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Computer-assisted assessment of the histological structure of the human sural nerve

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Background: *The aim of this study was to assess the histological structure (cross-sectional area — CSA, number of nerve bundles) of the human sural nerve at the level above the lateral malleolus, using computer-assisted image analysis.*
Materials and methods: *This study has been conducted using sural nerves dissected from cadavers during routine autopsies. The harvested tissues samples were dehydrated, embedded in paraffin, sectioned at 4 µm and stained with haematoxylin and eosin. Each cross-section was photographed (16 × magnification) and the images were analysed using Java ImageJ.*
Results: *The studied group comprised 12 women and 25 men (mean age 60.1 ± 15.7 years), yielding a total of 74 sural nerves (37 right vs. 37 left). The mean ± standard deviation CSA of the sural nerve was 0.14 ± 0.07 cm^2. The mean number of nerve bundles in the sural nerve was 10.5 ± 6.0. In terms of gender and side, neither the CSA ($p = 0.45$ and $p = 0.79$, respectively) nor the number of nerve bundles revealed any differences ($p = 0.34$ and $p = 0.47$, respectively). Strong negative correlations were noted between the age of the donors and the sural nerve CSA ($r = –0.69$, $p = 0.02$), as well as the number of nerve bundles ($r = –0.57$, $p = 0.06$).*
Conclusions: *This study shows that there are no statistical differences between the CSA and the number of nerve bundles in the sural nerve when compared by gender and side of the lower limb. This study also allows drawing the conclusion that the sural nerve degenerates with age in terms of both the CSA and the number of nerve bundles.* (Folia Morphol 2014; 73, 3: 292–297)

Key words: bundle, computer-assisted image analysis, histology, nerve, sural

INTRODUCTION

The sural nerve is a small, peripheral sensory nerve which derives from the S1 and S2 nerve roots [1]. The sciatic nerve at the level of the popliteal fossa divides into the tibial and common peroneal nerves. It has been determined that the sural nerve originates distally, in the place where the medial sural cutaneous nerve branches connect with the common peroneal nerve fibres [5, 16].

The sural nerve innervates the cutaneous part of the posterolateral aspect of the lower third of the leg, the lateral malleolus, the lateral side of the dorsal part of the foot to the fifth toe [1, 6].

According to Belsack et al. [1] this nerve can be easily found about 10 cm above the calcaneus. Here, slightly lateral to the Achilles tendon, this nerve adheres to the lesser saphenous vein. Distally from this point the sural nerve runs between the lateral

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malleolus and the calcaneus and continues to the fifth toe [5, 16].

The sural nerve is widely used for many therapeutic interventions — to determine nerve conduction velocity, for nerve biopsy and during nerve grafting procedures [4, 5, 10].

This nerve is the most commonly used donor nerve for peripheral nerve reconstruction [12]. The histological structure of the graft should be compatible with the nerve that will receive that graft.

The aim of this study was to assess the histological structure of the human sural nerve at the level above the lateral malleolus, using computer-assisted image analysis.

MATERIALS AND METHODS

This study has been conducted using sural nerves dissected from cadavers during routine autopsies performed at the Department of Forensic Medicine (Jagiellonian University Medical College).

There were no restrictions as to gender or age concerning inclusion into the study. Exclusion criteria included extensive damage to the sural nerve preventing proper sample acquisition.

The research protocol was approved by the Jagiellonian University Bioethics Committee (registry number KBET/119/B/2009).

Dissection technique

The incision was made 2 cm posteriorly from the apex of the lateral malleolus. The incision was 16 cm long — 14 cm proximally to the apex of the lateral malleolus and 2 cm distally. After dissecting the skin and the subcutaneous tissue, the tibial nerve and the common peroneal nerve were exposed at the level where they join to form the sural nerve. Next, the sural nerve was dissected 1 cm below the joining point of the 2 nerves mentioned above, but before it was able to give off any branches. Each time a 1 cm long fragment of the sural nerve was excised. The existing incision was closed using a running intradermal suture.

Preparing histological slide

The excised fragment of the sural nerve was placed in a glass container with a 10% solution of formaldehyde (pH 7.4). After 2–5 days a part of it was excised for further tissue processing. Tissues were dehydrated, embedded in paraffin, sectioned at 4 μm and stained with haematoxylin and eosin (Fig. 1).

Micromorphometry

The number of nerve bundles in the sural nerve was assessed using light microscopy (100 × magnification). Next, each cross-section was photographed (16 × magnification) and the images were analysed using Java ImageJ (version 1.46d) developed by Wayne Rasband (National Institute of Health) [11]. The cross-sectional area (CSA), transverse and longitudinal dimensions and the number of nerve bundles in the sural nerve were calculated.

Statistical analysis

Statistical analysis was conducted using computer software Statistica 10.0 PL by StatSoft Poland (licensed to the Jagiellonian University Medical College). To analyse the data, elements of descriptive statistics were used (mean, standard deviation [SD], percentage distribution). To assess differences between groups the t-test or the χ^2 test were used. The significance level was set at $p < 0.05$.

RESULTS

The studied group comprised 12 women and 25 men (mean age 60.1 ± 15.7 years), yielding a total of 74 sural nerves (37 right vs. 37 left).

The mean ± SD CSA of the sural nerve was 0.14 ± ± 0.07 cm^2. The mean number of nerve bundles in the sural nerve was 10.5 ± 6.0.

When compared by gender, the mean CSA of the sural nerve in women was 0.12 ± 0.06 cm^2 and in men 0.14 ± 0.08 cm^2 ($p = 0.45$). When compared by gender, the mean number of nerve bundles of the sural nerve in women was 10.3 ± 8.3 and in men 9.5 ± 9.2 ($p = 0.34$).

When compared by side, the mean CSA of the sural nerve in the left lower extremity was 0.13 ± ± 0.07 cm^2 and in the right 0.12 ± 0.09 cm^2 ($p = = 0.79$). When compared by side, the mean number of nerve bundles in the left sural nerve was 11.8 ± 6.9 and in the right 13.0 ± 6.6 ($p = 0.47$).

A strong negative correlation was noted between the age of the donors and the CSA of the sural nerve ($r = –0.69$, $p = 0.02$) (Fig. 2). Another strong negative correlation, with borderline statistical significance, was noted between the age of the donors and the number of nerve bundles in the sural nerve ($r = –0.57$, $p = 0.06$) (Fig. 3).

DISCUSSION

This study presents data obtained using computer-assisted image analysis of the histological stru-

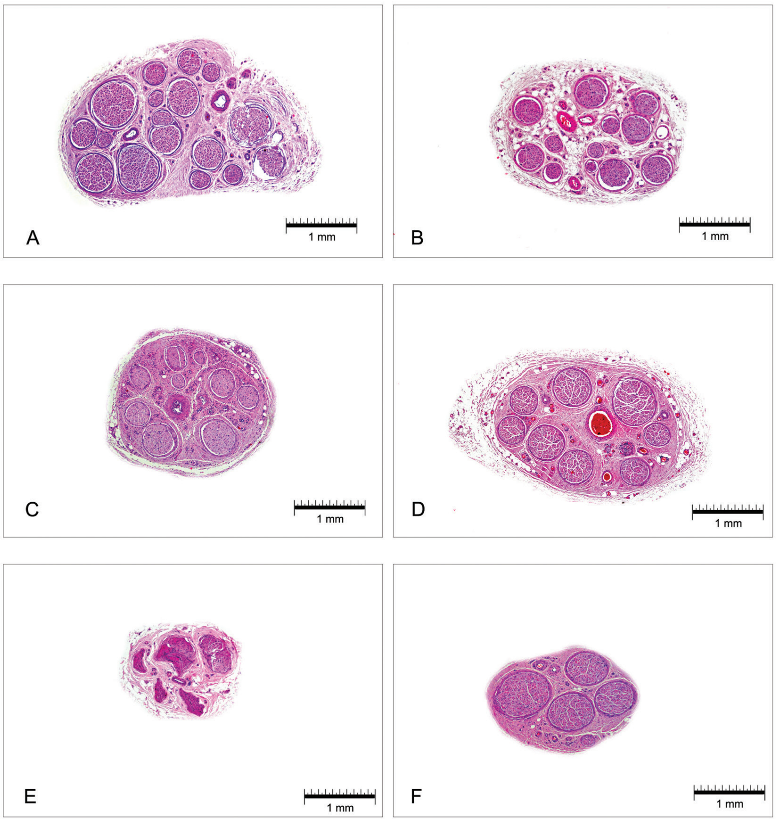

Figure 1. Cross sections of different sural nerves (haematoxylin and eosin staining). All cross sections were taken at the same level — 1 cm below the joining point of the tibial and the common peroneal nerves. All specimens were male. y.o. – years old. Varying number of nerve bundles can be seen: **A.** 17 (38 y.o.); **B.** 12 (50 y.o.); **C.** 9 (53 y.o.); **D.** 8 (55 y.o.); **E.** 5 (62 y.o.); **F.** 5 (69 y.o.).

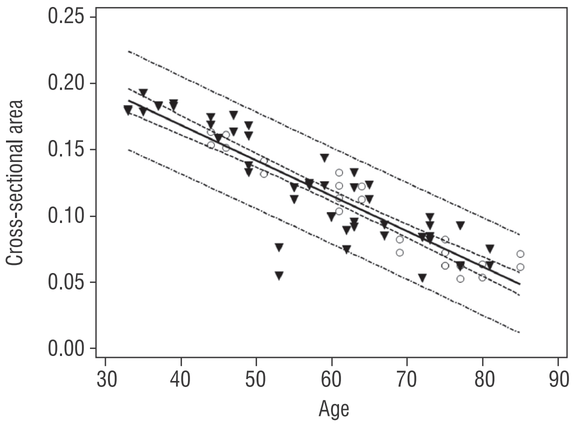

Figure 2. A scatter plot of participants' age vs. sural nerve cross--sectional area. Black triangles represent males, and white circles represent females. The continuous line represents regression. The dash lines represent the 95% confidence intervals of the regression. The dash-dot lines represent the 95% prediction intervals.

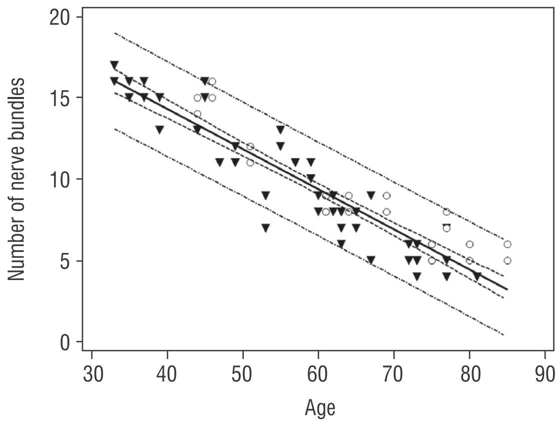

Figure 3. A scatter plot of participants' age vs. number of nerve bundles. Black triangles represent males, and white circles represent females. The continuous line represents regression. The dash lines represent the 95% confidence intervals of the regression. The dash-dot lines represent the 95% prediction intervals.

cture of the human sural nerve. Literature analysis shows that previous researchers focused mainly on its anatomical variations [1, 5, 9, 14–18]. A limited number of studies concentrated on the histological analysis of the sural nerve [3, 7, 8, 13]. Table 1 summarises the results of previous studies on the subject

Table 1. Studies on the subject of sural nerve structure

	Group (n)	CSA of the sural nerve [cm²]	Aim of the study	Type of study	Pathology of the experimental group	Population
Mizia et al. [this study]	Total: n = 37	0.14	To analyse the histological structure (CSA, number of nerve bundles) of the human SN at the level above the lateral malleolus	Cadaveric	–	European
Vuksanovic -Bozaric et al., 2013 [17]	Total: n = 1 Age = 56	–	Case report: atypical SN anatomical course	Cadaveric	–	European
Pazzaglia et al., 2013 [13]	Total: n = 57 EG: n = 20 CG: n = 37	0.34	To characterise the SN in a sample of Charcot- -Marie-Tooth 1A patients	US 18 MHz	Charcot-Marie-Tooth 1A disease	European
Ricci et al., 2009 [14]	Total: n = 20 EG: n = 5 CG: n = 15 MA = 59	–	To demonstrate that US visualisation of the SN can be achieved in routine clinical practice	US 14 MHz and 18 MHz	CVI	European
Hobson-Webb et al., 2013 [7]	Total: n = 50 EG: n = 25 MA = 60.6 CG: n = 25 MA = 49.2	EG: 0.53 CG: 0.49	To establish the US characteristics of the SN in diabetic polyneuropathy and correlating them with electrodiagnostic findings	US 18 MHz	Diabetic polyneuropathy	American
Cartwright et al., 2009 [3]	Total: n = 36 EG: n = 12 CG: n = 24	EG: 0.5 CG: 0.63	To compare the SN CSA in individuals with CMT 1B with normal, healthy controls	US 12 MHz	Charcot–Marie-Tooth 1A disease	American
Cartwright et al., 2008 [2]	Total: n = 60 MA = 45.9	0.53	To obtain reference values of SN CSA at the level of the distal calf	US 15 MHz	–	American
Eid and Hegazy, 2011 [5]	Total: n = 12	–	To determine the variations of the SN and its relations to the calcaneal tendon and lateral malleolus	Cadaveric	–	African
Belsack et al., 2013 [1]	Total: n = 10 MA = 53	0.15	To describe the normal US anatomy and examination technique of the SN and to present a case series of patients with different types of SN pathology	Cadaveric, US 12 MHz	Different pathologies	Asian
Liu et al., 2012 [8]	Total: n = 150 EG: n = 100 CG: n = 50	CG: 0.14 NNG: 0.15 NG: 0.18	To evaluate using US the morphological changes of the SN in patients with type 2 diabetes mellitus	US 22 MHz	Diabetes mellitus (type 2)	Asian
Wahee et al., 2010 [18]	Total: n=30	–	To classify the different patterns of cutaneous nerves found in Indian foetuses and comparing them with other populations	Cadaveric	–	Asian
Sekiya et al., 2006 [16]	Total: n = 18	–	To provide a qualitative description of the fibres constituting the cutaneous nerves of the calf, ankle and the dorsum of the foot	Cadaveric	–	Asian
Madhavi et al., 2005 [9]	Total: n = 130	–	To establish different patterns of cutaneous innervation of the SN on the dorsum of the foot	Cadaveric	–	Asian

CSA — cross-sectional area; CG — control group; CVI — chronic venous insufficiency; EG — experimental group; MA — mean age; NNG — non-neuropathic group; NG — neuropathic group; SN — sural nerve; US — ultrasonography of sural nerve structure. However this is the first study to assess the number of nerve bundles in the sural nerve at the level of the lateral malleolus.

In our study we obtained the CSA of the sural nerve, as well as the number of nerve bundles in its structure. The results were compared between males and females as well as in regard to the side of the lower limb. We found that the average CSA of the sural nerve (0.14 cm^2) was similar to the results obtained by Belsack et al. [1] — 0.15 cm^2 and Liu et al. [8] — 0.168 cm^2. However, our results significantly differed from those obtained by Pazzaglia et al. [13] — 0.34 cm^2. The reasons for such a discrepancy are most probably the fact that Pazzaglia et al. [13] assessed the CSA of the sural nerve at the midcalf instead of the lateral malleolus, and used an ultrasound assessment (18 MHz probe) for their study rather than performing a histological assessment of autopsy specimens. The methodology of most [7, 13] sural nerve ultrasound studies has been based on performing a cross-sectional diameter measurement by drawing a line across the widest point of the nerve, beginning and ending just inside the hyperechoic rim. Such a measurement method is very error-prone when compared with computer-assisted histological assessment. That is why we would like to point out that further studies on the subject of sural nerve structure should preferably adhere to CSA values given by histological studies, rather than ultrasound-based research to avoid overestimating the CSA.

It was noted that there were no differences in the histology (CSA, number of bundles) of the sural nerve in regard to the side of the lower extremity. Based on the achieved results, it can be concluded that a sural nerve graft may be taken from either the right or the left lower limb.

As our study suggests, the sural nerve considerably degenerates with age. This finding is consistent with other studies [7, 13]. The nerves' degeneration affects not only its CSA but the number of a bundles as well. Based on these results, we suggest that sural nerve grafts should be used only for autografts. However, if need arises for an allograft, such a procedure should be performed with the donor and the host being of a similar age. This finding could partially, but not entirely as the ultrasound measured CSA was over 2.5 times larger than the one obtained in microscopic assessment, explain the difference in CSA values between our study and that by Pazzaglia et al. [13]. The patients examined by Pazzaglia et al. [13] were considerably younger (mean age of the group 44.2 years) than in our study.

This study has been based on the analysis of histological material obtained from cadavers during routine autopsy. This is of great importance, as the majority of studies assessing the structure of the sural nerve were based on ultrasonography [1, 13, 14]. The accuracy of histological measurements greatly outweighs the ultrasonographic one as has been mentioned before.

However, this study has also several limitations. The number of examined cadavers was fairly small (n = 37). However, when compared with literature data, our findings are similar to the results obtained on larger groups [1, 8]. The mean age of the cadavers was 60 years. This shows the need to perform a similar study basing on material obtained from younger donors. However we believe that this study significantly contributes to the body of literature concerning sural nerve graft material selection methods, and is the first to report the number of nerve bundles present in the sural nerve at the level of the lateral malleolus.

CONCLUSIONS

Concluding, this study reveals that there are no statistical differences between the CSA and the number of nerve bundles in the sural nerve when compared by gender and by side of the lower limb. This study also allows drawing the conclusion that the sural nerve degenerates with age in terms of both the CSA and the number of nerve bundles.

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