Microsurgical Anatomy of the Spermatic Cord and Spermatic Fascia: Distribution of Lymphatics, and Sensory and Autonomic Nerves

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Purpose: An understanding of the microsurgical anatomy of the spermatic cord and spermatic fascia is important for surgeons during microsurgical varicocelectomy and denervation. We examined the distribution of the lymphatics, and the sensory and autonomic nerves of the spermatic cord.

Materials and Methods: We collected spermatic cords from 11 men undergoing orchiectomy for localized testicular tumors and we biopsied a third of the spermatic fascia from 36 men undergoing microsurgical varicocelectomy. Immunohistochemical staining of the pan-neuronal marker PGP 9.5 (protein gene product 9.5), the sensory nociceptor marker CPRP (calcitonin gene-related peptide), the sympathetic marker TH (tyrosine hydroxylase), the parasympathetic marker VIP (vasoactive intestinal polypeptide) and the lymphatic marker D2-40 was performed. We counted the number of nerves and lymphatics.

Results: PGP 9.5 staining revealed dense nerve distributions in the spermatic cord and fascia. Sensory and autonomic nerve fibers were basically co-localized in the same nerve. Of the nerves 50% were identified near the vas deferens and 20% were identified in the spermatic fascia. Sensory and sympathetic nerve fibers represented most of the nerves but a few parasympathetic nerve fibers were observed. Of the lymphatics 36 per patient were identified in the spermatic cord but only a few were identified in the spermatic fascia.

Conclusions: Sensory and sympathetic nerves accounted for the majority of the nerves. Although the functional aspects of the nerves remain undetermined, information on the distribution of nerves and lymphatics is useful when dealing with nerves and preserving lymphatics during microsurgical varicocelectomy or denervation.

Key Words: testis; spermatic cord; nervous system; lymphatic vessels; anatomy, regional

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THE spermatic cord contains the vas deferens, the testicular artery and veins, the lymphatics and numerous nerves.[1] The innervation around the spermatic cord includes the genital branch of the genitofemoral nerve, the ilioinguinal nerve and the iliohypogastric nerve,[2] which are all associated with chronic orchialgia.[3] Some information is available on intraoperative microanatomy, such as the numbers of vessels and lymphatics in the spermatic cord.[4] However, the distribution of undetectable microsurgical nerves and lymphatics, and the innervation density of sensory and autonomic nerves are unclear.

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Understanding the microanatomy of the spermatic cord and the spermatic fascia is important for urologists who perform surgery involving the spermatic cord, such as microsurgical varicocelectomy and denervation.[5] Indeed, surgical microscopes enable the identification and preservation of the lymphatics of the spermatic cord, which contributes to decreasing the risk of postoperative hydrocele.[6] However, the specific localization of lymphatics in the spermatic cord is unknown. Knowledge of the distribution and the characteristics of the nerves in the spermatic cord during microsurgical denervation contributes to the surgical outcome. In the genitourinary system the nerve subtypes have been determined in the vagina[7] and the testis.[8]

The objective of this study was to identify the locations, numbers and different types of nerves and lymphatics in the spermatic cord and spermatic fascia to advance the understanding of the surgical anatomy for microsurgical varicocelectomy and denervation in humans.

MATERIALS AND METHODS

Patients and Samples

We studied spermatic cords from 11 patients who underwent orchiectomy for testicular cancer. Cross sections were obtained from 3 parts of the cords, namely the internal inguinal ring, the inguinal canal and the external inguinal ring. We also studied spermatic fascia from 36 men who underwent varicocelectomy for infertility treatments (table 1). Biopsy specimens were obtained from the third of the spermatic fascia that contained the internal spermatic fascia, the cremasteric fascia and the external spermatic fascia.

Patients who underwent microsurgical varicocelectomy were divided into 2 groups, including a pain negative group of 25 and a pain positive group of 11. The latter included patients with dull pain or discomfort. Operations were performed by a single surgeon (KS). This study was approved by our institutional review board and written informed consent was obtained from all patients.

Table 1. *Patient characteristics*

No. varicocele pts	36
Median age (range)	32 (15–42)
Mean testicular vol (ml):	
Rt	16
Lt	14
% Varicocele grade:	
G2	58
G3	42
Mean luteinizing hormone (mIU/ml)	4.27
Mean follicle-stimulating hormone (mIU/ml)	7.50
Mean testosterone (ng/ml)	4.31
% Scrotal pain or discomfort pts:	
Pos	31
Neg	69
Median age (range)	48 (27–68)
% Tumor location:	
Rt	50
Lt	50
% Tumor stage I	100
% Histological type:	
Seminoma	67
Nonseminoma	33

Tissue Processing and Immunostaining

Immunohistochemical staining was done on formalin fixed, paraffin embedded blocks. The primary antibodies were anti-TH antibody as a marker of sympathetic nerve fibers (1:100, rabbit polyclonal IgG, sc-14007, Santa Cruz Biotechnology®), anti-PGP 9.5 antibody as a pan-neuronal marker (1:1,200, rabbit polyclonal IgG, ab10404), anti-CGRP antibody as a marker of sensory nerve fibers (1:100, goat polyclonal IgG, ab36001), anti-VIP antibody as a marker of parasympathetic nerve fibers (1:200, rabbit polyclonal IgG, ab43841) and anti-D2-40 antibody as a marker of lymphatics (1:100, mouse monoclonal IgG, ab77854, Abcam®).

The sections were deparaffinized and rehydrated. Endogenous peroxidases were blocked and the sections were treated with microwave boiling for antigen retrieval. After blocking with 3% bovine serum albumin in phosphate buffered saline the sections were incubated with several primary antibodies overnight at 4C. The sections were incubated with Histofine® Simple Stain MAX PO secondary antiserum for 30 minutes. Detection was performed with DAB (diaminobenzidine, No. 415172, Nichirei, Tokyo, Japan). Sections were counterstained for 1 minute with Mayer hematoxylin and mounted.

Quantitative Analyses

Under a microscope at 400× magnification we counted the nerves and lymphatics that were immunostained for several antibodies in 3 areas, ie around the vas deferens and the other areas in the spermatic cord and spermatic fascia. We recognized pan-neuronal marker PGP 9.5 positive nerves as whole nerves. We analyzed the innervation density of CGRP positive sensory nerves, TH positive sympathetic nerves and VIP positive parasympathetic nerves. Two urologists (SO and KS) independently evaluated immunostaining and the mean number was calculated per patient.

Statistics

Statistical analysis was performed with SPSS®, version 12.0. Data are expressed as the mean ± SD. The 2 groups were compared by the Mann-Whitney rank sum test for nonparametric data with $p < 0.05$ considered statistically significant. Relationships of parameters to the number of preserved lymphatics during microsurgical varicocelectomy and the number of lymphatics in the spermatic fascia obtained during the operations were examined by linear regression techniques using the Pearson correlation coefficient.

RESULTS

Table 1 lists patient characteristics related to testicular tumors and varicoceles. Micronerves immunostained for the pan-neuronal marker PGP 9.5 were abundant in the spermatic cord and the