | 7 | 2 | 9 |
| HSF and radius fracture | 4 | — | — | 4 |
| HSF and EJL | 2 | — | — | 2 |
| HSF and HJL | 2 | — | — | 2 |
| HSF and costa (I-II) fracture | 1 | — | — | 1 |
| HSF and ulnar nerve | 1 | — | — | 1 |
| HSF and subscapular muscle | 1 | — | — | 1 |
| Ulna fracture and epidural hematoma | — | — | 1 | 1 |
| Biceps and triceps muscle | 3 | — | — | 3 |
| Biceps and brachioradial muscle | — | 1 | — | 1 |
| Biceps tendon and brachioradial muscle | — | 1 | — | 1 |
| Three other associated injuries | 11 | — | — | 11 |
| HSF, radius, and ulna fracture | 4 | — | — | 4 |
| HSF, HJL, and ulnar nerve | 2 | — | — | 2 |
| HSF, EJL, and costa (I–III) fracture | 1 | — | — | 1 |
| HSF, costa (III–X), and vertebra (T8) fracture | 1 | — | — | 1 |
| HSF, spleen, and mesentery rupture | 1 | — | — | 1 |
| Femur, pelvis bones, and costa (V–III) fracture | 1 | — | — | 1 |
| Femur, pelvis bones, and tibia fracture | 1 | — | — | 1 |
| Four other associated injuries | 5 | 1 | — | 6 |
| HSF, EJL, radius, and ulna fracture | 1 | — | — | 1 |
| HSF, brachial plexus lesion, radius, and ulna fracture | 1 | — | — | 1 |
| HSF, costa (II–V), vertebra (C2, C3), and femur | 1 | — | — | 1 |
| HSF, vertebra (C2, C3), clavicula, and deltoideus | 1 | — | — | 1 |
| HSF, ulna, femur, and tibia fracture | 1 | — | — | 1 |
| Ulnar and radial artery, ulnar, and median nerve | — | 1 | — | 1 |
| Five other associated injuries | 3 | — | — | 3 |
| HSF, brachial artery, median and ulnar nerve, and hemothorax | 1 | — | — | 1 |
| HSF, median nerve, femur, and fibula fracture | 1 | — | — | 1 |
| HSF, ulnar nerve, ulna, femur, and fibula fracture | 1 | — | — | 1 |
| Total | 85 | 21 | 4 | 110 |

HSF, humeral shaft fracture; EJL, elbow joint luxation; HJL, humeral joint luxation.

TABLE 5 Causes and mechanisms of traumatic radial nerve injuries.

| *N* of patients (=93) | Traumatic nerve lesions | Gender | | Age groups | | | Total |
|---|---|---|---|---|---|---|---|
| | | Male | Female | 0–25 | 26–50 | 51–75 | |
| Cause of injury | Road traffic accident | 22 | 5 | 12 | 15 | — | 27 |
| | Occupational accident | 22 | 4 | 2 | 13 | 11 | 26 |
| | Fall from the standing position | 14 | 6 | 6 | 9 | 5 | 20 |
| | Compression due to bad posture during sleep | 5 | 3 | — | 8 | — | 8 |
| | Physical confrontation | 8 | — | 5 | 3 | — | 8 |
| | Heavy object crushing as a nonoccupational accident | 2 | — | 2 | — | — | 2 |
| | Gunshot wound | 1 | — | 1 | — | — | 1 |
| | Traction by a dog leash | — | 1 | — | — | 1 | 1 |
| Mechanism of injury | Fracture-related contusion with traction | 34 | 10 | 11 | 22 | 11 | 44 |
| | Laceration | 16 | 2 | 7 | 10 | 1 | 18 |
| | Contusion not related to fracture | 12 | 2 | 6 | 8 | — | 14 |
| | Compression | 5 | 3 | — | 8 | — | 8 |
| | Cut | 7 | — | 4 | — | 3 | 7 |
| | Posttraumatic fibrosis | — | 2 | — | — | 2 | 2 |

TABLE 6 Causes and mechanisms of iatrogenic radial nerve injuries.

| *N* of patients (=42) | Iatrogenic nerve lesions | Gender | | Age groups | | | Total |
|---|---|---|---|---|---|---|---|
| | | Male | Female | 0–25 | 26–50 | 51–75 | |
| Cause of injury | Internal fixation of the humeral shaft | 16 | 13 | 5 | 9 | 15 | 29 |
| | Osteosynthetic material removal | 2 | 3 | — | 1 | 4 | 5 |
| | Schwannoma resection | 1 | 3 | — | 4 | — | 4 |
| | Lipoma resection | 2 | — | — | 2 | — | 2 |
| | Repositioning under general Anesthesia | — | 2 | — | 2 | — | 2 |
| Mechanism of injury | Postoperative fibrosis | 13 | 13 | 2 | 11 | 13 | 26 |
| | Laceration | 5 | 3 | 1 | 4 | 3 | 8 |
| | Compression | 1 | 4 | — | 3 | 2 | 5 |
| | In-bone entrapment | 2 | 1 | 2 | — | 1 | 3 |

automatically referred, as well as the complex patients from the former Yugoslavia region. The present study is the largest in Europe and one of the largest published series on the surgical treatment of radial nerve lesions worldwide (8, 19).

Based on our experience, surgical treatment of radial nerve lesions demands the surgeon to meticulously analyze all aspects of the injury and be aware of the relevant surgical treatment options (17, 29, 30). Detailed insight into etiological and epidemiological characteristics (age, working-age, gender, mechanism, location, extent of an injury, etc.) lead to the clear and more accurate prognosis and recovery expectations. This allows us to achieve best possible outcomes and also to avoid additional surgeries, which may compromise the recovery or even lead to severe consequences.

According to the published literature (26, 31–37), the patients referred for peripheral nerve surgery usually belong to the working population, and majority of them are male. This has also been shown in a study of surgically treated radial nerve lesions in general (38) as well as a study of surgically treated radial nerve lesions associated with humeral shaft fractures (17). The results of our study are in accordance with the results of aforementioned studies.

Regarding some studies (39–49), gender of the patients may be associated with the cause of trauma, which is in line with the results of our study. Most of our patients injured during road traffic accidents, occupational accidents, and fights were males, while most of those who fell were females. These results may be explained by a greater chance for male population to participate in traffic (41–44), fights (48, 49), or work with heavy machines and objects (45, 46), unlike the females, which make them prone to these accidents. On the other hand, the lower body strength of females, in general, may be the reason why they suffer severe injuries in low/energy trauma (50).

Most of the patients in our study were traumatized, and the most common etiology of the nerve lesion was traumatic. These results are in accordance with the results of studies concerning surgically treated radial nerve lesions in general (8, 9), as well as radial nerve lesions associated with humeral shaft fracture (17).

The distribution of mechanisms of traumatic nerve injuries referred for surgery mostly depend on the affected nerve and at what location in the extremity the damage occurred (8, 13, 37). The median and ulnar nerve are more superficial (51), and therefore more exposed to laceration and cutting (13, 37), while the radial nerve, which lays close to the bones, is

usually subjected to fracture-related contusion (8, 16). The results of our study may be compared with that of the study by Kim et al. (8). A lower occurrence of our patients with gunshot wounds may be explained by the different firearms available in these two countries (52, 53), as well as the different global peace index (GPI) (54) and different shooting frequency during the study periods (55, 56).

The higher occurrence of iatrogenic nerve lesions in our study, comparing to the study of Kim et al. (8), may be due to different referral of patients in our country. Overall, patients with iatrogenic radial nerve lesions are commonly managed by the surgeons who performed the primary surgery (57). In our country, majority of iatrogenic nerve lesions referred for surgery are managed at our department (14).

Most of the iatrogenic nerve lesions in our study were a consequence of ORIF of the humeral shaft, which is in accordance with the published literature (14, 16, 58). The fact that iatrogenic nerve injuries are a common consequence of the extremity surgery (59) may explain why some of our patients acquired radial nerve lesion during resection of the tumor in the upper arm. The reported cases of iatrogenic radial nerve lesions due to repositioning under general anesthesia are described in the literature (60), and this happened in two of our patients during an emergency surgery. No injection injuries (8) and injuries due to blood pressure cap compression (7) were noted, probably due to the increased awareness of these injuries in the last few decades.

Idiopathic radial nerve lesions may occur due to the nerve entrapment at multiple sites in the upper extremity (7, 61, 62), out of which the most commonly described is at the supinator muscle arch, the arcade of Frohse, which is in accordance with our results. A lower occurrence of patients with radial nerve entrapment in our study, compared to the study of Kim et al., may be due to the low familiarity of treating physicians with this particular entity and a considerable part remaining underdiagnosed.

The most common PNST in the published literature are schwannoma and neurofibroma, usually occurring between the third and fifth decades of life, as solitary lesions, or within neurocutaneous syndromes (15). The schwannomas rarely involve intrafascicular growth of the tumor, unlike neurofibroma, which usually involves several fascicles (63). The results of our study concerning the age, frequency, and presence of infiltration are in accordance with the published literature.

The results of this study suggest that the etiology of radial nerve lesions demanding surgery is most often accidental, rather than health related, and the resources should be directed toward the prevention of such accidents (both traumatic and iatrogenic). Future studies should focus on all aspects of the lesion to better guide the management and potentially predict outcomes of surgical treatment.

The major limitations of this study are its retrospective nature and the involvement of only surgically treated patients. The former may also be the reason for somewhat later referral, as these patients initially seek help from their local medical care providers and get referred only after the definitive failure of conservative treatment. A decent amount of patients received surgical treatment in local centers, especially the iatrogenic cases; therefore, we lack some data that prevent us to analyze the whole patient population.

## Conclusion

The etiology of radial nerve lesion is most often traumatic, and almost all patients belong to the male working-age population. Iatrogenic nerve injuries were frequent and most often a consequence of open reduction and internal fixation of the humeral shaft. The nerve lesions of neoplastic and idiopathic entrapment origin are less frequent in our population.

Based on the fact that traumatic or iatrogenic injuries constitute the majority of cases, with their relevant mechanisms and upper arm predomination, it is crucial to raise awareness and understanding of the radial nerve injuries among orthopedic surgeons to decrease the numbers of these patients and properly preserve or treat them within the initial surgery.

When occurred, the radial nerve lesions may be associated with significant functional and socioeconomic consequences and should be managed by experienced specialists.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Faculty of Medicine in Belgrade. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

All authors have significantly contributed to the manuscript, in design of the study, data gathering, statistical analysis, interpretation of results, and deriving conclusions. The final manuscript was revised by the two senior authors LR and NL,

before submission. All authors contributed to the article and approved the submitted version.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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# A review of the diet, nutrients, and supplementation potential for the outcome augmentation in surgical treatment of peripheral nerve injuries

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**Objective:** Although the studies have shown the beneficial effects of diet, nutrition, and supplementation as an independent treatment modality, their roles are underestimated in the treatment of peripheral nerve injuries. This is in great part due to the development of efficient nerve repair techniques, combined with physical treatment and stimulation. To achieve the best possible functional recovery diet, nutrition, and supplementation should be implemented within a multidisciplinary approach. The aim of the study is to provide insight into the potentially beneficial effects of diet, nutrients, and supplementation, in the limitation of nerve damage and augmentation of the functional recovery after surgery in a review of human and animal studies.

**Methods:** The data relating to the diet, nutrients, and supplementation effects on peripheral nerve injuries and their treatment was extracted from the previously published literature.

**Results:** General balanced diet as well as obesity influence the initial nerve features prior to the injury. In the period following the injury, neuroprotective agents demonstrated beneficial effects prior to surgery, and immediately after the injury, while those potentiating nerve regeneration may be used after the surgical repair to complement the physical treatment and stimulation for improved functional recovery.

**Conclusions:** Standardized diet, nutrition, and supplementation recommendations and protocols may be of great importance for better nerve regeneration and functional recovery as a part of the multidisciplinary approach to achieve the best possible results in surgically treated patients with peripheral nerve injuries in the future.

KEYWORDS

nutrition, supplements, peripheral nervous system, injury, surgery, regeneration, functional recovery

# 1. Introduction

The development of peripheral nerve (PN) surgery after traumatic injuries had reached the limits of functional recovery through contemporary nerve repair techniques (1). The majority of studies dealing with surgical treatment are limited to the surgical perspective only, less commonly combined with physical treatment, and rarely with stimulation, while the medical and other treatment is usually reserved for those not being candidates for surgery (2).

The understanding of the Schwann cells response and the brain plasticity in PN injuries, together with modern nerve repair strategies had led to enviable functional recovery (3, 4). However, these results are in large part improved by mandatory physical treatment and stimulation (5). Diet, nutrients, and supplements impact on the other hand is observed as a sole treatment modality, usually in animal models with crush injury (6). Although the results are encouraging and the conclusions are extremely positive, human studies are lacking.

Complementary supplementation and nutrition are one more point where one can augment the outcome of repair; however, there are no guidelines, recommendations, or review studies to give nerve specialists another card to play with (7).

To understand the real-life impact of diet, nutrients, and supplementation on functional recovery in humans with injured nerves, we are not allowed to deprive the surgical treatment. Nevertheless, the two modalities are rarely combined on purpose. The augmentation of the functional recovery after a reconstructive surgical procedure, through the adjusted diet and nutrition, with additional supplementation is a perspective (8).

This review aimed to provide insight into the effects of diet, nutrients, and supplements related to PN preservation and regeneration after traumatic injury, as well as to imply the significance of outcome augmentation, in addition to the surgical repair in patients with PN injuries, through a review of animal models studies.

# 2. Methods

The data relating to the supplementation, nutrition and diet effects from the studies on PN injuries was extracted. Studies published in scientific literature included in the databases PubMed, Google Scholar, Science Direct, and Web of Science were evaluated. No limitations in terms of study design were applied. Both human and animal studies were included. Special attention was taken to the implications of outcome augmentation after surgical treatment.

To identify the nutritional factors impacting the recovery of the PN after injury, we have performed an initial search, to identify any review studies on diet, nutrients, and supplements' roles in PN injuries. This led to the identification of these factors for further literature search.

Articles of interest were found through the searches of PubMed, Science Direct, and Google Scholar databases using the keywords peripheral nerve OR brachial plexus OR peripheral nervous system AND injury OR trauma in combination with the common terms [e.g., "diet," "nutrient (s)," "supplement", etc.] and the identified factors keywords (e.g., "Vitamin D," "alcohol," etc.).

The analysis of cited references led to the inclusion of even more studies, which were omitted from the search results.

## 2.1. Inclusion criteria

- Nerve injury, including peripheral and cranial nerves,
- Either crush or transection injury, and
- Studies in patients or animal models.

## 2.2. Exclusion criteria

- Review papers and
- Combination with pharmacological agents (drugs).

# 3. Review and discussion

Out of the 42 identified publications, we included 34 relevant animal model experiments. Four reviews were excluded, and one human randomized controlled trial. Also, three animal studies were excluded for the combined use of targeted substances with pharmacological agents (drugs). The details of the studies, with the element in scope, the effect and the proposed mechanism are listed in **Table 1**.

## 3.1. Diet

### 3.1.1. Balanced diet

The lifelong alleviated diet was previously referred to as the attenuation of lipid peroxidation, inflammation, and immune cell infiltration, thus acting neuroprotective, preventing age-related damage to the nerves (9, 10).

The dietary components may have effects on PN alone or in combination. The synergistic effect may be enhanced with the consumption of nutrients and bioactive components together, also supported by the production of endogenous neurotrophic factors that increase environmental nerve repair (6).

### 3.1.2. Energy value

Studies on the brain, spinal cord, and nerve regeneration have previously related low-calorie diet and hunger to

TABLE 1 Diet, nutrients, and supplements playing a role in peripheral nerve injuries with their effects and proposed mechanisms.

| | Author(s), date | Subjects | Injured nerve | No. of cases | Effect | Mechanism observed |
|---|---|---|---|---|---|---|
| Diet | | | | | | |
| Balanced diet | Opalach et al. (2010) | Rats | Sciatic nerve | 12 | Maintains a younger state in peripheral nerves | Age-related oxidative damage defy. |
| Energy value | Rangaraju et al. (2009) | Rats | Sciatic nerve | N/A | The improvements in nerve architecture with diet restriction. | By sustained expression of protein chaperones and markers of the autophagy–lysosomal pathway. |
| Obesity | Bekar et al. (2014) | Rats | Sciatic nerve | 24 | Obesity may affect peripheral nerve structure and regeneration negatively after crush injury.<br>Schwann cells were stained darkly and appeared damaged.<br>Myelinated axons had irregular myelin sheaths:<br>Endoneurium was more pronounced<br>Regenerated axons were smaller | Obesity may reduce the amounts of growth factors (GAP-43) or their effectiveness, which results in a delay in regeneration and recovery. |
| Alcohol | Ertem et al. (2009) | Rats | Posterior tibial nerve | 32 | Alcohol intake has negative influences on peripheral nerve regeneration:<br>Severe axonal loss<br>Myelin degeneration<br>Regenerative clusters<br>Endoneural fibrosis | Direct toxic effect of alcohol is suggested, although subsequential malnutrition may play a more important role. |
| Nutrients | | | | | | |
| Carbohydrates | Singer and Mehler (1983) | Rats | Hypoglossal nerve | 12 | A relatively greater amount of glucose is taken up into the regenerating nucleus at the time of 2-deoxyglucose infusion in fasting patients. | Probably due to increased glucose use during fasting, a deficit of glucose occurs in the regenerating nucleus. |
| Lipids | Liskiewicz et al. (2016) | Rats | Sciatic nerve | 51 | Regeneration of sciatic nerves was improved in ketogenic diet preconditioned rats. | Ketogenic diet may influence growth of mature nerve fibers, thus improving regeneration. |
| | Michael-Titus (2007) | Mice | Facial nerve | 12 | Dietary administration of docosahexaenoic omega-3 fatty acid expresses neuroprotective and pro-regenerative effects after nerve injury. | Significant effect on the response of neurons and microglia to injury, and appears to promote a pro-regenerative response. |
| | Gladman et al. (2012) | Mice | Sciatic nerve | 22 | Polyunsaturated fatty acids mediate neuroprotective and pro-regenerative effects, while also inhibiting neuroinflammation and oxidative stress | The exact mechanism is not known, and it is considered to be a combination of targets, from ion channels to nuclear receptors. |
| | Silva et al. (2017) | Mice | Sciatic nerve | N/A | Oral administration of combined eicosapentaenoic and docosahexaenoic acids accelerates regeneration, prevents neuropathic pain, and possibly expresses protective properties after peripheral nerve injury. | Modulation of glial cells, which are considered the main producers of tumor necrosis factors, may be involved in these effects. |
| | Avila-Martin et al. (2015) | Rats | Spared Nerve Injury Model[a] | 47 | Omega-9 fatty acids reduce noxious hyperreflexia and pain-related anxiety behavior following peripheral nerve injury. | Decreases the COX-2/COX-1 ratio in lipopolysaccharide-activated macrophage cells and OX-42 expression within the ipsilateral lumbar spinal dorsal horn |
| Alpha-lipoic acid | Demir et al. (2014) | Rats | Sciatic nerve | 24 | Alpha-lipoic acid has a protective effect through the improvement of regeneration of the injured nerve. | Antiapoptotic and anti-inflammatory effects. |
| | Haidar et al. (2020) | Rats | Sciatic nerve | 126 | Implatation of composite nanofiber sheets incorporating alpha-lipoic acid and atrovastatin improved both motor and sensory recovery | This novel formulation uses multiple neuroprotective drugs presented together, but with the different release profiles on nerve regeneration. |

(*continued*)





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TABLE 1 Continued

| | Author(s), date | Subjects | Injured nerve | No. of cases | Effect | Mechanism observed |
|---|---|---|---|---|---|---|
| | Wang et al. (2021) | Rats | Sciatic nerve | 48 | Alpha-lipoic acid may be used to treat neuropathic pain caused by peripheral nerve injury | Significantly shortened paw withdrawal threshold and latency, improved morphologic changes in the dorsal root ganglia, reduced the aggregation and proliferation of satellite glial cells, and decreased numbers of P53 + cell |
| Proteins | Pan et al. (2009) | Rats | Sciatic nerve | 122 | Oral intake of natto increases regeneration (improvement of motor function and in electrophysiological study), decreases vacuole number, increased angiogenesis and axon counts and improves expression of myelin | Decreases injury-induced fibrin deposition, improves injury-induced disruption of blood-nerve barrier and loss of matrix component such as laminin and fibronectin. Attenuates the production of TNF-alpha and apoptosis. |
| Leucin | Singer and Mehler (1983) | Rats | Hypoglossal nerve | 12 | Regenerating neurons leucine uptake in fasted animals is increased. | Either due to the deficit of amino acid produced by fasting, inducing or derepressing enzymes which transport leucine, or amino acid metabolism depression because of the deficit of glucose. |
| Carnosine | Mirzakhani et al. (2018) | Rats | Sciatic nerve | 72 | The regenerating effect of carnosine on the muscle mass is likely through: acceleration of functional recovery improvement of histological and ultrastructural alterations | Suppression of lipid peroxidation, provokes antioxidant enzyme activity and amelioration of cytokine production. |
| Acetyl-L-carnitine | Avsar et al. (2014) | Rats | Sciatic nerve | 24 | Functional recovery of rats treated with acetyl-L-carnitine significantly improved in walking track analysis, and the latencies of the somatosensory evoked potentials components were significantly decreased. | Acetyl-L-carnitine accelerates sciatic nerve regeneration by reducing apoptosis and lipid peroxidation and promoting myelinization. |
| | Mohammadi et Amini (2017) | Rats | Sciatic nerve | N/A | Acetyl-L-carnitine loaded in a silicone tube bridging the nerve defect improves functional recovery and quantitative morphometric indices of sciatic nerve. | Faster recovery of the axons led to the statistically significant difference between the muscle weight ratios, and morphometric indices showed that the number and diameter of the myelinated fibers was higher in the treated group. |
| | Kokkalis et al. (2009) | Rats | Distal to end-to-side transfer | 25 | The ability of the acetyl-L-carnitine to enhance nerve regeneration after end-to-side neurorrhaphy in combination with various types of donor nerve injury distal to the coaptation site | Administration of acetyl-L-carnitine alone did not prove to be a stimulus, but in an injury model of the donor nerve (crush injury) proved to be a significantly more potent stimulus for regeneration. |
| | Wilson et al., 2010 | Rats | Sciatic nerve | 10 | Adjuvant acetyl-L-carnitine treatment after transection and repair may improve both sensory and motor outcomes and merits further investigation. | Increases the number of regenerating nerve fibers but also morphologically improves the quality of regeneration and target organ weight and reinnervation signs. |
| Vitamin B group | Altun and Kurutas (2016) | Rats | Sciatic nerve | 80 | Tissue levels of vitamin B complex and vitamin $B_{12}$ vary with progression of crush-induced peripheral nerve injury. | |
| | Kong et al. (2004) | Rats | Optic nerve | 24 | Intramuscular injections immediately after crush injury and then every 2 days demonstrated the potential for vitamin $B_{12}$ as a neuroprotective agent after optic nerve crush injury. | Axons preservation was noted and more axons and retinal ganglion cells remained in the treated group. |
| | Kang et al. (2019) | rats | Sciatic nerve | 9 (11) | Folic acid might improve peripheral nerve injury repair. | Promotes Schwann cell proliferation, migration, and secretion of nerve growth factor |
| Vitamin D | Chabas et al. (2013) | Rats | Peroneal nerve | 36 | Cholecalciferol induces a significant locomotor and electrophysiological recovery. Cholecalciferol increases number of preserved or newly formed axons in the proximal end mean axon diameter in the distal end neurite myelination in both distal and proximal ends | Acts on myelination *via* the activation of several myelin-associated genes involved in axogenesis and myelination. |
| Vitamin E | Tamaddonfard et al. (2014) | Rats | Sciatic nerve | 60 | Vitamin E (from Safranal) produced improving effects on crushed-injured sciatic nerve functions. | These effects may be mediated through antioxidant effects by reducing MDA level. |

(*continued*)





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TABLE 1 Continued

| | Author(s), date | Subjects | Injured nerve | No. of cases | Effect | Mechanism observed |
|---|---|---|---|---|---|---|
| | Lu et al. (2011) | Mice | Spared Nerve Injury Model[a] | 32 | Neuropathic pain developed after peripheral nerve injury may be inhibited by combination of vitamin C and vitamin E. | Probably through the antioxidative and anti-inflammatory effects. |
| Magnesium | Pan et al. (2011) | Mice | Sciatic nerve | 18 | Improved neurological function recovery and enhanced nerve regeneration were found in mice with a sciatic nerve injury. Schwann cells may have been rescued from apoptosis by the suppression of inflammatory responses. | Mg supplementation improves: neurobehavioral, electrophysiological functions, enhanced regeneration marker, and reduced deposits of inflammatory cells as well as expression of inflammatory cytokines. Reduces Schwann cell apoptosis was in line with the significant expression of bcl-2, bcl-X (L) and down-regulated expression of active caspase-3 and cytochrome C. |
| Supplementation | | | | | | |
| Epigallocatechin-3-gallate (in Green tea) | Renno et al. (2006)<br>Kian et al. (2018) | Rats<br>Rats | Sciatic nerve<br>Sciatic nerve | 30<br>56 | Epigallocatechin-3-gallate therapy has neuroprotective effects against trauma induced degeneration. | The mechanism of action of GT in reducing locomotion deficits and hyperalgesia that are often associated with peripheral nerve injury, is yet to be discovered. |
| Evening primrose oil | Ramli et al. (2017) | Rats | Sciatic nerve | 72 | Evening primrose oil supplementation improved peripheral nerve recovery in rats, by preserving: the shape of the axons the thickness of the myelin sheath the diameter of the axons | Acts *via* substrate for production of vasodilator prostanoids such as prostacyclin to improve nerve perfusion. |
| Sesame oil | Hsu et al. (2016) | Mice | Sciatic nerve | 30 | Sesame oil improved electrophysiological and functional assessments in mice with sciatic nerve crush injury, having beneficial effects on sciatic nerve regeneration and functional recovery. | Sesame oil significantly decreased lipid peroxidation and increased Nrf2 and GAP43 expression in sciatic nerve. |
| Melatonin | Rateb et al. 2017 | Rats | Sciatic nerve | 40 | Melatonin had a significant role in improvement of the recovery of damaged sciatic nerves in rats. Especially when given at the dark period. | Through its antioxidant, antiapoptotic, anti-inflammatory effects and nerve growth factor stimulation. |
| | Pan et al. (2021) | Rats | Sciatic nerve | 48 | Proliferation and migration abilities of schwann cells in the melatonin group were significantly higher than those of Schwann cells in the control group | Bioinformatics analysis showed that Shh may be the key gene for the promotion of peripheral nerve regeneration by melatonin |
| Creatine | Helvacioglu et al. (2018) | Rats | Sciatic nerve | 15 | In rats, supplementation with creatine had a positive effect on the regeneration of injured sciatic nerve. | Probably diminishes the harmful effects of peripheral nerve crush injury. |

[a]The Spared Nerve Injury model involves ligation of two of the three branches of the sciatic nerve (the tibial nerve and the common peroneal nerve), while the sural nerve is left intact.





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improved regeneration (11). Limitation of energy intake in these patients is however complicated, and advising the patients to limit their energy intake is at least controversial.

### 3.1.3. Obesity

Obesity may negatively affect PN regeneration after injury, through the reduction of growth factors amounts or their effectiveness, resulting in the delay of the regeneration and recovery.

The study of morphological features revealed significant differences in nerve structure and regeneration caused by the negative effects on axon number, myelin thickness, nerve area, the amplitude of compound action potential, and reduction in the number of growth factors in the sciatic nerve-injured rats due to fat-diet induced obesity (12).

### 3.1.4. Alcohol

Neuropathy due to alcohol abuse is a known entity (13). Painful peripheral neuropathy resulting from the excessive and chronic use of alcohol occurs due to an unknown pathophysiological mechanism (14). Apart from malnutrition and nutrient deficiency occurring due to malabsorption, the direct neurotoxic effect was shown to be an independent factor in the development of the disease (15).

The same factors influence the regeneration of the nerve after injury. The study on rat models has demonstrated the negative impact of alcohol in rats with transected nerves (16).

These effects were previously evaluated in the Danish study by Behse and Buchtal who have compared the groups with alcoholic neuropathy and malnutrition neuropathy (17). In this study, Danish beer was the predominant alcoholic beverage, fortified with thiamine and Vitamin B6 at that time, and resulted in an absence of malnutrition, further development of symptoms and even led to weight gain. Alcoholic neuropathy group symptoms were related to pain, while the malnutrition group experienced progressive weakness, casting a decent shadow on the direct neurotoxic effect of alcohol *per se* (18).

## 3.2. Macronutrients

### 3.2.1. Carbohydrates and lipids

The type and quantity of lipids may influence the regeneration of the PN by several mechanisms including pro-regenerative and neuroprotective, as well as pro-inflammatory and neurodegenerative effects when intake exceeds reasonable amounts.

A ketogenic diet had been attributed to a neuroprotective effect on PN, although more clinical trials are needed to prove the effects (19).

There is no specific recommendation, but the lipid content type and appropriate $n-6/n-3$ ratio seem to make a significant influence on nerve regeneration. Omega-3 and omega-9 polyunsaturated fatty acid's positive effects were demonstrated.

#### *3.2.1.1. Omega-3 fatty acids*

Experimental studies support the use of polyunsaturated fatty acids as a promising pharmacological approach in PN injuries.

Polyunsaturated fatty acids, such as eicosapentaenoic and docosahexaenoic acids, mediate neuroprotective and pro-regenerative effects, while also inhibiting neuroinflammation and oxidative stress. The exact mechanism is not known, and it is considered to be a combination of targets, from ion channels to nuclear receptors (20).

In mice, oral administration of combined eicosapentaenoic and docosahexaenoic acids had regenerative and possibly protective properties after PN injury (21).

#### *3.2.1.2. Omega-9 fatty acids*

CIS-monounsaturated omega-9 fatty acid—oleic acid, administered in combination with albumin or as 2-hydroxyoleic acid, promotes antinociception and anxiolytic effects following both central and PN injury. Although these results are generally positive, the impact on regeneration is questionable. Motor function improvement and spasticity reduction were observed in patients with spinal cord injury, implicating the possibility of a positive effect on PN (22).

#### *3.2.1.3. Alpha-lipoic acid*

In rats with sciatic nerve injury, alpha-lipoic acid has a protective effect through the improvement of regeneration of the injured nerve by its antiapoptotic and anti-inflammatory effects (23), while the implantation of composite nanofiber sheets incorporating alpha-lipoic acid and atorvastatin contributed to the recovery of the motor and sensory function and nerve regeneration (24). As a treatment for neuropathic pain caused by PN injury potentially is considered alpha-lipoic acid, which requires further verification (25).

### 3.2.2. Proteins and amino acids

The consumption of protein resources with high biological quality, especially including essential amino acids must, be maintained at a certain level to meet organism requirements (6).

The specific role in augmentation is reflected by the effects of natto (extracts of fermented soybeans) on the improvement of motor function and in electrophysiological results, through a complex nerve preservation mechanism (26).

#### *3.2.2.1. Leucin*

The study of Singer and Mehler, also tried to explain the increased leucine uptake in fasted animals. The two options included (1) deficit of amino acids produced by fasting, inducing or derepressing enzymes, which transport leucine, and (2) amino acid metabolism depression because of the

deficit of glucose (resulting in a deficit of metabolic products of amino acids metabolism which could accelerate uptake) (27).

#### *3.2.2.2. Carnosine*

The gastrocnemius muscle mass reduction from a sciatic nerve crush injury may be improved near to its normal value with carnosine supplementation. The beneficial effects may be associated with the acceleration of functional recovery and the improvement of histological and ultrastructural alterations through the mechanisms of suppression of lipid peroxidation, provoking of antioxidant enzyme activity and amelioration of cytokine production (28).

### 3.2.3. Acetyl-L-carnitine

The modified amino acid is one of the most researched nutrients in peripheral nerve injuries and their surgical treatment. Several studies carried out in animals were published on the various applications of acetyl-L-carnitine for nerve preservation (29), regeneration (30), and augmentation of surgical treatment by both topical (31) and systemic administration (32), even in the delayed fashion (33).

The results of all these studies demonstrated beneficial effects on regeneration; however, the neuroprotective effects were not as pronounced, especially when the acetyl-L-carnitine was given in a delayed fashion (7 days after injury). A recent study evaluated individual and combined effects of erythropoietin and acetyl-L-carnitine; however, regardless of the positive impact from both, combined improved efficacy was not found (34).

The only human study related to nutritional therapy failed to stress the importance of diet nutrition and supplementation in nerve regeneration, although in a chronic entrapment, not the injury. In this double-blinded, randomized, placebo-controlled study, which included adult patients with severe carpal tunnel syndrome acetyl-L-carnitine did not improve nerve regeneration (35).

## 3.3. Micronutrients

### 3.3.1. Vitamins B6 and B12

The beneficial effects of group B6 and B12 vitamins in peripheral neuropathies are well known. The possibility and impact of the improvement of nerve regeneration after injury are not sufficiently clarified. It was previously shown that the levels of the vitamin B complex are significantly lower immediately after the injury, with progression through time Supplementation of vitamin B complex in the acute period of PN injury deserves to be considered as an option, which may be useful for the acceleration of nerve regeneration (36). However, the mechanism of action in injured nerve regeneration is different, due to the different types of nerve lesions. Namely, the immediate damage, and the role of supplements is induction of regeneration, not protection (9).

Vitamin B12 (methylcobalamin) has an analgesic effect which may be explained by improving nerve conduction velocity and regeneration of injured nerve. Also, in neuropathic pain states, methylcobalamin inhibited the ectopic spontaneous discharges from peripheral sensory neurons (37).

The underlying pathophysiological mechanism is probably neuronal protection by promotion of regeneration of injured nerves (38) while antagonizing glutamate-induced neurotoxicity (37).

### 3.3.2. Folic acid

Supplementation with folic acid improved the organoleptic features of spinal axons in *in vivo* grafted PN segments of adult Sprague-Dawley rats. The same positive effects were noted in spinal cord contusion injuries, emphasizing that the folic acid supplementation should not be limited only to the embryonic period and prevention of neural-tube defects, but also to augmentation of functional recovery in surgically treated PN injuries (39).

In rats, folic acid might improve PN injury repair. Namely, Schwann cells' proliferation and migration, as well as nerve growth factors' secretion were promoted by folic acid (40).

### 3.3.3. Vitamin D

Vitamin D is known for its potential in immune response regulation in various diseases. In PN injury, the positive effect on myelination was demonstrated in rat models. This specific effect is intended to be used solely, although the use with the surgical repair might lead to a better functional recovery.

Chabas et al. on animal models have proven the potential of ergocalciferol (Vitamin D2) and cholecalciferol (Vitamin D3) in the augmentation of the spinal cord and PN regeneration. An analysis of the gene, which regulates Vitamin D3 in the ganglia of the back roots and Schwan's cells, was also performed.

Cholecalciferol is more effective than ergocalciferol and, when administered at a high dose (500 IJ/kg/day), cholecalciferol induces significant locomotor and electrophysiological recovery by increasing the number of preserved and newly formed axons at the proximal end, the mean diameter of the axon at the distal end, and induction of myelination at both distal and proximal ends.

A modified expression of several genes involved in axonogenesis and myelination was also found, after 24 h of vitamin D3 introduction, which leads to the conclusion that Vitamin D acts on myelinization by activating several associated genes (41).

#### 3.3.4. Vitamin E

One study, analyzing the impact of vitamin E, found some improving effects on motor impairment, pain hypersensitivity, Wallerian degeneration, and muscular atrophy induced by a sciatic nerve crush injury. The effect is probably due to the inhibition of the oxidative stress pathway by reducing the malondialdehyde level.

Due to the high contents of Vitamin E, safranal, a major component of saffron, is recommended as a dietary supplement in patients with nerve injury (42).

It was also previously demonstrated that neuropathic pain developed after PN injury may be inhibited by the combination of vitamin C and vitamin E, probably through the antioxidative and anti-inflammatory effects (43).

#### 3.3.5. Magnesium (Mg)

Magnesium supplements significantly improve functional recovery in various neurological disorders, especially in cerebrovascular disease through the decrease in systemic vascular resistance and improvement of cardiac function (44). Not as much data are available on PN injuries related effects, and even those studies involve filaments and wires, rather than supplementation (45, 46). In a study of the injury of the sciatic nerve in an animal model, the diet with high magnesium content significantly increased plasma and plasma magnesium concentrations. In addition, magnesium supplements improved neurobehavioral and electrophysiological functions, improved regeneration markers, and reduced inflammatory cell deposits, as well as the expression of inflammatory cytokines. Schwan cell cellular apoptosis was also reduced in accordance with significant expression of Bcl-2, Bcl-KSL and decreased expression of active Caspase-3 and cytochrome C. It was concluded that magnesium positively influences neurological regeneration and improves neural regeneration, while also preserving Schwan's cells of apoptosis by suppressing inflammatory response (47).

### 3.4. Supplementation

#### 3.4.1. Green tea

The intake of green tea may assist nerve recovery after traumatic injuries. Although more studies should explore the cellular and molecular mechanisms, which mediate such effect, initial studies with a green tea polyphenol epigallocatechin gallate led to some promising conclusions (48).

##### *3.4.1.1. Epigallocatechin gallate*

Prior to the study on PN, a possible therapeutic effect in spinal cord injury was noticed through the improvement in the flat beam test. Furthermore, at an early stage of spinal cord injury, inflammatory cytokines were modulated and axonal sprouting was higher (49).

The study on sciatic nerve transection injury revealed biochemical, histopathological, and immunohistochemical evidence that epigallocatechin gallate therapy may have neuroprotective effects against injury-induced degeneration (50).

#### 3.4.2. Sesame oil

Polyunsaturated (omega-6), and monounsaturated (omega-9) fatty acids account for more than 80% of the total fat contents of sesame oil. Together with the natural antioxidant sesamol and vitamin E, the content assures a neuroprotective effect.

In a study by Hsu et al. sesame oil improved electrophysiological and functional assessments in mice with sciatic nerve crush. The beneficial effects are based on significantly decreased lipid peroxidation and increased Nrf2 and GAP43 expression in the sciatic nerve (51).

#### 3.4.3. Evening primrose oil

Supplementation with evening primrose oil might be significant in the therapy of PN injury. It was shown that evening primrose oil supplementation improved PN recovery in rats (52).

#### 3.4.4. Melatonin

Melatonin may improve the proliferation and migration of Schwann cells *via* the Sonic Hedgehog signaling pathway after PN injury and in that way promote PN regeneration (53). Melatonin had a significant role in the improvement of the recovery of damaged sciatic nerves in rats, through its antioxidant, antiapoptotic, anti-inflammatory effects, and nerve growth factor stimulation. Giving melatonin during the dark period had better results than giving melatonin during the light period (54).

#### 3.4.5. Creatine

In rats, supplementation with creatine had a positive effect on the regeneration of injured sciatic nerve, which was also verified by electronic microscopy (55).

### 3.5. Implications and limitations

Reviewed studies comprised different animal models and different options involving the dietary and nutritional interventions as well as the supplements used for the improvement of nerve preservation and regeneration after injury, as well as for the outcome augmentation following surgical repair.

Since the whole review is based on animal studies, we could not derive clear conclusions or recommendations for

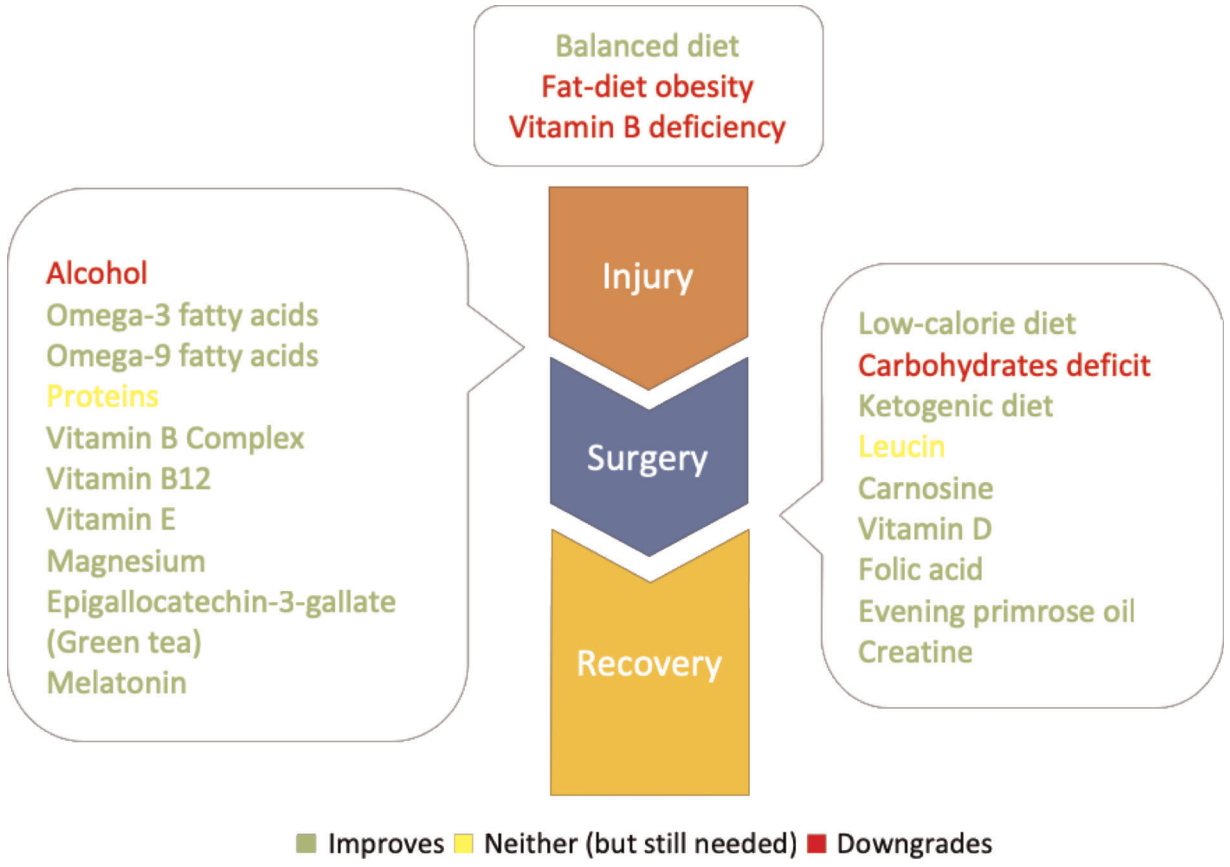


FIGURE 1
Diet, nutrients and supplements with their respective target points: up: predisposing factors; left: predominately neuroprotective (could be taken from the injury onset); right: predominately neuroregenerative (could be taken after the surgery).

surgical treatment augmentation in the human population, but focused to give an insight into every possible dietary, nutritional or supplement-related influence.

Possible mechanisms of action are shown in Table 1 in detail and give a significant contribution to a deeper understanding of the general aspects of diet, nutrition, and supplementation effects on the peripheral nervous system.

To provide some initial waypoint, based on our review and the presumed mechanism of action, the specific target points for the introduction or activity of the individual nutrients and supplements were marked in Figure 1.

## 4. Conclusions

Standardized diet, nutrition, and supplementation protocols may be of the greatest importance for better nerve regeneration and functional recovery, as a part of the multidisciplinary approach to achieve the best possible results in patients with PN injuries in the future.

The augmentation of functional recovery, as a part of the multidisciplinary approach, is far less controversial than the treatment of PN injuries based entirely on conservative options. Further human studies should focus on the augmentation of functional recovery, in addition to the surgical treatment of PN injuries, to clarify the definite underlying mechanisms and give clear recommendations and guidelines for better outcomes.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

## Author contributions

All authors have contributed significantly to the paper. ML and SM-R designed the study, SL and NB gathered the data, and LR revised the manuscript as an expert in peripheral nerve

surgery. All authors contributed to the article and approved the submitted version.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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# Useful functional recovery and quality of life after surgical treatment of peroneal nerve injuries

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Closed injuries to the peroneal nerve recover spontaneously in about a third of patients, but surgery may be needed in the remaining 2/3. The recovery after surgery is not always satisfactory and the patients may need an orthosis or a walking aid to cope with regular daily activities. This study aimed to evaluate the useful functional recovery and quality of life (QoL) in surgically treated patients with peroneal nerve (PN) injuries. The study involved 51 patients who have undergone surgical treatment due to PN injury in our department, within a 15-year period (2006–2020). Thirty patients (59%) were treated with neurolysis, 12 (23%) with nerve repair techniques, and 9 (18%) with tendon transfer (TT). Neurolysis is employed in the least extensive nerve injuries when nerve continuity is preserved and yields a motor recovery ratio of almost 80%. Nerve repairs were followed by 58.33% of patients achieving M3+ recovery, while 41.66% recovered to the useful functional state (M4 or M5) With the use of TTs, all patients recovered to the M3+, while 66.7% recovered to M4. All our results correspond to the results of previous studies. No statistically significant differences were found regarding the QoL of the groups. There is an apparent advantage of neurolysis, over nerve repair, over TT procedure, both in terms of useful functional recovery, and foot-drop-related QoL. However, when involving all aspects of QoL, these advantages diminish. The individual approach leads to optimal results in all groups of patients.

KEYWORDS
peroneal nerve, trauma, outcome, quality of life, surgery

## Introduction

Peripheral nerve injuries account for 2%–3% of all patients admitted to primary-level trauma centers (1). According to the previously published data, they are present in less than 2% of patients with limb injuries, while less than 1% of cases involve a peripheral nerve injury in the lower extremity (2). Although rare, the injuries may have a

devastating impact on all aspects of living and significantly decrease quality of life (QoL) (3).

Peroneal nerve (PN) palsy presented as "foot drop", is the most common mononeuropathy, but the majority of cases are related to radiculopathy and herniated discs, with favorable natural history and treatment outcomes (4), and rarely entrapment neuropathy or injury (5). Closed injuries to the PN may recover spontaneously, without specific treatment, in about a third of patients, but in the remaining 2/3, a permanent foot drop remains (6, 7). These patients are usually candidates for surgery, followed by a prolonged period of physical treatment and methods for the augmentation of nerve recovery in a multidisciplinary setting (8). The recovery is not always sufficient, and even M3 according to the Medical Research Council (MRC) scale for muscle strength (9), may end up as insufficient for some patients. Sometimes, a traditional or high-tech orthosis or walking aid may be needed to cope with regular daily activities (10).

Surgical options to treat these patients include procedures aimed at the exploration and various forms of neurolysis (external, internal, intrafascicular) (11), and nerve repair options (direct repair, grafts, or artificial conduits) (12, 13). Nerve transfers do not have the same beneficiary effect in the lower as they do in the upper extremity (14, 15), while tendon transfers (TTs) may be applied with success when recovery capacity is compromised (16, 17). Previous studies reported different results of motor recovery with the use of these techniques (18), and their combined use efficacy (19).

Motor recovery is the most commonly measured outcome, and there are some tools to asses useful functional recovery and QoL, however, with some applicable limitations. Patients treated for lower extremity nerve injuries were rarely evaluated in light of QoL before, while the foot drop is usually evaluated in terms of QoL with a focus on the need for braces, most commonly with the Stanmore system (20, 21).

This study aimed to evaluate useful functional recovery and QoL in surgically treated patients with PN injuries, as well as the evaluation of chosen general QoL inventories in terms of lower limb and PN affection.

# Methods

The study involved a retrospective series of patients who have undergone surgical treatment due to the PN injury at our department in a 15-year period from January 2006 to December 2020.

All procedures performed were in accordance with the institutional ethical standards and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants included in the study.

## Inclusion criteria

- Patients with surgically treated PN injury
- Traumatic and iatrogenic nature of injury
- Common stem or branches involvement

## Exclusion criteria

- Bilateral PN injury
- Associated tibial nerve injury

Out of 57 surgically treated patients fulfilling the inclusion criteria, six patients were excluded: one due to the bilateral injury, and five due to the associated tibial nerve injury.

## Management

Within the preoperative evaluation, patients and injury characteristics were recorded, including the details on previous surgical interventions and associated injuries. Muscle strength was evaluated using the MRC, sensibility of the affected region using the Mackinnon–Dellon scale (MDS) (22), and the visual-analog scale (VAS) for the assessment of pain.

The individually tailored approach and decision-making on the modality of surgical treatment depended on two important features: (1) nerve continuity and (2) reinnervation capacity; based on the clinical and neurological examination, electrophysiology, imaging, and the time passed from injury to our initial examination.

Neurolysis was performed in patients with preserved continuity and reinnervation capacity (preserved efferent muscles); nerve repair with graft when the nerve was interrupted in continuity, but with preserved reinnervation capacity; and TT when there was no reinnervation capacity (usually due to the long-standing nerve lesion), regardless of the continuity.

The right timing is of utmost importance in peripheral nerve surgery. Immediate repair may be performed in open injuries with clear cuts. Open injuries involving the proximal and/or distal ends (e.g., laceration), are repaired in a delayed fashion, usually 3–4 weeks after the injury, while the initial surgery usually includes identification of the proximal and distal roots and their marking. In closed injuries, electrophysiological studies to confirm the lesion are carried out 3–4 weeks after the injury, but surgical treatment is indicated only in cases with no signs of recovery in electrophysiological studies performed 12 weeks after the injury (13). TTs use is usually not affected by the time passed from injury to surgery.

Neurolysis is performed through the popliteal approach when PN continuity is preserved. After skin incision, soft

tissue dissection was performed to reveal and identify the PN. External neurolysis is performed when intraoperative findings showed that the nerve was compressed by surrounding scar/fibrous tissue. In situations when intraneural fibrosis was found, internal neurolysis is performed. After satisfactory deliberation of the nerve, hemostasis is performed and the wound is closed in a layered fashion. Usually, drainage was not needed.

The same popliteal approach is used for nerve repair as well, but, in situations when nerve continuity was not preserved. After identification and preparation of proximal and distal ends of PN, either direct (suture) or repair with various graft types was performed.

Direct repair (with 9/0 interrupted sutures) is possible when the nerve defect was less than 3 cm in length and adequate coaptation without tension could be achieved. When a longer nerve defect is found, it was repaired with sural nerve grafts, usually from the same-sided leg. Cable grafting was performed when the nerve defect was proximal to the ending branches and interfascicular autografting when the nerve defect included ending branches. The wound is then closed in a layered fashion.

For TTs, we usually used the tibialis posterior muscle tendon, which is divided into two slips. One slip is attached to the tibialis anterior muscle and the second one to the extensor digitorum longus muscle and extensor hallucis longus muscle. Suturing is performed with 2/0 sutures, followed by hemostasis and wounds closure in a layered fashion. Immobilization with above knee cast, and foot and fingers in extension, is set and kept for 6 weeks postoperatively.

After the surgery and wound healing (and plaster removal in patients with TTs), all patients were referred to physical treatment in dedicated rehabilitation centers and local health service providers for at least 6 months.

Follow-up included neurological examinations by an independent neurologist, and also functional assessment: monthly, during the first 3 months, and every 3 months later on. Postoperative recovery was recorded with MRC and the residual pain was graded on VAS of pain. The use of orthosis or walking aid was also recorded.

QoL evaluation was performed when no further recovery was expected. Since there is no dedicated tool to asses QoL in patients with PN injuries, we opted for the use of three questionaries including the Ulm questionnaire as a dedicated tool for peripheral nerve injuries (23), the Short Form 36 (SF-36) health survey as a general QoL inventory (24), and Stanmore questionnaire focusing on foot-drop correction (25).

## Statistical analysis

In the case of normally distributed variables, mean and standard deviation are shown in the tables, while the differences are tested using the *t*-test; in case of two groups, and ANOVA in the case of more than two groups. For variables not falling under the normal distribution, median, minimum, and maximum values are reported in tables, while the differences between groups are tested using the Man–Whitney–Wilcoxon test, for two groups, or the Kruskal–Wallis test in case more than two groups are present. Categorical variables are presented by the number of observations and the percentage, while $\chi^2$ or Fisher test is used to compare frequencies between groups. All data were analyzed using R 3.4.2. [R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria].

This case series has been reported in line with the PROCESS Guideline (26).

# Results

This study included 51 patients: 30 (59%) were treated with neurolysis, 12 (23%) with nerve repair, and 9 (18%) with TT. Patients were mostly male (70%) with a median age of 41 (19–69) years old, with an urban residence (80%), and medium education (86%). All patients were Caucasian. **Table 1** shows the sociodemographic characteristics and preoperative status of the patients by surgery class.

Characteristics of the surgery and postsurgical treatment, and of the Ulm questionnaire are presented in **Table 2**. The majority of patients reported that they had experienced an improvement due to the surgery (63%), that they were satisfied with the results of the surgical treatment (76%), most would undergo the surgery again if they had known the results (88%), and just above one-third of patients noticed a significant pain relief (37%).

**Figure 1** shows the comparison between the preoperative and postoperative functional status according to the MRC scale. Thirty-three of the 51 included patients achieved useful functional recovery (M4 or M5), and 18 remained without significant improvement ($p < 0.001$) (≦M3). Neurolysis yields good results with an M3+ ratio nearing 80%, while useful functional recovery was achieved in 22 (73.3%) of 30 patients. Nerve repairs were followed by 58.33% of patients achieving M3+, while 41.66% recovered to a useful functional state. With the use of TT, all patients recovered to the M3+, while 66.7% recovered to M4.

**Figure 2** shows the differences between the preoperative and postoperative VAS in patients treated with different surgical approaches. The dashed lines represent the difference in single patients. Overall, in the majority of patients, the pain intensity reduced significantly; although, in each class of surgery, there were cases where the pain remained the same or even increased. The reduction in pain, as a difference in

TABLE 1 Sociodemographic characteristics and preoperative status of patients by surgery class.

| | All participants $N = 51$ | Neurolysis $N = 30$ | Nerve repair $N = 12$ | Tendon transfer $N = 9$ | $p$-value |
|---|---|---|---|---|---|
| Gender | | | | | 0.112 |
| Male | 36 (70.6%) | 18 (60.0%) | 11 (91.7%) | 7 (77.8%) | |
| Female | 15 (29.4%) | 12 (40.0%) | 1 (8.3%) | 2 (22.2%) | |
| Age | 41.0 (19.0–69.0) | 43.5 (19.0–69.0) | 42.0 (20.0–62.0) | 38.0 (24.0–53.0) | 0.542 |
| Residence | | | | | 0.546 |
| Rural | 10 (19.6%) | 5 (16.7%) | 2 (16.7%) | 3 (33.3%) | |
| Urban | 41 (80.4%) | 25 (83.3%) | 10 (83.3%) | 6 (66.7%) | |
| Education | | | | | NA |
| High school | 44 (86.3%) | 25 (83.3%) | 11 (91.7%) | 8 (88.9%) | |
| College | 6 (11.8%) | 5 (16.7%) | 0 (0.0%) | 1 (11.1%) | |
| University | 1 (2.0%) | 0 (0.0%) | 1 (8.3%) | 0 (0.0%) | |
| Nature of injury | | | | | NA |
| Trauma | 38 (74.5%) | 20 (66.7%) | 12 (100.0%) | 6 (66.7%) | |
| Iatrogenic | 13 (25.5%) | 10 (33.3%) | 0 (0.0%) | 3 (33.3%) | |
| Class of injury | | | | | NA |
| Primary | 41 (80.4%) | 23 (76.7%) | 12 (100.0%) | 6 (66.7%) | |
| Secondary | 10 (19.6%) | 7 (23.3%) | 0 (0.0%) | 3 (33.3%) | |
| Nerve continuity | | | | | NA |
| Yes | 37 (72.5%) | 30 (100.0%) | 0 (0.0%) | 7 (77.8%) | |
| No | 14 (27.5%) | 0 (0.0%) | 12 (100.0%) | 2 (22.2%) | |
| No. of assoc. injuries | | | | | NA |
| 0 | 15 (30.0%) | 6 (20.0%) | 6 (50.0%) | 3 (37.5%) | |
| 1 | 25 (50.0%) | 19 (63.3%) | 4 (33.3%) | 2 (25.0%) | |
| 2 | 7 (14.0%) | 4 (13.3%) | 2 (16.7%) | 1 (12.5%) | |
| 3 | 2 (4.0%) | 1 (3.3%) | 0 (0.0%) | 1 (12.5%) | |
| 5 | 1 (2.0%) | 0 (0.0%) | 0 (0.0%) | 1 (12.5%) | |
| MDS (preop.) | | | | | NA |
| S0 | 49 (96.1%) | 28 (93.3%) | 12 (100.0%) | 9 (100.0%) | |
| S1 | 2 (3.9%) | 2 (6.7%) | 0 (0.0%) | 0 (0.0%) | |

MDS, Mackinnon–Dellon scale; NA, not available.

VAS scores, was statistically significant [Friedman test, $\chi^2_{(1)} = 31.8$, $p < 0.001$].

Figure 3 shows the results of the SF-36 QoL questionnaire in the three groups of patients. The highest (best) scores across the three groups are seen in the social functioning (SF) and role-emotional (RE) scales, followed by pain index (BP). Lower scores are seen on the physical functioning (PF), role-physical (RP), general health (GH) perceptions, and vitality (VT) scales. The lowest scores are seen on the mental health (MH) scale. Standardized physical component scales had values of 50 for neurolysis and nerve reparation, and 45 for TT, while for the standardized mental component scales the values were 47, 49, and 50, for neurolysis, nerve reparation and TT, respectively.

Figure 4 shows the Stanmore score by surgery class. There was no statistically significant difference in the Stanmore score (ANOVA, $F = 0.419$, $p = 0.66$), or the Stanmore grades (Figure 5) (weak, correct, good, and very good) among the three surgical treatment modalities.

## Discussion

The study evaluated the outcomes and QoL of 51 patients with PN injuries, who received one of three surgical treatment options (neurolysis, nerve repair, or TT), based on preoperative characteristics and individually tailored approaches.

PN injuries require surgical treatment in approximately 2/3 of cases (6), with the usual indication for surgery being more than 3 months after injury without recovery for closed injuries, and immediate or as soon-as-possible repair for open

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TABLE 2 Surgery characteristics and results of the Ulm questionnaire by surgery class.

| | All participants $N = 51$ | Neurolysis $N = 30$ | Nerve repair $N = 12$ | Tendon transfer $N = 9$ | $p$-value |
|---|---|---|---|---|---|
| Timing of surgery (months) | 4.5 (1.5–349.5) | 4.5 (1.5–14.8) | 3.0 (1.5–7.6) | 18.6 (5.2–349.5) | <0.001 |
| Physical treatment | 6.0 (0.0–60.0) | 6.0 (0.0–60.0) | 6.0 (0.0–24.0) | 4.0 (1.0–18.0) | 0.414 |
| Supplements | | | | | 0.211 |
| No | 3 (5.9%) | 1 (3.3%) | 2 (16.7%) | 0 (0.0%) | |
| Yes | 48 (94.1%) | 29 (96.7%) | 10 (83.3%) | 9 (100.0%) | |
| Orthosis | | | | | 0.030 |
| Preop. | 8 (22.9%) | 4 (23.5%) | 3 (33.3%) | 1 (11.1%) | |
| Postop. | 13 (37.1%) | 10 (58.8%) | 2 (22.2%) | 1 (11.1%) | |
| Preop./Postop. | 14 (40.0%) | 3 (17.6%) | 4 (44.4%) | 7 (77.8%) | |
| Did anything improve due to surgery? | | | | | 0.435 |
| Not at all | 3 (5.9%) | 1 (3.3%) | 2 (16.7%) | 0 (0.0%) | |
| Slightly | 6 (11.8%) | 2 (6.7%) | 3 (25.0%) | 1 (11.1%) | |
| Moderate | 10 (19.6%) | 5 (16.7%) | 2 (16.7%) | 3 (33.3%) | |
| Quite a bit | 11 (21.6%) | 7 (23.3%) | 2 (16.7%) | 2 (22.2%) | |
| Very much so | 21 (41.2%) | 15 (50.0%) | 3 (25.0%) | 3 (33.3%) | |
| How satisfied are you with the result of surgery? | | | | | 0.157 |
| Not at all | 4 (7.8%) | 1 (3.3%) | 3 (25.0%) | 0 (0.0%) | |
| Only slightly | 4 (7.8%) | 2 (6.7%) | 1 (8.3%) | 1 (11.1%) | |
| Moderately | 4 (7.8%) | 2 (6.7%) | 2 (16.7%) | 0 (0.0%) | |
| Quite a bit | 11 (21.6%) | 6 (20.0%) | 1 (8.3%) | 4 (44.4%) | |
| Very satisfied | 28 (54.9%) | 19 (63.3%) | 5 (41.7%) | 4 (44.4%) | |
| If you know the current result, would you undergo the procedure again? | | | | | 0.476 |
| Yes, without any doubt | 36 (70.6%) | 22 (73.3%) | 7 (58.3%) | 7 (77.8%) | |
| Yes, very likely | 9 (17.6%) | 4 (13.3%) | 3 (25.0%) | 2 (22.2%) | |
| No most likely not | 3 (5.9%) | 1 (3.3%) | 2 (16.7%) | 0 (0.0%) | |
| No certainly not | 3 (5.9%) | 3 (10.0%) | 0 (0.0%) | 0 (0.0%) | |
| Did your pain change since surgery? | | | | | 0.063 |
| Not at all | 8 (15.7%) | 1 (3.3%) | 4 (33.3%) | 3 (33.3%) | |
| Slightly | 12 (23.5%) | 6 (20.0%) | 2 (16.7%) | 4 (44.4%) | |
| Moderately | 12 (23.5%) | 9 (30.0%) | 2 (16.7%) | 1 (11.1%) | |
| Quite a bit | 15 (29.4%) | 10 (33.3%) | 4 (33.3%) | 1 (11.1%) | |
| Very much so | 4 (7.8%) | 4 (13.3%) | 0 (0.0%) | 0 (0.0%) | |

(sharp injuries or lacerations) (27). Timing for surgery is established as the most important factor that predisposes the recovery potential and also plays a major role in the choice of surgical technique ($p < 0.001$), together with the nerve continuity status and nature of injury (28).

These same principles were applied to our patients, although we have no data on patients who recovered or started to recover during the 3 months period, as these were not referred to the neurosurgical department. On the other hand, some patients reported for an initial exam when the reinnervation capacity was lost (more than 12 months without recovery after the injury, and with obvious target muscles atrophy). In these cases, we opted for TT rather than palliative bracing to achieve functional restoration (18).

The use of TT increased the percentage of surgically treated patients, but it is possible that it justified the rates for those patients who were not treated on time due to referral issues. The use of TT in patients with failed recovery after neurolysis or nerve repair is advised as a salvage procedure, although in our study, no patients underwent this kind of augmentation (29).

Neurolysis is employed in the least extensive nerve injuries when nerve continuity is preserved and yields good results with a motor recovery ratio nearing 80% (30, 31). These results correspond to ours, with 88% of patients achieving M3+, and 72.2% recovering to the M4+.

Nerve repairs of PN lesions were previously reported to have a roughly half (50%) motor recovery rate with the use of

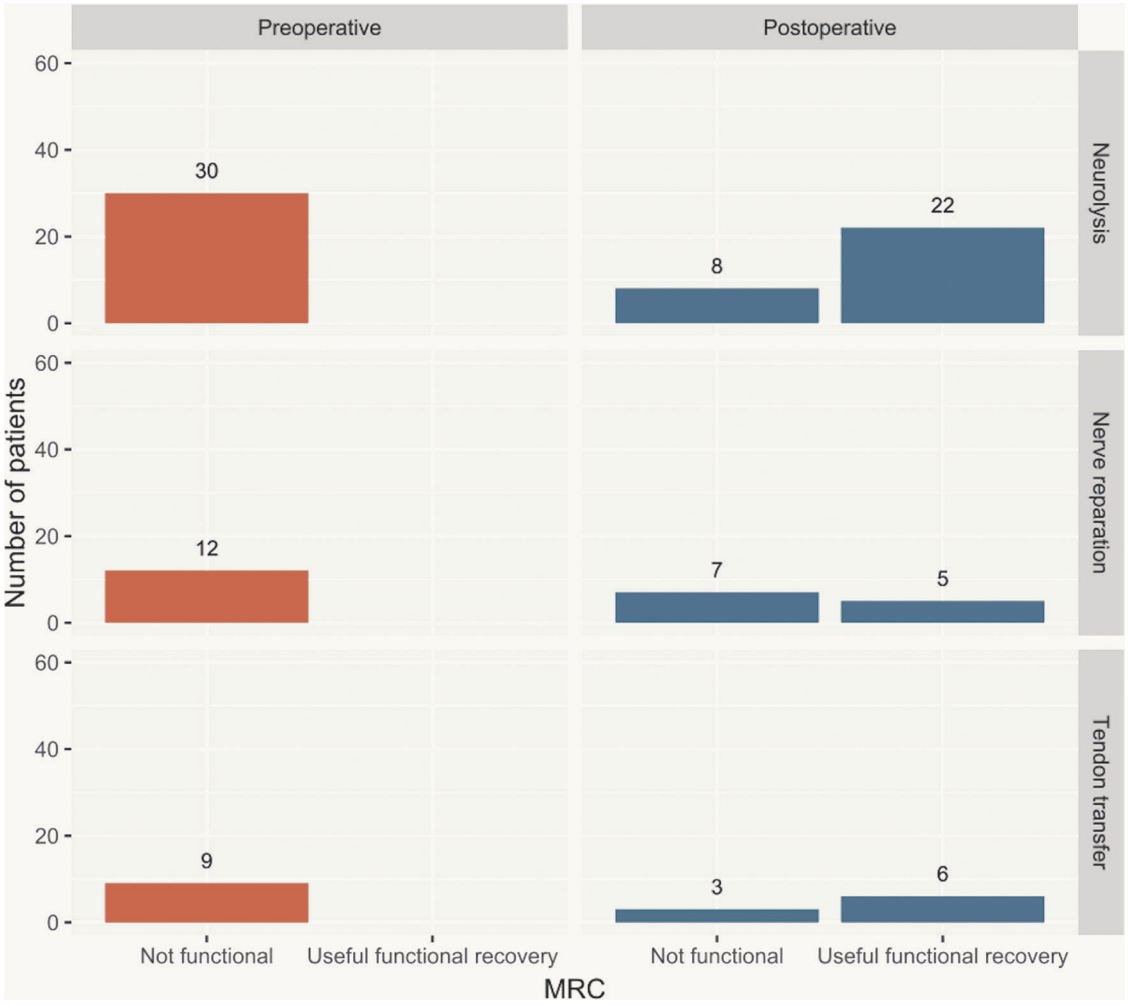


FIGURE 1
Preoperative vs. postoperative useful functional recovery according to the MRC scale by surgical technique applied. MRC, Medical Research Council.

grafts, while the direct repair carries a somewhat higher rate of 60%–80% (30–32), in our study there was a slight increase as 58.33% of patients achieved M3+ recovery, while 41.66% recovered to the useful functional state with M4 and M5. It should be mentioned that 10 of 12 patients who underwent nerve repair received sural nerve grafts for the repair, one patient's nerve was directly sutured and one patient received an artificial conduit. Since the patients with nerve repair achieve satisfactory outcomes in roughly half of cases, it was proposed that these patients may undergo TT as a salvage procedure (12), and some authors even proposed to perform the one-stage nerve repair and TT immediately (33).

TT have very good results when only motor strength recovery is observed with recovery rates over 80% (almost 85% when concurrent posterior tibial TT was employed in a systematic review, and up to 100% in single studies (16, 31). However, this procedure is indicated as salvage, for isolated PN palsy with good ankle mobility, good strength of the posterior tibial muscle and poor prognosis of spontaneous recovery in order to decrease dependence on brace for walking, and to improve hip and knee function with improved gait kinematics (16, 34). All our patients recovered to the M3+, while 66.7% recovered to M4, but there were no cases who recovered to M5 which corresponds to the results of previous studies.

Pain was not an indication for surgery in our patients, but the common pattern of pain decrease was noted regardless of the surgical strategy. Previously, the authors have reported performing (internal or external) neurolysis to treat neuropathic pain (29, 35), especially in patients with gunshot wounds (36). Based on our results, we can hypothesize, that the origin of pain in our patients was not neuropathic in the majority, but rather chronic foot pain due to the instability and the arch flattening.

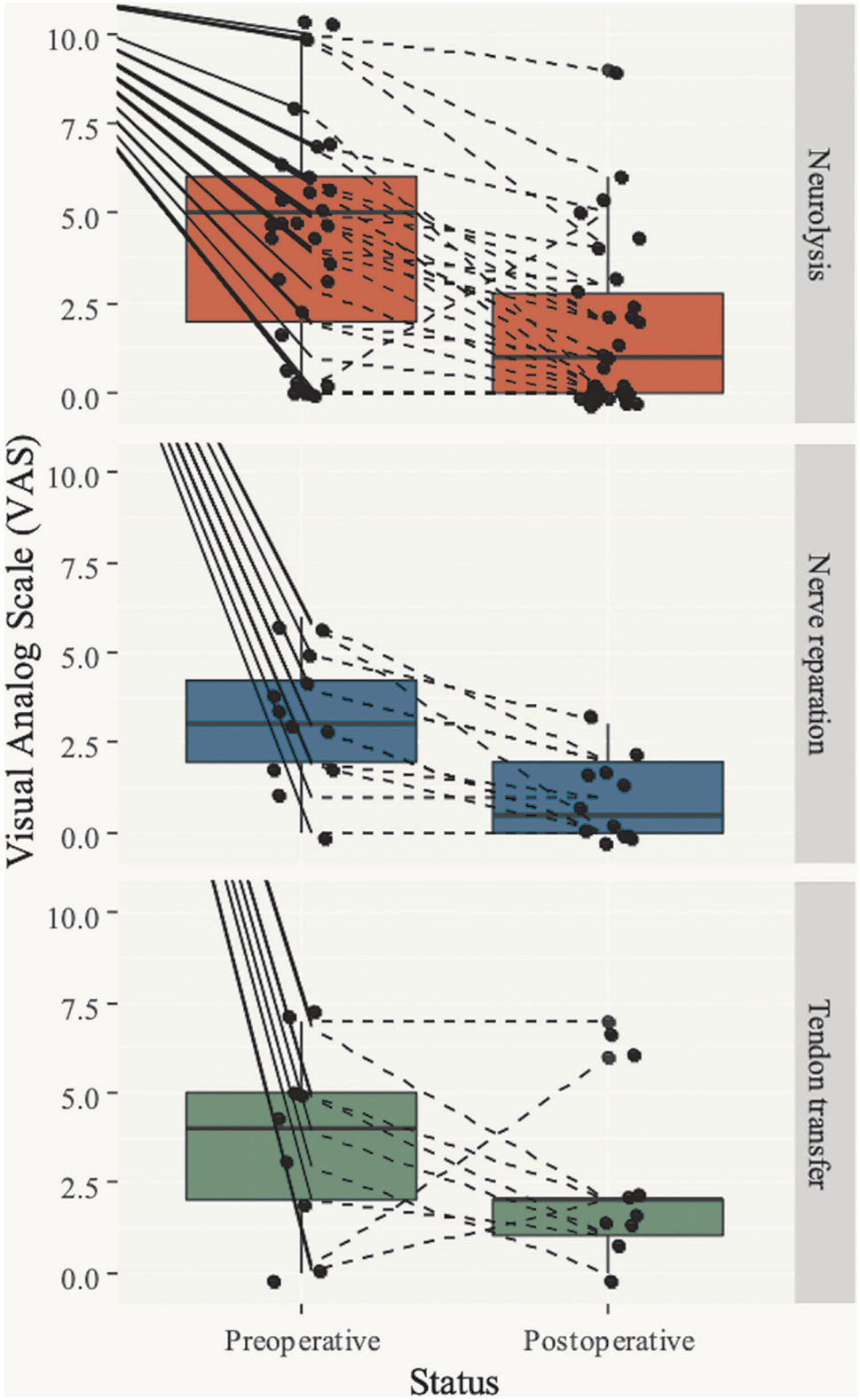


FIGURE 2
VAS pain scale difference between preoperative and postoperative status by class of surgery. VAS, visual-analog scale.

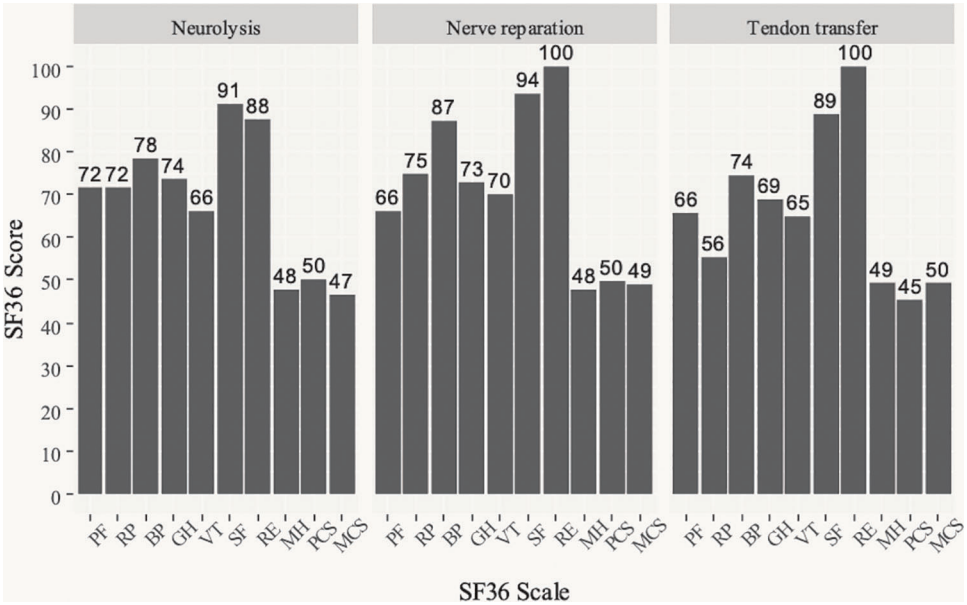


FIGURE 3
Results of the SF-36 quality of life questionnaire.

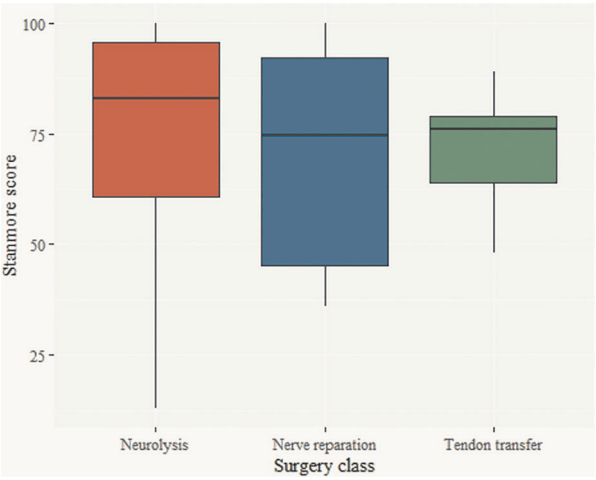


FIGURE 4
Stanmore scores among different surgical treatment modalities.

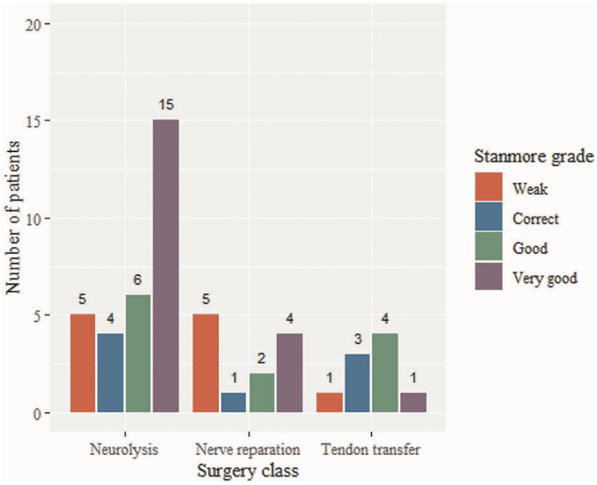


FIGURE 5
Stanmore grades among different surgical treatment modalities.

The most dedicated foot drop inventories focus on the use of bracing as a main aim of the treatment, and the use of TT, rather than the functional recovery following nerve release or repair, and actual QoL (20, 21, 37). Although in a relatively small cohort, there were no statistically significant differences in the QoL scores between the three treatment options, suggesting that no surgical technique influences the QoL by itself, but rather the right approach allows to achieve a similar QoL. A previous study discovered that patients with chronic foot drop had a reduced QoL with significantly poorer scores in the physical and psychosocial domains (38). This was not the case in our study, as the majority of patients recovered satisfactorily, leading to better overall scores.

While results from the three questionnaires focused on the overall QoL are consistent, when employing the Stanmore system, and assessing purely motor outcomes and the need for prosthesis, we found an apparent advantage of neurolysis, over nerve repair, over TT. Probably, due to the different

regeneration potential, but also nerve injury severity, leading to favorable outcomes, compared to the previously reported 69% of patients with chronic foot drop in the need for bracing (38).

The insufficient number of patients (for a more powerful statistical analysis) overall, and especially in the TT and nerve repair groups is a usual limitation of studies on peripheral nerve injuries, and it is similar or even advantageous to other studies on the topic (16, 30–32).

There is no specific tool for the evaluation of QoL in patients with PN injuries, but we have shown that readily available tools can capture the QoL well, and quite consistently.

Future studies should focus on the improvement of all three surgical procedures, and a unified guided surgical decision-making process, as every procedure has its place in specific patients. Larger cohorts of patients should be recruited in a multidisciplinary fashion and merged within prospective trials leading to high-quality recommendations and guidelines.

## Conclusion

There is an apparent advantage of neurolysis, over nerve repair, over nerve transfer procedure, both in terms of useful functional recovery, and foot-drop-related QoL. However, when involving all aspects of QoL, these advantages diminish.

Individual approach to patients with severe PN injuries, involving all features and aspects of both injury and the patient, leads to the achievement of optimal results in all groups of patients, regardless of the regeneration potential and injury severity, but these should be considered as primary guides in choosing the surgical approach.

QoL system focusing on the peripheral nerve injuries is detrimental to a better understanding of the actual patient's state, recovery and satisfaction as present inventories lack specificity.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

## Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade, Belgrade, Serbia. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

All authors contributed to the article and approved the submitted version.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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