

## COMPARATIVE STUDY OF THE SCIATIC NERVE REPAIR TECHNIQUE OF WISTAR RATS WITH AND WITHOUT THE USE OF VEIN WRAPPING

### ESTUDO COMPARATIVO DA TÉCNICA DE REPARO DO NERVO ISQUIÁTICO DE RATOS WISTAR COM E SEM O USO DE INVÓLUCRO VENOSO

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#### RESUMO

**Introdução:** Partindo do ponto de vista da qualidade da função motora, os autores compararam diferentes técnicas de reparo de nervos periféricos (nervo isquiático) com a técnica de envolver a neurografia primária com invólucro venoso. Conforme descrito por outros autores, esta técnica vem apresentando resultados promissores em relação à redução da dispersão das fibras nervosas, aderência perineural e formação de neuroma. Contudo, ainda não eram claros os dados referentes à recuperação motora dessa técnica. Este trabalho pretende avaliar a qualidade da reabilitação motora ao utilizar o invólucro venoso envolvendo a neurografia primária.

**Métodos:** O presente trabalho consiste em um estudo experimental, de desenho prospectivo, controlado e randomizado com animais da linhagem Wistar. Para isso, foram criados quatro grupos: controle (C), neurografia (N), tubulização venosa (V) e neurografia com invólucro venoso (NV). O nervo isquiático foi então seccionado e reparado utilizando a técnica referente a cada grupo e, no período pós-operatório, os espécimes tiveram a função motora avaliada por meio do *Sciatic Function Index* (SFI) na 4ª, 8ª e 12ª semana.

**Resultados:** Considerando a avaliação motora dos grupos N e NV, estes apresentaram um resultado superior de SFI em relação ao grupo C e V ( $p < 0,0001$ ). Quando comparados entre si, estes grupos apresentaram resultados idênticos do ponto de vista estatístico, ou seja, não foram encontradas diferenças significativas ( $p > 0,005$ ).

**Conclusão:** Em modelo animal as técnicas de neurografia primária isolada e a associada ao invólucro venoso são semelhantes no quesito recuperação da função motora.

**Palavras chave** – Nervo isquiático; Enxerto autólogo; Regeneração nervosa.

#### ABSTRACT

**Background:** Considering the quality of motor function, the authors compared different peripheral nerve repair techniques with the end-to-end neurography technique associated with the venous wrapping. According to other authors, the vein wrapping technique has shown promising results regarding the reduction of nerve fiber dispersion, perineural scar and neuroma formation. However, data regarding motor recovery has not been clear yet. Therefore, the aim of this study is to evaluate the motor recovery of the neurography with vein wrapping.

**Methods:** This article is an experimental study with randomized control trials. We used a rat sciatic nerve injury model in which we completely transected and repaired the right sciatic nerve. Therefore, 4 groups were created: control (C), end-to-end neurorrhaphy (N), vein tube (V) and the group of end-to-end neurorrhaphy associated with vein wrapping (NV). In the postoperative period, the specimens had motor function assessed by the *Sciatic Function Index* (SFI) in the 4th, 8th and 12th week.

**Results:** Considering the motor function of the groups N and NV, these have shown a higher SFI score in relation to group C and V ( $p < 0.05$ ). Comparing N with NV both groups were identical, in other words, significant differences have not been found ( $p > 0.05$ ).

**Conclusions:** Considering statistical data, groups N and NV were strictly equal. Therefore, the techniques of isolated end-to-end neurorrhaphy or associated with the vein wrapping were similar in terms of motor function recovery, and they may be used interchangeably.

**Keywords** – Sciatic Nerve; Autologous Graft; Nerve Regeneration.

## INTRODUCTION

The repairing of traumatic injuries in peripheral nerves is frequent in the area of microsurgical plastic surgery. If left untreated, they result in reduction or even loss of the sensitivity and motor function of the innervated segment.<sup>1,2</sup> Despite the fact that it is quite frequent, it still constitutes a great surgical challenge, since the postoperative results are, frequently, unsatisfactory.<sup>3</sup>

The gold standard treatment is the end-to-end neurorrhaphy, which consists on the coaptation of damaged fascicular groups, indicated when there is no tension during the approach of the extremities.<sup>4</sup> However, when this tension exists, the most indicated approach is the autologous nerve graft.<sup>5</sup> Nonetheless, there is also the option of repairing it by tubulization, either through artificial or biological material, as a way to allow a bridge for the regeneration of the fascicular groups.<sup>6,7</sup> The postoperative results, as previously mentioned, are still unsatisfactory, mainly in relation to the time and the quality of the recovery. Supporting this statement, there is the fact that it takes months to achieve the improvement of the sensory and motor function and, furthermore, the occurrence of painful mass formation in the region of the trauma, known as neuroma.<sup>8,9</sup>

Considering such results, the aim of this study is to improve the repairing procedure. Therefore, a technique already described in literature, which consists of utilizing a vein wrapping to protect the neuroanastomosis region, but generally applied for the resolution of nerve compressions, was used.<sup>10</sup> However, this technique has been tested by several authors with other objectives, mainly to reduce the dispersion of nerve fibers and the formation of neuroma, since the wrapping allows neuronal growth only in the direction of its extremities union, possible lateral growth is blocked by venous tissue<sup>11,12,13</sup>.

Since this technique may be applied in order to reduce neuromas, the aim of this study is to find out if the end-to-end neurorrhaphy associated with vein wrapping shows improvement in motor function when compared to the control group and the vein conduit technique.

## METHODS

The present study consists of an experimental, prospective, controlled and randomized study, approved by the Commission of Ethics in the Use of Animals in the State University of Ponta Grossa (case 032/2017). In total, 40 male 80-day-old Wistar rats (*Rattus norvegicus*) weighing an average of 250g were obtained from the Vivarium of the State University of Ponta Grossa. The animals were kept in appropriate boxes where they received water and were fed “*ad libitum*”, without restrictions in the movement, respecting cycles of 12 hours of light, in average temperature of 24 °C. All the 40 the animals were distributed in 4 groups of 10 rats each: Control group (C group); Group with only neurorrhaphy (N group); Group with venous graft only (V group); Group with neurorrhaphy associated with vein tube (NV group).

All rats were weighed and submitted to general anesthesia by intraperitoneal injection of ketamine at a dose of 50 mg/kg in combination with 5 mg/kg of Xylazine. For a better comparison between the groups studied, the standardization of the procedure was performed in all rats, all of them had the sciatic nerves and the external jugular vein dissected and sectioned. Therefore, the trichotomy of the lateral dorsal region of the right pelvis was performed in all groups as well as in the ventral region of the neck. After the preparation of the skin with 10% iodopovidone topical solution, the ventral portion of the right side of the neck was submitted to a paramedian incision, approximately 2 centimeters long, following the direction of the muscle fibers of the sternocleidomastoid muscle, to withdraw the external jugular vein. Subsequently, the lateral dorsal side of the right posterior limb was sectioned with scalpel, an incision of 3 centimeters along the imaginary line between the knee joint and the hip joint, compromising the skin and the subcutaneous area with posterior divulsion of the fascia between the biceps femoris and gluteus maximus muscles, in order to avoid muscle trauma. The sciatic nerve was, then, dissected and sectioned with a scalpel and, immediately, repaired or not depending on the group:

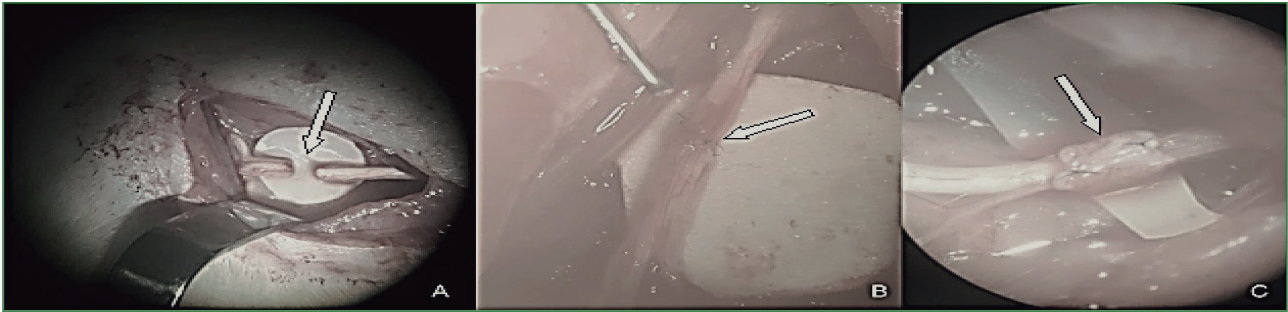
Group C: was submitted to sciatic nerve section, however without repair (Figure 1-A);

Group N: was submitted to epineural end-to-end neurorrhaphy with 10-0 monofilament nylon (Ethicon Inc. Somerville, NJ, USA) (Figure 1-B);

Group V: vein graft was performed in order to allow a conduit for neuronal growth, without end-to-end neurorrhaphy (Figure 1-C);

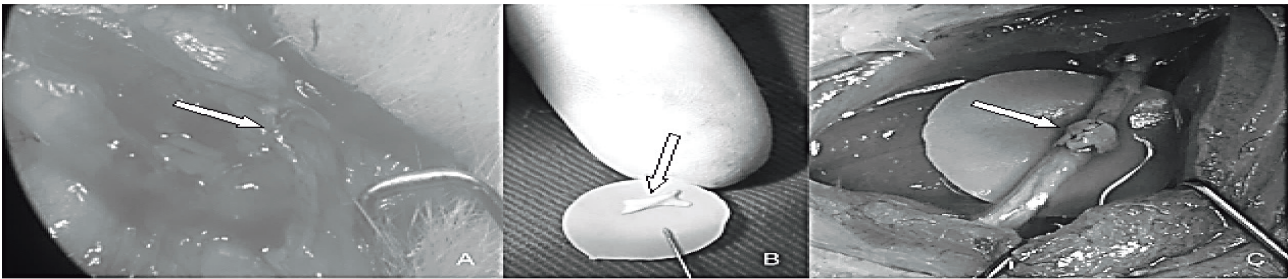
Group NV: After removal of the external jugular vein (Figure 2-A) it was sectioned longitudinally (Figure 2-B), because, without this procedure, the vein was not able to properly involve the nerve, causing constriction of the nerve and potential ischemia of the nerve endings. It was then submitted to epineural end-to-end neurorrhaphy with 10-0 monofilament nylon (Ethicon Inc. Somerville, NJ, USA) and it was involved by the tunica intima of the external jugular vein (Figure 2-C).

**Figure 1.** A) Cross-sectioned sciatic nerve in group C (arrow); B) group N sciatic nerve repaired with 10-0 monofilament nylon suture (arrow); C) repair of group V using only the vein wrapping, without end-to-end neurorrhaphy (arrow).



Reference – author

**Figure 2.** A) Identification of the external jugular vein (arrow); B) jugular vein sectioned in its longitudinal direction and opened to view its lumen (arrow) with dimensions of approximately 3.6 x 0.6 mm when tension-free; C) Demonstration of the NV group with vein wrapping performed after end-to-end neurorrhaphy (arrow).



Reference – author

The entire procedure was performed with the help of the stereoscopic microscope DF Vasconcelos with a four-fold increase. The fascia between the muscles was closed with 4-0 Catgut (continuous suture) and the skin had its edges coapted with intermittent suture with 3-0 Catgut.

The fascia between the muscles was then closed using 4-0 Catgut (Ethicon Inc. Somerville, NJ, USA) thread (continuous simple suture) and the skin had its edges coaptized using 3-0 Catgut (Ethicon Inc. Somerville, NJ, USA). Each group had its sciatic nerve evaluated by the walking track method, which uses a transparent surface where the rats traveled in order to have their legs photographed for later analysis with the Sciatic Function Index (SFI). The rats had their sciatic nerve evaluated in the 4th, 8th and 12th week. Between these weeks, the rats just received water and were fed “*ad libitum*”.

The distance between the posterior limb impressions was assessed according to the equation described by Medinaceli<sup>14</sup> (Figure 4) and the formula items are shown in Figure 5:

- EPL- distance from the heel to the third toe of the experimental paw;
- NPL- distance from the heel to the third toe of the normal or control paw;
- ETS- distance from the first to the fifth toe of the experimental paw;
- NTS- distance from the first to the fifth toe of the normal or control paw;
- EIT- distance from the second to the fourth toe of the experimental paw;

NIT- distance from the second to the fourth toe of the normal or control paw.

In the 12th week after SFI evaluation, the rats were euthanized, with anesthetic overdose.

**Figure 3.** Photograph of the rat's paw (arrow) with a millimeter ruler (arrowhead) for proper calculation of the SFI.



Reference – author

**Figure 4.** SFI formula as a method of assessing motor function described by Medinaceli.

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

**Figure 5.** Representation of the measured distances to be used in the SFI formula.



Reference – author



The values obtained by the SFI formula close to zero represent the complete function of the sciatic nerve, whereas values close to -100 mean total loss of function.

The analyzes between the groups, such as the data related to the functional analysis of the sciatic nerve of the animals, were submitted to the ANOVA statistical test and Tukey post-hoc analysis. For all the analyzes, values of  $p < 0.05$  were considered statistically significant. Each group had its means compared in each test period, in order to evaluate the possible differences between the research groups.

**RESULTS**

Table 1 shows the results of SFI mean values for each group over the analyzed period. Figure 6 and Figure 7 were made up considering the data on Table 1, they show the means of the SFI during the analyzed period. It may be observed that in all groups there is loss of sciatic function and that in Group C, it has slight worsening over time, and in the other groups there is gradual improvement. This improvement was statistically significant ( $p < 0.05$ ) in all groups when compared to Group C.

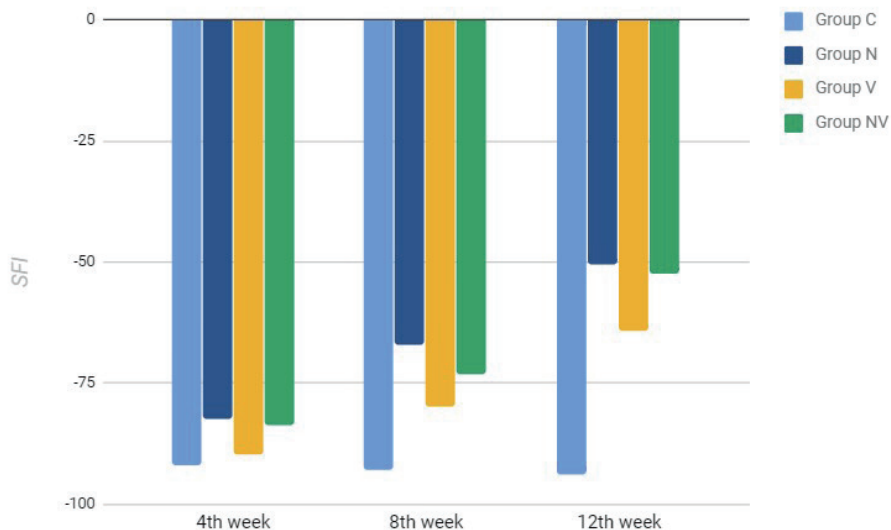
**Table 1.** SFI values – means of SFI and standard deviations. Equal letters represent absence of statistical significance. Different letters represent  $p < 0.05$ .

	Group C	Group N	Group V	Group NV
4th week	-92,08 (+- 3,14) <sup>a</sup>	-82,38 (+- 1,31) <sup>b</sup>	-89,70 (+-1,00) <sup>a</sup>	-83,76 (+- 1,83) <sup>b</sup>
8th week	-92,96 (+- 3,56) <sup>a</sup>	-66,91 (+-1,44) <sup>b</sup>	-79,83 (+-1,57) <sup>c</sup>	-73,27 (+-1,70) <sup>d</sup>
12th week	-93,95 (+- 3,30) <sup>a</sup>	-50,42 (+- 1,12) <sup>b</sup>	-64,05 (+- 1,64) <sup>a</sup>	-52,24 (+- 0,97) <sup>b</sup>

Reference – author

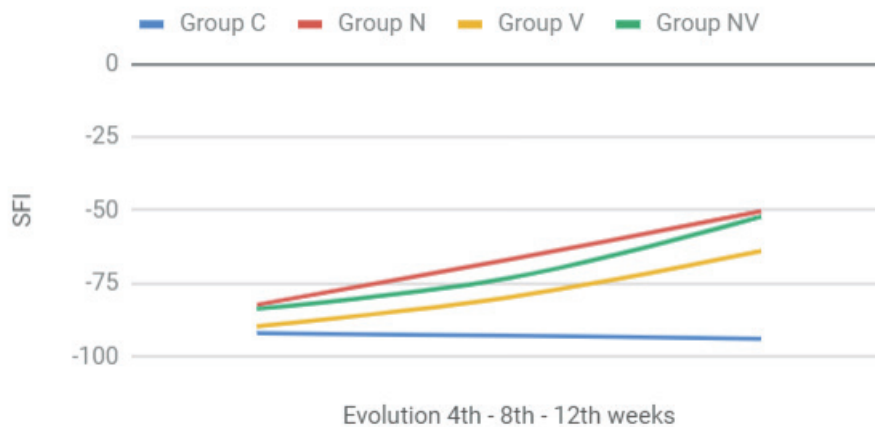
It may be observed in the Figure 6 and 7 that group V presents a significant improvement in relation to Group C ( $p < 0.05$ ); however, group V, compared to the others, presents the worst recovery.

**Figure 6.** Graph showing the values obtained by calculating the SFI.



Reference – author

**Figure 7.** Graph showing the improvement of SFI values. Attention given to the similarity of the curves of groups N and NV, which did not present statistical differences ( $p > 0.05$ ) and both were superior when compared to groups C and V ( $p < 0.05$ ).



Reference – author

Both N and NV groups had a higher SFI score when compared to group C and V ( $p < 0.05$ ), however when compared to each other, Group N was superior to Group NV only in week 8 ( $p < 0.05$ ), but during the remaining weeks, even at the end of the study, these differences were not found in week 4 ( $p > 0.05$ ) and week 12 ( $p > 0.05$ )

## DISCUSSION

The results of this study show a significant improvement of the groups N and NV in relation to the other groups and without significant difference between them. Thus, when only the vein was used as a conduit to protect the injured area (group V), there was recovery of the motor function superior only to the control group ( $p < 0.05$ ), in other words, the use of the vein as protection and conduit allows the regeneration of the neuronal tissue, but its use alone is not sufficient for adequate regeneration, requiring the use of end-to-end neurorrhaphy, which was compatible with a review article<sup>15</sup>. Accordingly, it is evident in the result of N and NV, which present superior results of SFI when compared to C and V ( $p < 0.05$ ), in other words, the performance of end-to-end neurorrhaphy must occur in order to produce proper motor recovery which is compatible with a review article<sup>16</sup>.

In surgical practice, the result of any neurorrhaphy is multifactorial and does not only involve motor recovery, but also the time for rehabilitation, type of affected nerve, age of the patient, tension between the injured extremities, type of trauma and also the formation of fibrosis in the neuroanastomosis region. This fibrosis is the result of the inflammatory cascade in the region mediated by macrophages, lymphocytes, mast cells and the Schwann cells themselves, which release adhesion factors and cytokines, which may eventually lead to the formation of the neuroma<sup>17, 18, 19, 20</sup>.

Therefore, studies using the vein wrapping technique in order to reduce this inflammatory reaction have already been done<sup>11, 12, 13, 20</sup>. The results of such studies have shown that when the nerve is surrounded by venous tissue there is less dispersion of its nerve fibers. The probable explanation is because of the formation of a channel that allows the nerve regeneration in a single direction, which occurs through the migration of proximal fibers in an attempt to reach the distal extremity<sup>19</sup>. On the other hand, when this channel does not exist, the growth does not occur properly, since the

proximal fibers may take a false path, growing laterally or even backwards, rarely finding the distal extremity. This explains the unsatisfactory motor recovery of the control group when compared to the other groups ( $p < 0.05$ ), on this account, as there was no coaptation of the edges, the neural growth is impaired and the edges do not meet each other.

In addition to the low motor recovery of the control group as demonstrated by this study, lesions that make a false path and do not find the distal extremity end up showing a disorderly cell proliferation pattern, especially in the region where the fibers make a false path<sup>21</sup>. In these cases, extremely fibrotic scar tissue, known as a neuroma, is formed. The formation of this scar has been shown to be reduced with the use of protective tissue involving the traumatized region<sup>22, 23, 24</sup>. Therefore, when just the vein wrapping was used to protect the injured area (group V), there was a recovery, considering motor function, superior only to the control group ( $p < 0.05$ ), in other words, the use of the vein as protection allows the regeneration of neuronal tissue, but its use alone is not sufficient for adequate regeneration, making the use of end-to-end neurorrhaphy necessary. Proof of this is found in the results of groups N and NV, which showed superior results of SFI in relation to groups C and V ( $p < 0.05$ ), in other words, the performance of end-to-end neurorrhaphy is necessary for adequate recovery of the motor function, which is compatible with a review article<sup>25, 26</sup>.

Considering that it has already been demonstrated by several studies the superiority of end-to-end neurorrhaphy as a gold standard, studies have sought to improve this technique by applying the vein wrapping to the neuroanastomosis region in order to reduce the inflammatory response of the perineural tissue adherence and reduce the formation of neuroma<sup>18, 23, 24</sup>. However, in addition to the better neuronal growth, already found in the literature, it was necessary to verify if the use of the vein wrapping associated with end-to-end neurorrhaphy showed better recovery of motor function or at least similar recovery compared to the end-to-end neurorrhaphy alone.

Therefore, the aim of this study was reached by demonstrating that the neurorrhaphy technique associated with the vein wrapping is superior, in matters of motor function, compared to groups C and V ( $p < 0.05$ ). Furthermore, it was evidenced that the NV group did not present statistically significant differences when compared to the N group ( $p > 0.05$ ), in other words, the quality of motor recovery was identical, therefore, the technique of involving the neuroanastomosis with venous tissue, in order to form a protective layer, may be interchangeable with the gold standard one (end-to-end neurorrhaphy) to treat nerve injuries.

Consequently, this study was able to demonstrate an adequate rehabilitation of motor function with the end-to-end neurorrhaphy technique associated with vein wrapping, when contrasted with other forms of repair. This was verified by the superior recovery of the NV group when compared to the control group and to the group vein wrapping alone (group C and group V -  $p < 0.05$ ). When compared with group N, the recovery of motor function with the use of vein wrapping was strictly the same from the statistical point of view. However, in addition to equal motor recovery, the association of the vein wrapping still has the ability to reduce the formation of neuromas and other complications described in the literature<sup>11-13, 22-24</sup>, therefore, it may be considered as a superior technique compared to the conventional form of repairing peripheral nerve injuries.

Considering the involvement of neuroanastomosis with venous tissue presented a statistical result strictly equal to the end-to-end neurorrhaphy technique (gold standard). The future perspectives of the present work refer to the need for greater application and study of the use of the vein wrapping, with the purpose of presenting greater evidence regarding the interchangeability of these techniques.



For that, clinical trials are the next step to implement the technique that has already proved efficient in this animal experimental study.

## CONCLUSION

Groups N and NV showed no statistically significant differences in an experimental study with an animal model. Therefore, the techniques of isolated end-to-end neurorrhaphy and associated with the vein wrapping are similar considering the recovery of motor function.

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