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Comparison of the histological structure of the tibial nerve and its terminal branches in the fresh and fresh-frozen cadavers

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ABSTRACT

Background: The aim of this study was to compare the histological structure (cross-sectional area (CSA) and number of nerve fascicles) of the distal part of the tibial nerve (TN) and its terminal branches (medial plantar nerve [MPN], lateral plantar nerve [LPN]) in the fresh and fresh-frozen cadavers using computer assisted image analysis.

Materials and methods: The tibial nerve with terminal branches (medial and lateral plantar nerves) were dissected from the fresh and fresh-frozen cadavers. Each nerve was harvested 5 mm proximally and respectively 5 mm distally from the tibial nerve bifurcation, marked, dehydrated, embedded in paraffin, sectioned at 2 μm slices and stained with haematoxylin and eosin. Then the specimens were photographed and analyzed using Olympus cellSens software.

Results: The fresh cadavers group comprised 60 feet (mean age 68.1 ± 15.2 years). The mean CSA and the number of nerve fascicles were respectively 15.25 ± 4.6 mm^2, 30.35 ± 8.45 for the tibial nerve, 8.76 ± 1.93 mm^2, 20.75 ± 7.04 for the medial plantar nerve and 6.54 ± 2.02 mm^2, 13.40 ± 5.22 for the lateral plantar nerve. The fresh-frozen cadavers group comprised 21 feet (mean age 75.1 ± 9.0 years). The mean CSA and the number of nerve fascicles were respectively 13.71 ± 5.66 mm^2, 28.57 ± 8.00 for the tibial nerve, 7.55 ± 3.25 mm^2, 18.00 ± 6.72 for the medial plantar nerve and 4.29 ± 1.93 mm^2, 11.33 ± 1.93 for the lateral plantar nerve. Only lateral plantar nerves showed statistical differences in the CSA and the number of

nerve fascicles between examined groups ($p = 0.000$, $p = 0.037$ respectively). A positive correlation was found between donors age and tibial nerve CSA in the fresh cadavers group ($r = 0.44$, $p = 0.000$). A statistical difference was found between the medial and lateral plantar nerves both in the CSA and the number of nerve fascicles ($p < 0.001$, $p < 0.001$ respectively).
Conclusions: The CSA and the number of nerve fascicles of the tibial and medial plantar nerves were similar in the fresh and fresh-frozen cadavers whilst different in the lateral plantar nerve. The tibial nerve showed increasing CSA with the advanced age in the fresh cadavers. The medial plantar nerve had larger CSA and more nerve fascicles than the lateral plantar nerve.
Key words: tibial nerve, cross-sectional area, medial and lateral plantar nerves, fresh cadavers, fresh-frozen cadavers

INTRODUCTION

The tibial nerve is a peripheral sensorimotor nerve arising as a branch of sciatic nerve bifurcation in the popliteal fossa [40]. It runs vertically on the tibialis posterior muscle together with the posterior tibial vessels. Postero-inferiorly to the medial malleolus it terminates emitting medial plantar nerve and smaller lateral plantar nerve [28]. The tibial nerve bifurcation level shows a great variability as so depending on the study its localization is referred to the medial or lower located lateral malleolus [24, 43]. Most commonly it is described below the tip of the medial malleolus, inside the tarsal tunnel [42]. Tibial nerve and its branches provides innervation to the posterior lower leg, the muscles and skin of the sole of the foot [21].

For many years ankle arthroscopy has proved to be a useful diagnostic and therapeutic procedure for ankle and foot disorders. Although it is a minimally invasive surgery neurological complications are most frequently reported referring to the tibial, sural, superficial peroneal and deep peroneal nerves [1, 45, 47]. According to Freedman et.al [13] all neurovascular impairments are caused by distractor pin or portal placement. In order to avoid iatrogenic injuries and to perform safe and reproducible arthroscopy constant training is highly recommended.

Nowadays necessity of constant practicing of surgical skills is emphasized by professionals [2]. They clearly highlight the superiority of fresh cadavers among any frozen or anatomically preserved. However, due to ethical and technical problems as well as limited access to the fresh bodies, fresh-frozen cadavers proved to be convenient surgical training model [35]. Because of their most lifelike features they are used by surgeons, orthopedics, radiologists and anesthesiologist to practice and improve operating skills [12, 17]. Fresh-frozen bodies also found application in the research and bioengineering, allowing development of new instruments and procedures.

The aim of the this study was to compare the histological structure of the tibial nerve and its terminal branches in the fresh and fresh-frozen cadavers.

MATERIALS AND METHODES

The study was conducted on 60 lower limbs of the fresh cadavers and on 21 lower limbs of the fresh-frozen cadavers in the Department of Anatomy between December 2016 and March 2019. The group of fresh-frozen cadavers composed of already amputated lower limbs at the level of the knee originating from mixed donors with known medical record. The exclusion criteria were any deformation of the lower limb or the lower limb trauma, surgical or radiotherapeutic procedures of the lower limb, chronic disease of the lower limb in the medical record of the donor.

The research protocol was approved by the local Ethics Committee (Registry No. 122.6120.315.2016). The study has been performed in accordance with the ethical standards established in the 1964 Declaration of Helsinki and its later amendments.

Dissection technique

The incision was made in the midline between the tip of the medial malleolus and the Achilles tendon. It continued 10 cm proximally along the Achilles tendon and 10 cm distally curving anteriorly 2 cm below the tip of the medial malleolus. Upon dissecting the skin and the subcutaneous tissue the tibial nerve was visualized together with the posterior tibial artery and two posterior tibial veins. After meticulous dissection the tibial nerve, its bifurcation and lateral and medial plantar nerves were exposed. The plantar nerves were marked 2 cm distally

from the tibial nerve bifurcation point with the following pattern: blue thread - lateral plantar nerve, white thread - medial plantar nerve. The tibial nerve was left without any marking. Then 3 cm proximally to the bifurcation the tibial nerve was cut out from the main nerve trunk. Accordingly, 3 cm distally the medial and lateral plantar nerves were cut out. The excised tibial nerve and its terminal branches were removed en bloc from the cadaver. The incision was closed with the running subcuticular suture. In the group of fresh-frozen cadavers the dissection was performed after thawing of the specimens overnight at room temperature. The harvesting was carried out by the same surgeon.

Preparation of histological slide

The excised block of nerves was fixed in a 10% solution of the formaldehyde (pH 7.4). After 2-5 days it was removed from the formaldehyde. The tibial nerve was cut transverse to the nerve axis 5 mm and 10 mm proximally to the tibial nerve bifurcation point as were the medial and lateral plantar nerves 5 mm and 10 mm distally to the tibial nerve bifurcation point. Obtained 5 mm long nerve fragments were dehydrated separately and embedded in paraffin according to its initial marking. Each paraffin cube was transverse sectioned with the microtome providing one 2 μm thick slice. Subsequently each slice was stained with haematoxylin and eosin (Figure 1).

Micromorphometry

The CSA and the number of nerve fascicles of the tibial nerve, medial plantar nerve and lateral plantar nerve were assessed using a light microscope (Olympus BX53, 20 x magnification). Each cross-section was photographed (20 x magnification), afterwards the CSA was measured semi-automatically using Olympus cellSens Standard 2.3 software with the producers precision of 10 μm, whilst the number of nerve fascicles was calculated manually. Each slice was assessed once by the same pathologist. Then the values of the CSA and the number of nerve fascicles were tabulated according to the group (fresh or fresh-frozen cadavers).

Statistics

Obtained data were statistically processed using descriptive statistics such as percentage, mean, median, standard deviation, upper and lower quartiles. A p-value of < 0.05 was considered as statistically significant. Two groups were compared using the Mann-Whitney test or t-test depending on normal distribution. To compare CSA and number of nerve fascicles between TN, MPN and LPN paired t-test or Wilcoxon rang test were used depending on whether data was normally distributed. Correlation coefficients were calculated to establish any statistical dependence between parameters. All analyses were performed using MedCalc version 16.8.

RESULTS

There were thirty fresh cadavers dissected (n = 60 lower limbs) with a mean age of 68.1 ± 15.2 (range from 27 to 91 years). 28 feet were female (46.7%) and 32 were male (53.3%). In the group of fresh-frozen cadavers twenty one lower limbs were dissected with a mean age of 75.1 ± 9.0 (range from 60 to 92 years). 12 feet were female (57.1%) and 9 were male (42.9%). The mean CSA and number of nerve fascicles of the tibial, medial plantar and lateral plantar nerves in the fresh and fresh-frozen groups are presented in Table I. Gender differences between examined groups are presented in Table II. In both examined groups males' tibial nerves showed larger CSA and more nerve fascicles than females'. Only lateral plantar nerves showed statistical differences in the CSA and number of nerve fascicles between examined groups. The lateral plantar nerve also proved statistical difference among males (CSA and number of nerve fascicles) and females (CSA) in fresh and fresh-frozen cadavers. In the fresh cadavers no statistically significant differences between right and left foot of the individual were found ($p > 0.05$). Such comparison was not possible to perform in the fresh-frozen cadavers as the examined lower limbs originated from different individuals. There is statistically significant difference between medial and lateral plantar nerve in CSA and number of nerve fascicles in both groups ($p < 0.001$). CSA of the medial plantar nerve confirmed to be 1.3 times and 1.8 times larger than the lateral plantar nerves' in the fresh and fresh-frozen specimens respectively. The medial plantar nerve also proved to have more nerve fascicles than the lateral plantar nerve in both examined groups. A positive correlation was noted between the age of donors and the CSA of the tibial nerve in the fresh cadavers group ($r = 0.44$, $p = 0.000$) (Table III).

DISCUSSION

The present study compares histological structure (CSA and number of nerve fascicles) of the distal part of the tibial nerve and its terminal branches (medial and lateral plantar nerves) in the fresh and fresh-frozen cadavers assessed using computer-assisted measurements. Literature analysis shows that in the previous studies the CSA of the tibial nerve was evaluated by ultrasound or magnetic resonance imagining on the living patients or volunteers [5, 15, 22]. To the best of authors knowledge this is the first publication analyzing histological differences in peripheral nerves obtained from the fresh and fresh-frozen cadavers. It is also the first study revealing tibial, medial and lateral plantar nerves CSA measured directly on the nerves harvested from the fresh cadavers. Furthermore no reference values for the CSA of the medial and lateral plantar are available in the literature.

In the present study the tibial, medial plantar and lateral plantar nerves harvested from the 60 fresh cadavers were compared to 21 collected from the fresh-frozen cadavers. The fresh cadavers group composed of younger donors (mean age: 68.1 vs 75.1) and presented slightly higher values of CSA (tibial nerve: 15.25 vs. 13.71; medial plantar nerve 8.76 vs 7.55; lateral plantar nerve: 6.54 vs. 4.29) and more nerve fascicles (tibial nerve: 30.35 vs. 28.57; medial plantar nerve 20.75 vs 18.00; lateral plantar nerve: 13.40 vs. 11.33). Nevertheless tibial nerve CSA measured in both groups is in line with results of ultrasound and magnetic resonance imaging performed on living patients (Table IV). The statistical analysis proved that the tibial and medial plantar nerves are similar in the fresh and fresh-frozen groups. On the other hand the lateral plantar nerves appeared to be statistically different. Such discrepancy may be the result of anatomical differences of the examined nerves. The lateral plantar nerve is the smaller terminal branch of the tibial nerve bifurcation [21]. Because of that it may be suggested that freezing process does not alter larger nerves (TN, MPN) whilst impacts smaller ones (LPN). Although the differences proved to be statistically insignificant (except for LPN) their slightly decreased values in fresh-frozen cadavers is worth noticing. Besides micromorphometric assessment some differences between two examined groups appeared during its histological preparation. Fresh-frozen specimens showed grater stiffness and hardness of the nerve trunks, poorly stained with haematoxylin and eosin and revealed more artifacts in the microscopic analysis.

Decreased CSA of the assessed nerves may be explained by Bakhach [4] who described changes occurring in biological tissues during freezing using thermodynamic and

biophysical laws. Emphasizing that water may reach up to 70% of tissues volume he examined its transfer between intra and extracellular compartments throughout crystallization process. Intracellular formation and aggregation of ice crystals destroy its structures and cause mechanical stress on the cell walls resulting in deformation and fragmentation. Moreover water transition into a solid state leads to changes in extracellular chemical composition with the increased ion accumulation. Such concentration gradient between cell membrane makes water run out of the intracellular space causing its dehydration. These may elucidate rigidness of the nerve samples, artifacts in the microscopic assessment and slightly decreased CSA of the fresh-frozen cadavers registered in the present study.

Although fresh cadavers retain biomechanical features and are most suitable for the surgical training, they putrefy and are available only for the short time [3]. Searching for the best fresh body equivalent brought to many studies on its preservation [9, 12]. Along proved advantages each method revealed some limitations, as so: formalin fixation makes the specimens stiff and discolored, Thiel embalming requires infrastructure for the process and is not suitable for all tissues, fresh-freezing brings the risk of infection and needs time for thawing [39]. Nevertheless fresh-frozen cadavers seems to be the most flexible and realistic [19]. They appeared to be even better than the virtual reality stimulator [34].

While literature provides comparative analysis of the fresh and fresh-frozen tendons [18, 6], bones [10, 26, 41], osteochondral allografts [29] there is lack of such comparison for the human peripheral nerves. Hohmann et al. [18] revealed that the long head of biceps tendons showed higher loads to failure and lower elasticity in the fresh-frozen samples when compared to the fresh specimens. At the same time fresh tendons were wider and presented larger CSA. On the contrary Bitar et al. [6] state that fresh-frozen tendons of the semitendinosus muscle show no histological differences referring to the fresh ones. Similarly Panjabi et al. [30] deny any physical or histological changes in the fresh-frozen specimens. Opposite to that Giannini et al. [14] noted an increased CSA in the fresh-frozen tendons of the posterior tibial muscles as well as increased stiffness and decreased ultimate load. An interesting study was performed by Zarb et al. [46] who analyzed the quality of the Magnetic Resonance (MR) images of a live patients', fresh-frozen and Thiel embalmed bones, ligaments, tendons and muscles of the ankle. The image quality of the fresh-frozen specimen appeared to be higher when compared to the live patient. Unfortunately no nerves of the ankle were included in the research which might have been beneficial for the present study reference.

Fresh-frozen peripheral nerves were examined mostly in relation to their biomechanical properties [8, 44]. Stouthandel et al. [37] compared Thiel embalmed and fresh-frozen median nerves showing slight increase of CSA in the embalmed group, no significant difference in elasticity and similar biomechanical patterns. Enlarged CSA of the nerves preserved with the Thiel method is interpreted to be the result of the embalming fluid uptake. Sargon et al. [32] counted the myelinated nerve fibers of the fresh-frozen facial nerve terminal branches concluding that both fresh and fresh-frozen human specimens are better than formalin fixed in order to perform the anatomic dissection and find tiny nerves.

To the best of authors knowledge there has not been any publication which compared histological structure of the fresh-frozen human nerves to the fresh ones. As so, such analysis of the peripheral nerves together with biomechanical experiments may constitute a valuable subject for the future studies.

Albeit there were relatively high number of lower limbs examined in the present study (81 feet) their uneven distribution among the compared groups (60 vs. 21) and low number of fresh-frozen cadavers might have influenced the results. Only nine males in the fresh-frozen cadavers group would have significantly hindered the gender comparison. Second limitation is the fact that lower limbs included in the group of fresh-frozen cadavers originated from different donors which impeded the intra-individual left-right comparison. Another restriction is the various age of the analyzed groups which is proved to correlate with peripheral nerves CSA [15, 27]. Narrow range of age in the fresh-frozen cadavers (from 60 to 92 years) might have also biased the age correlation which was confirmed for the tibial nerve CSA in the fresh cadavers (range of age from 27 to 91 years). Therefore, for the sake of future studies, the authors would recommend to collect and compare specimens from the contralateral sides of the individual (followed by the left-right difference exclusion).

CONCLUSIONS

To conclude, the authors of the present study proved that freezing process alters tissue properties of the smaller nerves on top of impacting biomechanical features of the peripheral nerves. Histological structure of the larger nerves remains uninfluenced by the freezing process.

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Table I. Measured nerve parameters for TN, MPN and LPN - comparison between fresh and fresh-frozen cadavers

Measurement		Fresh cadavers n	Fresh cadavers Mean ± SD	Fresh cadavers Median	Fresh cadavers Lower quartile (Q1)	Fresh cadavers Upper quartile (Q3)	Fresh-frozen cadavers n	Fresh-frozen cadavers Mean ± SD	Fresh-frozen cadavers Median	Fresh-frozen cadavers Lower quartile (Q1)	Fresh-frozen cadavers Upper quartile (Q3)
Cross-sectional area [mm^2]	tibial nerve	60	15.25 ± 4.65	14.66	11.77	17.29	21	13.71 ± 5.66	12.84	9.50	16.15
	medial plantar nerve		8.76 ± 1.93	8.45	7.19	9.90		7.55 ± 3.25	7.53	4.61	10.36
	lateral plantar nerve		6.54 ± 2.02	6.44	5.12	7.41		4.29 ± 1.93	4.31	2.52	5.76
Number of nerve fascicles	tibial nerve	60	30.35 ± 8.45	31.00	25.00	35.25	21	28.57 ± 8.00	31.00	22.00	35.00
	medial plantar nerve		20.75 ± 7.04	20.00	16.00	25.00		18.00 ± 6.72	18.00	12.00	22.00
	lateral plantar nerve		13.40 ± 5.22	13.50	10.75	15.00		11.33 ± 1.93	11.00	7.00	14.00

Footnotes: numbers in bold indicate statistically significant differences between fresh and fresh-frozen cadavers ($p < 0.05$).

Table II. Measured nerve parameters for TN, MPN and LPN - comparison by gender between fresh and fresh-frozen cadavers

Gender	Measurement		Fresh cadavers n	Fresh cadavers Mean ± SD	Fresh cadavers Median	Fresh cadavers Lower quartile (Q1)	Fresh cadavers Upper quartile (Q3)	Fresh-frozen cadavers n	Fresh-frozen cadavers Mean ± SD	Fresh-frozen cadavers Median	Fresh-frozen cadavers Lower quartile (Q1)	Fresh-frozen cadavers Upper quartile (Q3)
Women	Cross-sectional area [mm^2]	tibial nerve	28	12.27 ± 2.45	11.85	10.35	14.31	12	12.70 ± 3.90	13.46	9.28	15.[illegible]
		medial plantar nerve		7.81 ± 1.41	7.37	6.70	9.10		7.77 ± 3.38	7.41	5.88	10.[illegible]
		lateral plantar nerve		5.83 ± 1.25	5.77	4.61	6.86		4.47 ± 2.05	4.56	2.70	5.[illegible]
	Number of nerve fascicles	tibial nerve	28	26.32 ± 8.87	25.00	19.50	34.00	12	28.08 ± 9.13	31.50	20.50	34.[illegible]
		medial plantar nerve		17.71 ± 5.28	18.00	14.50	20.50		16.50 ± 7.23	17.00	12.00	19.[illegible]
		lateral plantar nerve		11.50 ± 3.72	12.00	9.00	14.00		11.42 ± 7.23	9.00	6.00	14.[illegible]
Men	Cross-sectional area [mm^2]	tibial nerve	32	17.86 ± 4.57	17.10	15.02	19.90	9	15.06 ± 7.45	12.57	10.09	16.[illegible]
		medial plantar nerve		9.58 ± 1.95	9.16	8.40	10.66		7.26 ± 3.25	7.64	4.61	9.[illegible]
		lateral plantar nerve		7.17 ± 2.36	7.08	5.18	8.35		4.05 ± 1.86	3.35	2.28	5.[illegible]
	Number of nerve fascicles	tibial nerve	32	33.88 ± 6.31	34.00	28.50	38.00	9	29.22 ± 6.67	30.00	25.00	35.[illegible]
		medial plantar nerve		23.41 ± 7.37	22.50	17.50	29.50		20.00 ± 5.77	20.00	17.00	24.[illegible]
		lateral plantar nerve		15.06 ± 5.81	14.50	12.50	16.50		11.22 ± 2.73	12.00	9.00	13.[illegible]

Footnotes: numbers in bold indicate statistically significant differences between males and females ($p < 0.05$).

Table III. Association between age and measured nerve parameters for TN, MPN and LPN in fresh and fresh-frozen cadavers

Measurement		Fresh cadavers n	Fresh cadavers R	Fresh cadavers p	Fresh-frozen cadavers n	Fresh-frozen cadavers R	Fresh-frozen cadavers p
Cross-sectional area	tibial nerve	60	**0.439**	**0.000**	21	0.112	0.629

[mm^2]	medial plantar nerve		0.083	0.531		0.040	0.862
	lateral plantar nerve		0.110	0.401		-0.045	0.847
Number of nerve fascicles	tibial nerve	60	0.086	0.512	21	-0.161	0.485
	medial plantar nerve		-0.224	0.085		-0.140	0.545
	lateral plantar nerve		-0.104	0.428		-0.204	0.376

Footnotes: numbers in bold indicate statistically significant age correlation ($p < 0.05$).

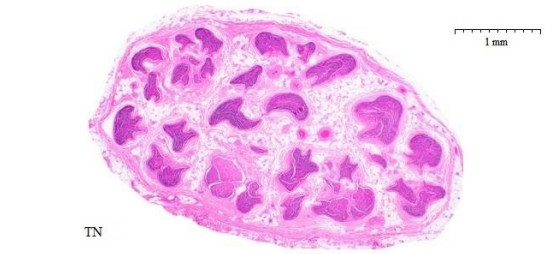
Table IV. Studies of the tibial nerve CSA measured at the level of medial malleolus

	Group (n)	Mean age	CSA of the tibial nerve at the level of medial malleolus [mm^2]	Reference range [mm^2]	Type of study
He et al., 2019 [16]	n = 40	55.2	11.6 ± 1.6	-	US 4 - 15 MHz
Lothet et. al., 2019 [25]	n = 15	21.7	12.3	-	US 18 MHz
Bedewi et al., 2018 [5]	n = 138	38.3	12.7 ± 4.5	2.0 - 30.0	US 18.5 MHz
Grimm et al., 2018 [15]	n = 100	51.2	10.2 ± 2.0	-	US 14 MHz
Kronlage et al., 2017 [22]	n = 60	30.5	* 8.1 ± 2.0	4.0 - 12.1	MRI
Singh et al., 2017 [36]	n = 75	39.5	12.4 ± 1.1	10.0 - 14.0	US 7 - 18 MHz
Kang et al., 2016 [20]	n = 20	65.0	12.4 ± 2.9	-	US 7 - 12 MHz
Boehm et al., 2014 [7]	n = 56	50.2	9.6 ± 2.2	9.0 - 10.2	US 12 - 15 MHz
Seok et al., 2014 [33]	n = 94	43.9	12.1 ± 3.1	8.5 – 22.8	US 5 - 12 MHz
Riazi et al., 2012 [31]	n = 43	46.8	17.7 ± 6.5	-	US 6 - 13 MHz
Tagliafico et al., 2012 [38]	n = 58	47.0	9.6 ± 4.0	7.2 - 13.7	US 17.5 MHz
Cartwright et al., 2008 [11]	n =60	45.9	13.7 ± 4.3	5.1 - 22.3	US 15 MHz
Lee et al., 2005 [23]	n = 24	57.4	12.0	-	US 10 - 12 MHz

* measured at the proximal third of the calf

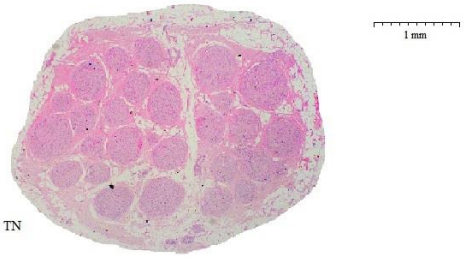
CSA - cross-sectional area; US - ultrasonography; MRI - magnetic resonance imaging

Figure 1. Cross-section of tibial nerve (TN), medial plantar nerve (MPN) and lateral plantar nerve (LPN) of the fresh cadaver (on the left) and fresh-frozen cadaver (on the right). Haematoxylin and eosin staining.

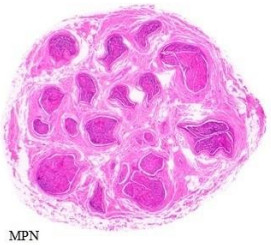


TN

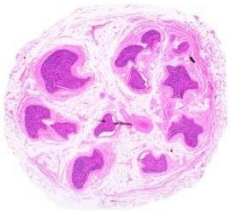
VS.



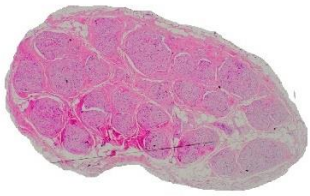
TN



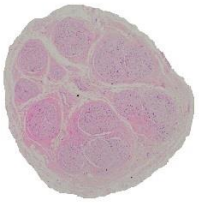
MPN



LPN



MPN



LPN