HISTOLOGIC EVALUATION OF END-TO-SIDE NEURORRHAPHY. EXPERIMENTAL STUDY IN RATS

FABIANO INÁCIO DE SOUZA1, GUSTAVO BISPO DOS SANTOS1, CIRO FERREIRA DA SILVA2, RAMES MATTAR JÚNIOR1, ARNALDO VALDIR ZUMIOTTI1

ABSTRACT
Objective: The aim of this study was to histologically compare the axonal sprouting after end-to-side neurorrhaphy with or without epineurotomy. Methods: twenty male Wistar rats were used, divided into two groups of 10 rats each. A 1.0cm segment of the tibial nerve E was dried and sutured on the opposite side, where it was sutured into the sciatic nerve D. In Group I, the suture was made directly in the epineurium and in Group II, epineurotomy was performed. After 4 weeks, histological evaluation was carried out of the transposed segment and the sciatic nerve distal to the suture. Results: the results showed a small number of remyelinated fibers, varying from 7 to 51 fibers in Group I and from 10 to 91 fibers in Group II. The Mann-Whitney U test was used, with p=0.311, showing there is no statistically significant difference between the two groups. There was no positive relation between the number of remyelinated fibers in the graft and in the suture site distal to the sciatic lesion. Conclusion: lateral-ending neurorrhaphy, with or without epineural window, does not promote efficient remyelination. Level of Evidence: Level II, prospective comparative study.

Keywords: Sciatic nerve. Nerve regeneration. Rats, Wistar.

INTRODUCTION
Peripheral nerve lesions are common and treatment methods are not yet able to foster fully satisfactory results, which awakens the interest of the scientific community. Among the techniques for microsurgical repair of injured nerves, end-to-side neurorrhaphy, which consists of the coaptation of an injured nerve segment, on the lateral surface of another adjacent intact nerve,1,2 is the most controversial in medical literature. The first reports on end-to-side neurorrhaphy date back to the beginning of the 20th century, where Kennedy et al. (1901) apud Al-Qattan3 and Ballance et al. (1903) apud Pienaar 4, respectively, suggest its effectiveness in the treatment of facial spasm and in facial nerve paralysis. Krivolutskaia et al.5 and Viterbo et al.6 reintroduced this technique almost a century later, affirming that there is lateral axonal sprouting to the receptor nerve without causing injury to the donor nerve. Since then, numerous articles have been published both by advocates and by critics of this technique, transforming end-to-side neurorrhaphy into one of the main controversies of microsurgery.7,8 The aim of this study is to perform a histological evaluation of axonal myelination in end-to-side neurorrhaphy, with and without epineurectomy.

MATERIALS AND METHODS
The study subjects were 20 young male Wistar rats, split into 2 groups of 10 animals. We anesthetized the animals with pentobarbital, in the concentration of 45mg/kg, administered intraperitoneally in the lower third of the abdomen. We promoted trichotomy, antisepsis and arrangement in the prone position. We made the longitudinal incision on the posterior side of the left paw, with dissection by planes and exposure of the sciatic nerve and its terminal branches. (Figure 1) We resected a 1.0cm segment of the left tibial nerve. On the contralateral side, the right sciatic nerve was dissected proximally, with the suturing of the right tibial nerve segment on its lateral wall. (Figure 2) We did not perform epineurectomy in Group I, while an epineurial window was created in Group II, using four stitches to suture the graft, applied at 3, 6, 9 and 12 hours. We used Mononylon 10.0 suture threads (Ethicon-Ethilon®, BV 130-4) and a DF

All the authors declare that there is no potential conflict of interest referring to this article.
Vasconcelos® microscope (Mod.MC-M3107), with 21x zoom. The sutures were performed avoiding axonal lesion of the right sciatic nerve. After 4 weeks, we euthanized the animals through the intraperitoneal administration of pentobarbital (150mg/kg). We made 1µm cuts in the graft segments and immediately distal to the suture in the sciatic nerve (Figure 3), where we conducted the histological analysis by means of the total count of the number of myelinated fibers.

RESULT

Groups I and II presented an extremely low number of remyelinated fibers. In Group I, the total number of remyelinated axons varied from 7 to 51 fibers, while Group II presented variation from 10 to 91 fibers. We employed the Mann-Whitney U test, presenting p=0.311, demonstrating that there was no statistically significant difference between the two groups. (Table 1) We verified that there was no positive correlation between the number of myelinated fibers and the presence of sciatic nerve lesion focuses (caused during the suturing). (Figure 4)

DISCUSSION

Nowadays, the best treatment after complete peripheral nerve injuries is direct coaptation of the stumps in the acute phase, using microsurgical techniques. In chronic lesions or those with segment loss, it is impossible to achieve stump coaptation without excessive tension. In these cases, nerve grafts are widely indicated. This technique does not produce full functional results, and causes injury to intact structures, condemning the patient to permanent sequelae, such as paresthesia, scars, risk of neuroma formation and chronic pain. This picture motivates researchers to seek treatment alternatives with better results. There are descriptions of several techniques, including tabulations through veins or the use of silicone tubes and end-to-side neurorrhaphy.
Theoretically, end-to-side neurorrhaphy is said to promote, from a complete nerve, lateral sprouting of the axons reaching the target organ and restoring its sensitivity and motor function. It is a fast, simple and safe procedure, since it allegedly does not provoke lesions in the intact donor nerve, and would not injure another intact site.\textsuperscript{6,10} It would be an excellent strategy, if it were not surrounded by contradictions.\textsuperscript{11} Viterbo \textit{et al.} propose that lateral sprouting occurs irrespective of the performance of prior epineurectomy. Other authors believe that an epineural window would be essential for sprouting, as without this window it would not be possible for an intact nerve to emit a sufficient quantity of axons to transpose the epineurium and promote myelination of the “receptor” segment.\textsuperscript{12,13} Some researchers believe that the myelinated fibers contained in the receptor nerve result from the lateral sprouting secondary to the lesion provoked in the surgical procedure. This lesion apparently occurs during the performance of the epineurectomy or in the suturing process.\textsuperscript{14} Aiming to investigate this hypothesis, we prepared slides for histological evaluation at the site immediately distal to the coaptation of the receptor nerve. In some cases, the presence of axonal sprouting secondary to the small lesion area was demonstrated. However, there was no positive correlation between these lesions and a larger number of myelinated fibers. In the count, we verified that the total number of myelinated fibers, between 7 and 51 in group 1 and between 10 and 91 in group 2, is practically negligible when compared to the total number of fibers, 5133, of the normal tibial nerve of Wistar rats.\textsuperscript{15} While several articles demonstrate good results of end-to-side neurorrhaphy in humans,\textsuperscript{10,16} others do not suggest its indication.\textsuperscript{17,18} For example, Fernandes \textit{et al.}\textsuperscript{9}, after reviewing literature, affirmed the nonexistence of sufficient experimental or clinical evidence for the indication of end-to-side neurorrhaphy in humans. The same opinion is shared by Pienaar \textit{et al.}, Pannicucci \textit{et al.}\textsuperscript{11} and Beris \textit{et al.}\textsuperscript{17} Due to the extremely favorable and tempting concepts of end-to-side neurorrhaphy and of the significant difference of opinions in literature, the performance of new experimental studies with the addition of neurotrophic factors is imperative.

\textbf{CONCLUSION}

In the experiment performed, we concluded that end-to-side neurorrhaphy, with and without epineural window, does not promote efficient remyelination.

\textbf{REFERENCES}