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How to Optimize Autonomic Nerve Preservation in Total Mesorectal Excision: Clinical Topography and Morphology of Pelvic Nerves and Fasciae

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Abstract
Background Urogenital dysfunction after rectal and pelvic surgery was significantly decreased with the introduction of nerve-preserving dissection and total mesorectal excision (TME). Profound topographic knowledge of the pelvic connective tissue spaces is indispensable for identification and preservation of autonomic pelvic nerves. The purpose of this cadaver study was to highlight the course of important autonomous nerve structures and to identify potential injury sites.
Methods Eleven cadavers were dissected according to TME with subsequent preparation of the pelvic nerves. The pelves of further three cadavers were sliced horizontally and cubed. Specimens were harvested and processed for light microscopy and immunohistochemistry to analyze both fascia and the types of nerves and their localization.
Results The neurovascular bundle, arising from the inferior pelvic plexus, shows the highest nerve density. At the lateral edge of Denonvilliers' fascia, it pierces the parietal pelvic fascia. Several fine nerve branches spread into the loose periprostatic tissue up to the prostate or pass the prostate toward the urinary bladder. En route, we consistently find perikarya of autonomic nerves. Within the mesorectum, nerve fibers are distributed heterogeneously with laterally high densities, ventrally and dorsally low densities.
Conclusion The highest risk for pelvic nerve damage—apart from lesions of the superior hypogastric plexus itself—is anterolaterally of the rectum where the neurovascular bundle releases from the pelvic sidewall. Careful dissection helps to identify and protect these nerve structures. The retroprostatic Denonvilliers' fascia contains no important nerve structures.

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Introduction

The concept of total mesorectal excision (TME) enables both total tumor excision with regard to fascial and lymphatic anatomy and a nerve-preserving surgery with reduction of urogenital dysfunctions. Various studies reflect significant decreases of local recurrence in curative surgery to 6–10% [1, 2]. Camillieri-Brennan and Steele evaluated meta-analytically all relevant papers listed in MEDLINE, published between 1970 and 1997, that focused on the quality of life after treatment for colorectal cancer [3]. Sexual dysfunction was described in 39% and urinary dysfunction in 27%. More recent studies applying the principles of TME describe a decrease of urinary dysfunction to 9% [4]. Sexual dysfunctions decreased to 26% [4], which is still too high but demonstrates the potential of TME.

Japanese surgeons combined pelvic autonomic nerve preservation with lateral lymph node dissection [5–8]. Depending on the Dukes stage, they found recurrence rates of 5–6% [8]. Urogenital disturbances were as low as 7% [8] when autonomic nerves were widely preserved. Only in patients with complete resection of the pelvic autonomic

nerves, severe urogenital dysfunction was observed in 31% [8].

The key to a successful TME is the definition of the intrapelvic fascial anatomy and the courses of the pelvic nerves. The excavation of the sacral bone is covered by the parietal pelvic fascia, a subperitoneal continuation of the transversal fascia, whereas rectum, prostate, seminal vesicles, and urinary bladder are covered by visceral pelvic fasciae, which are named accordingly (e.g., rectal fascia = mesorectum). Denonvilliers' fascia separates as urorectal septum the rectum from prostate/vagina (septum rectoprostaticum/-vaginal/-vesical). No clearly defined layers and no definable lateral edges are known today and the function of the nerves scattered throughout the fasciae has not been properly ascertained [9].

Two distinct compartments are found in the lower pelvis, separated by the levator ani muscle (Fig. 1). In the infralevatory pathway, the pudendal nerve arises from the sacral plexus and runs via the ischioanal fossa to the genitals as dorsal penis nerve, respectively, clitoris nerve. The pudendal nerve provides motoric and sensoric fibers. The supralevatory pathway nerves originate both from the hypogastric nerves, predominantly containing preganglionary sympathetic fibers from Th8 to L2, and the pelvic splanchnic nerves. They run, as well as numerous visceroafferent neurons, alongside the rectum, covered by the parietal pelvic fascia. The hypogastric nerve fuses with the parasympathetic splanchnic nerves originating from S2–S4 to the inferior hypogastric plexus. Some parasympathetic fibers innervating the descending colon reach the inferior mesenteric ganglion via the hypogastric nerve. The cavernous nerve originates periprostatically from the inferior hypogastric plexus and pierces the pelvic floor running toward the corpora cavernosa penis. The fibers running via the lateral ligament are at risk during TME and, likewise, at radical prostatectomy [10].

This study was designed to highlight the course of important neuronal structures and to differentiate the individual autonomic nerves and ganglia by means of immunohistochemistry.

Immunohistochemistry aids in distinguishing different nerve types. Studies by Weihe and Eiden [11], Eiden [12], Butler-Martin et al. [13], Wanigasekara et al. [14], Arvidsson et al. [15], and Kaleczyc et al. [16] describe markers for the sympathetic and parasympathetic system. Neurotransmission depends on the regulated exocytotic release of transmitter molecules. This requires the packaging of these substances into the specialized secretory vesicles, a process mediated by specific vesicular transporters. VMAT2 (vesicular monoamine transporter 2) can be found in serotoninergic, noradrenergic, dopaminergic, histaminergic, and adrenergic neurons. TH (tyrosine hydroxylase) is a catecholamin-synthesizing enzyme, which is located in sympathetic neurons. A marker for parasympathetic neurons is the colocalization of VAChT (vesicular acetylcholine transporter and VIP (vasoactive intestinal peptide), a neuropeptide, synthesized in the

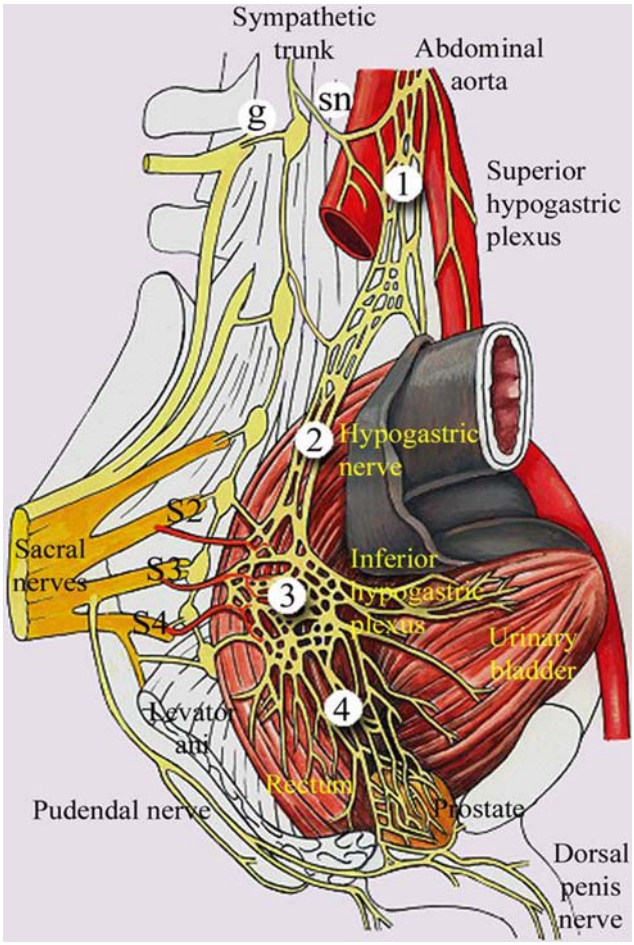

Fig. 1 Synopsis of the autonomic pelvic nerve system. View onto a hemisagitally cut male pelvis with exposure of the lumbosacral plexus. Sympathetic efferences and all afferences in bright yellow; parasympathetic efferences in red. The lower lumbar and pelvic sympathetic chain ganglia receive their fibers by interganglionary branches; white communicating branches do not exist. The gray communicating branches **[g]** to the sacral nerve roots contain efferences for the lower limb vasculature and sweat glands (as well as visceroafferent fibers). The superior hypogastric plexus **[1]** is made up by preganglionary fibers of the lumbar splanchnic nerves **[sn]** originating from the lumbar sympathetic trunk, and the aortic plexus. In addition, numerous visceroafferent fibers course back to the spine, and parasysmpathetic fibers ascend to the aortic and inferior mesenteric plexus. The mostly bilateral hypogastric nerve **[2]** connects the superior hypogastric plexus with the inferior hypogastric plexus **[3]**, into which parasympathetic fibers run from S2–S4. The urogenital neurovascular bundle **[4]** runs to the periprostatic and perivesical plexuses. Parts of this bundle perforate the pelvic floor as cavernous nerve running into the cavernous body. The connections to the sensomotory pudendal nerve are postulated based on clinical findings after nerve lesions. Visceroafferences may run in the pudendal nerve or via the cavernous nerve

perikarya of parasympathetic neurons, e.g., in the gastrointestinal tract.

Methods

In total, 14 human cadavers (7 males; median age, 79 (range, 61–93) years) donated to the Anatomical Institute were examined. Eleven cadavers were dissected within 48 hours after death. To obtain a gross overview, the bony pelves of two embalmed cadavers were cut parasagitally. After careful dissection of the viscera, the course and topography of the pelvic nerves was studied. In nine cadavers, the nerves were dissected from the superior hypogastric plexus up to the neurovascular bundle after midline abdominal incision and the preparation steps of TME. A liposuction device (Normed Medizintechnik GmbH; Tuttlingen, Germany) originally modified for radical, nerve-sparing hysterectomy [17] equipped with a sharp abrasor allowed better dissection of the nerves. All procedures were photo documented with a digital camera (Nikon Coolpix, Nikon, Düsseldorf, Germany), and specimens were collected from all exposed areas.

For anatomical reconstruction of fasciae and immunohistochemistry of nerves, the entire pelves of three cadavers (1 male) were cut transversely into cross-sections of 4-mm thickness. After photo documentation, sections were cubed into blocks of 10 × 20 × 4 mm to facilitate sectioning. The cubes were embedded in paraffin and whole mount tissue sections were performed by transverse sections with 5-μm thickness. Sequential cross-sections of each block were stained with hematoxylin and eosin or polyclonal antibodies directed against neuronal peptides or transporters according to the ABC-method (Table 1). Light microscopy was performed by using a Zeiss axiophot microscope (Zeiss, Jena, Germany), and morphometry was performed by using the software Diskus 4.15 (Technisches Büro Hilgers, Königswinter, Germany). In particular, diameters of nerves, width of mesorectum, and cross-sectional area of autonomic nerve ganglia were assessed. Data were collected in a Microsoft Excel spreadsheet. Statistical analysis was performed with the software package SPSS 10.0.7 (SPSS Inc., Chicago, IL).

Results

Lateral ligament and mesorectum

After finishing the preparation of the mesorectal-covering fascia, the lateral rectal ligament is the only remaining connection between lateral pelvic side and the mesorectum (Fig. 2a). The caudal margin of the rectal stalk has a close topographic relation both to the parasympathetic splanchnic nerves originating from S2–S4 (Fig. 2b) and the urogenital neurovascular bundle. Within the lateral ligament, fine nerve bundles with maximum diameters of 0.3 mm arising from the inferior hypogastric plexus and small calibre vessels and lymph capillaries pass to and from the rectum (Fig. 2c). Transverse sectional series through the ligament display the existence of autonomic ganglia only in the outer third close to the pelvic wall (Fig. 2d).

The nerve fibers are distributed heterogeneously around the mesorectum. Ventrally, around Denonvilliers' fascia, we find very low densities, laterally high densities. The median diameter of the pararectal nerves embedded in the mesorectum is 57 μm (SD 41.9 μm).

Denonvilliers' fascia

The rectal fascia fuses with the prostatic fascia forming a tissue layer between prostate, seminal vesicles, and rectum called Denonvilliers' fascia (Fig. 3a). Light microscopy does not allow for a clear separation into prostatic and rectal fascia. Anterolaterally to the rectum, the fascia recti is dispersed in multiple connective tissue fascicles travelling toward the parietal pelvic fascia (Fig. 3b). A comparable situation is seen in the level of the rectovesical septum (Fig. 3c). Accurate TME splits up the embryologically fused tissue layers, which initially separated the urogenital space from the pararectal space.

Urogenital neurovascular bundle

From its origin in the distal inferior hypogastric plexus, the neurovascular bundle runs caudally of the lateral ligament close to the pelvic floor, covered by the parietal pelvic fascia (Fig. 4a). At the lateral edge of the Denonvilliers' fascia,

Table 1 Primary antisera

Antigen	Code	Dilution	Species	Provider
VAChT	sc-7717	1:100	Goat	Santa Cruz Biotechnology, Santa Cruz, CA
VMAT2	sc-7721	1:100	Goat	Santa Cruz Biotechnology
VIP	sc-7841	1:100	Goat	Santa Cruz Biotechnology
SP	PC481T	1:100	Rabbit	Oncogene Research Products, San Diego, CA
NPY	PC223L	1:10	Rabbit	Oncogene Res. Products
TH	AB151	1:1,000	Rat	Chemicon, Temecula, USA

parietal and visceral fascia split up. The thickness of the neurovascular bundle is in the range of 10–12 mm. It contains numerous ganglia and nerve fibers embedded in loose connective tissue (Fig. 4b and c). The neurovascular bundle pierces the parietal pelvic fascia and a part of the nerves spreads medially within the fascicles of Denonvilliers' fascia toward the prostate and urinary bladder. The remainder passes around the prostate base toward the genitals.

Cavernous nerve

Figure 5a shows the localization of the bilateral cavernous nerve with a diameter of approximately 150 μm in a cross-section; 15 mm anterolaterally to the urethra, it pierces the pelvic floor and innervates the cavernous bodies (Fig. 5b and c). Thus, at least the efferent autonomous fibers do not travel together with the pudendal nerve in the infralevatory compartment.

Distribution of autonomous ganglia

The inferior hypogastric plexus is made up both by efferent preganglionary sympathetic and parasympathetic nerve fibers and afferent neurons. In addition, numerous ganglia cells are scattered in the inferior hypogastric plexus and both in the outer third of the lateral ligament and in the urogenital bundle (4a), where preganglionary neurons relay. Most of the ganglia (88.4%) are combined noradrenergic and cholinergic. Figure 6a-d show an example of a mixed ganglion of the inferior hypogastric plexus. Only exceptionally we found single noradrenergic (4.7%) or cholinergic (7.0%) perikarya. As expected, the cross-sectional areas of the autonomic ganglia decrease in size toward the periphery (data not shown).

Discussion

More than two decades of TME have proven the efficacy and benefits of this radical concept in rectal cancer surgery. Nevertheless, there still seems to be controversy about the anatomical basis, especially with regard to the pelvic fasciae and connective tissue spaces as well as to the course of autonomic nerves, which should be preserved (for review, see Lindsey et al. [18]). This is conceivable insofar as the topography and organization of the pelvic connective tissue

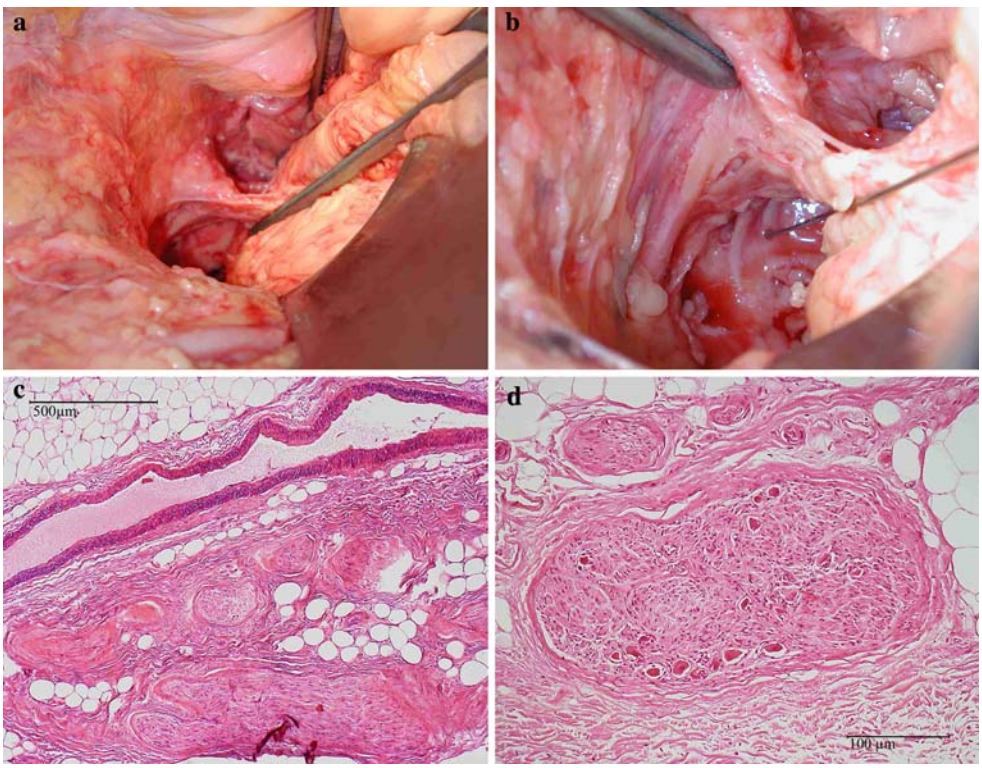

Fig. 2 Gross anatomy and histology of the lateral rectal ligament. After dissection of the mesorectum and careful deflection of the rectum to the right (**a**) the rectal stalk is visible. The upper forceps in (**a**) deflects the left deferent duct. In (**b**), the forceps highlights the insertion of the lateral ligament to the pelvic fascia. The needle points to the left sacral spinal roots on the pelvic floor well below the lateral rectal ligament. Close to the rectal wall (position of the pointer in (**b**)) the lateral ligament contains several transversely and longitudinally cut peripheral nerves (arrows) without ganglion cells (**c**). The nerves are surrounded by loose connective tissue and fat. Close to the pelvic wall (slightly medially of the forceps in (**b**)), some ganglion cells (**o**) are scattered into the nerves within the lateral ligament (**d**)

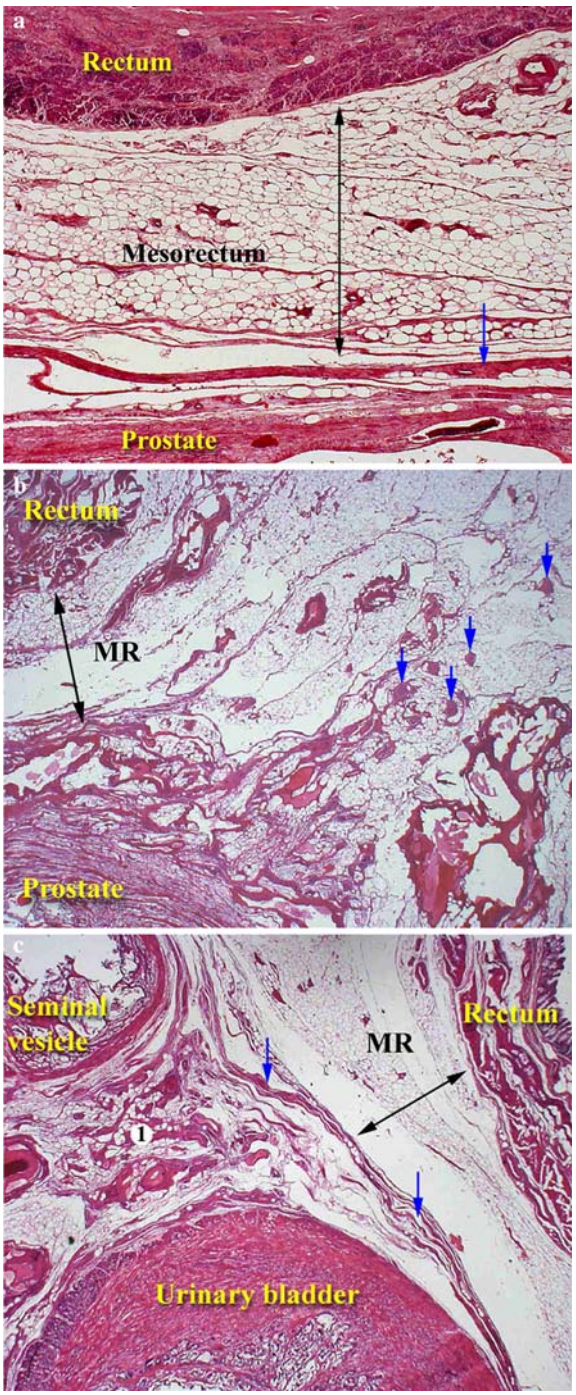

Fig. 3 Histology of Denonvilliers' fascia. (**a**) The mesorectum narrows in the ventral aspects down to 1.5 mm. It consists mainly of fatty tissue and loose connective tissue. No significant nerves are found. Denonvilliers' fascia (blue arrow) consists of a single, thin layer layer of connective tissue, which is in close contact to the prostatic fascia. The fixation induced shrinkage spaces (*) between mesorectum and Denonvilliers' fascia show that the fibers are not interwoven. Toward the lateral aspects (**b**), there is no clear cut demarcation of the fascia but, instead, a split-up of the fibers. In this region several fascicles engulf tiny nerve fibers (arrows). Cranially (**c**) Denonvilliers' fascia continues into the rectovesical septum, which also covers the seminal vesicle

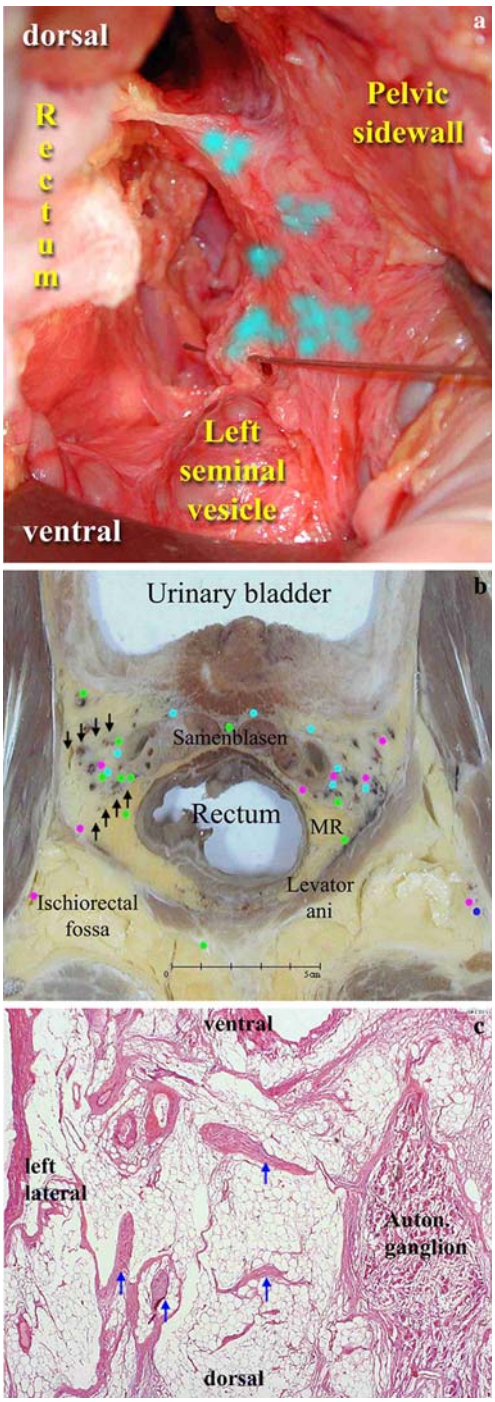

Fig. 4 Urogenital neurovascular bundle. Below the rectal stalk the fibers supplying the sexual and continence organs are bundled in a neurovascular bundle elevated by the pointer in (**a**). The localization and extent of autonomic ganglion cells are sprayed on in cyan. The cross-section in (**b**) depicts the localization of the urogenital neurovascular bundle (arrows) in the level of the seminal vesicles. The sizes of the color-coded individual nerves were determined in subsequent histologic examinations. Green dots represent nerves with diameters <150 μm, violet 150–300 μm, blue >300 μm, whereas cyan dots indicate perikarya of ganglion cells. Histology in (**c**) shows again that nerves (blue arrows) and autonomic ganglia are scattered in the loose connective tissue

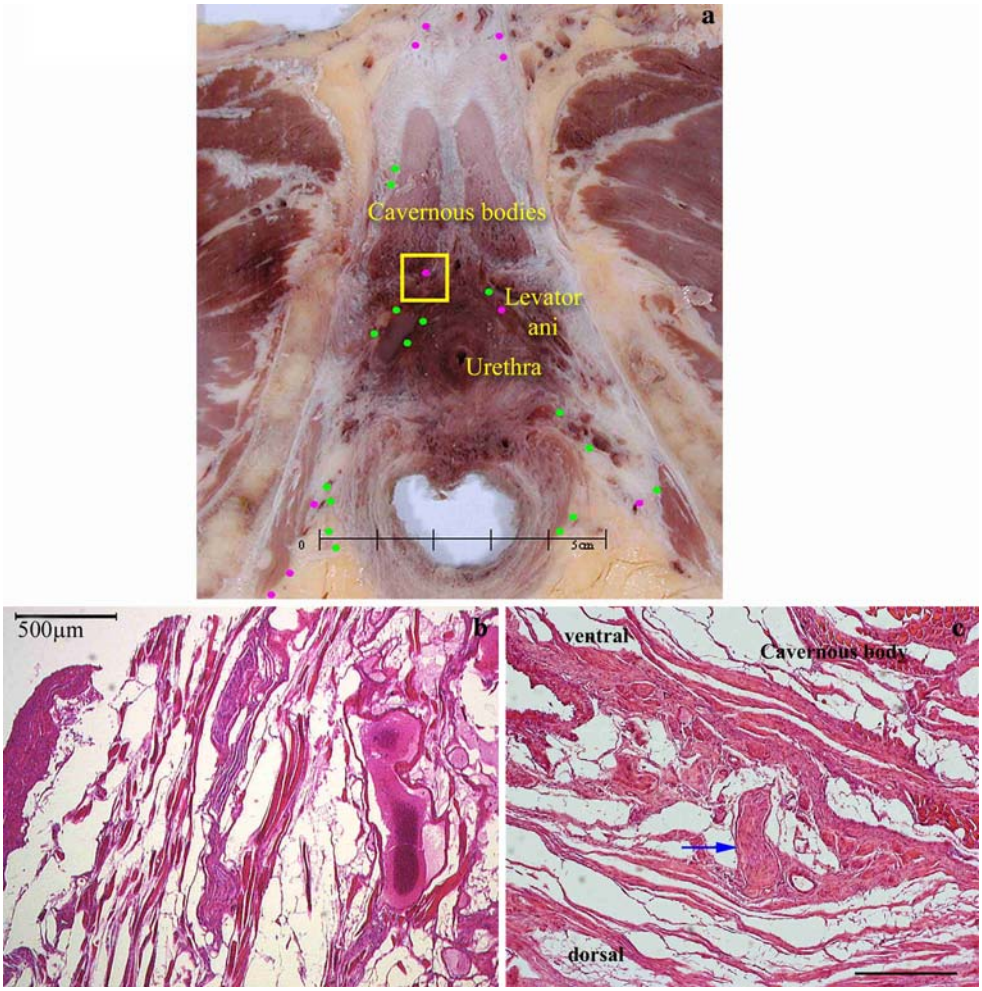

Fig. 5 Cavernous nerve. Cross-sections of the lower pelvic floor (**a**) with perforation of the cavernous nerve branches (color coding like in Fig. 4). Histology of the squared area shows the nerve fibers (blue arrows) crossing through the musculature (red arrows, (**b**)) into the cavernous body (**c**)

belongs to the most complex chapters of functional and surgical anatomy [19]. The individual connective tissue spaces, originating from the urogenital and rectal mesenchymal tissue, are discernable as individual spaces only after careful blunt or sharp dissection. In addition, a part of the pelvic visceral fascias is fused in adults, so that the clear demarcations found during embryogenesis are not preserved [20]. However, successful TME necessitates the identification of landmarks to preserve autonomic splanchnic nerves without impairing radicality.

Damage of the sympathetic superior hypogastric plexus when transecting the inferior mesenteric artery proximally (high-tie) may result in significant ejaculation disturbances. Urinary dysfunctions also may result from lesions of the sympathetic efferent and autonomic afferent fibers.

The rectal stalks, as a lateral continuation of the mesorectum, need to be resected. They originate as lateral rectal ligament from the lateral aspect of mesorectum at the middle third of the rectum at 3 o'clock and 9 o'clock positions. It has a trapezoid shape with a width from 30 to 37 mm at its ends [21]. The primary function of this extension of the mesorectum is to anchor the rectum to the endopelvic fascia and to serve as a pathway for rectal nerves and vessels, e.g., the middle rectal artery (if existent at all [22]). However, they appear as discrete structure only after careful dissection of fat and loose connective tissue in the surrounding spaces. Cross-sectional anatomy of the lower pelvic floor and subsequent histology does definitely not allow for a clear demarcation of the lateral ligaments because there is no compact connective tissue layer that separates the lateral ligament from the surrounding tissue. This might explain for the questioning of the existence as discrete anatomical structure even in recent cadaver studies [23].

TME necessitates the dissection of the lateral ligament, which runs cranially and close to the inferior hypogastric plexus. According to our cadaver study findings, an injury of autonomic urogenital nerves is most likely when not

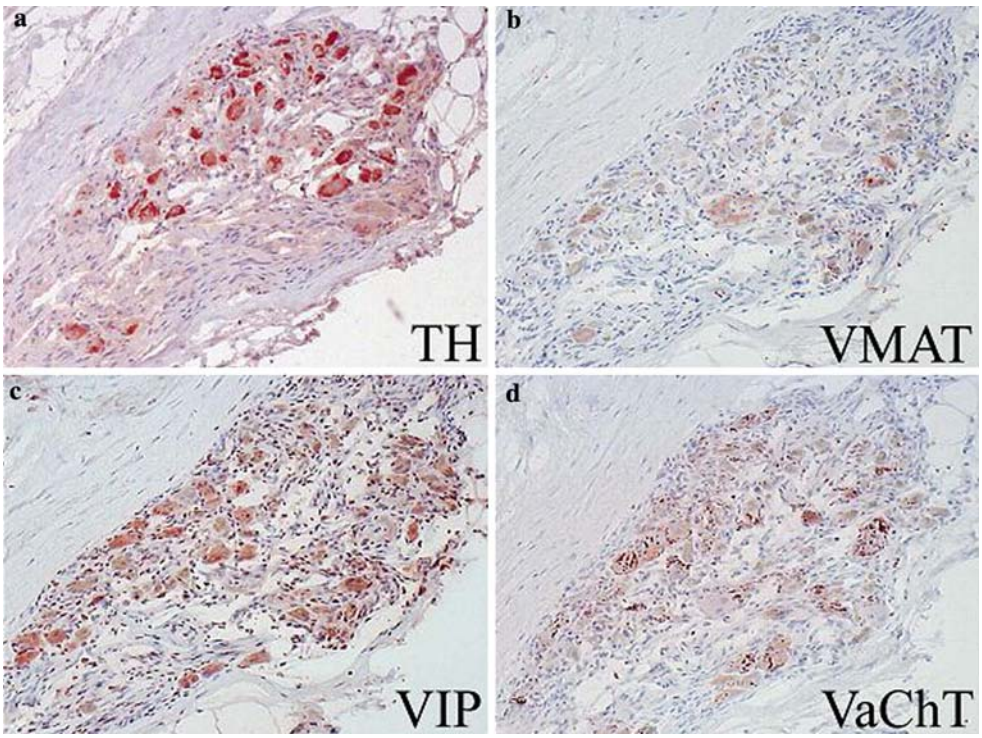

Fig. 6 Immunohistochemistry of the ganglion cells in the inferior hypogastric plexus. Sections (**a**–**d**) derive from the same area and display heterogeneous positive reactions toward tyrosine hydroxylase (TH), vesicular monoamine transporter 2 (VMAT2), vasoactive intestinal polypeptide (VIP), and vesicular acetylcholine transporter (VaChT). From the combination of the markers used, it is evident that the shown ganglion is made up both by sympathetic and parasysmpathetic fibers

respecting the more caudally located urogenital neurovascular bundle or when the ligament is dissected too close to the deflection of the lateral ligament into the parietal pelvic fascia with the underlying inferior hypogastric plexus. It should be pointed out that extensive lateralization or cranialization of the rectum by manual pulling displaces the inferior hypogastric plexus from the pelvic wall with subsequent risk of nerve damage [18]. Because all lymphatics are located within the mesorectum [19] with primary lymph nodes arranged around the superior rectal artery, there is no oncological need to dissect the lateral ligament laterally of the insertion in the parietal pelvic fascia. This anatomical conclusion is further substantiated by the low occurrence of positive lymph nodes in radical pelvic lymphadenectomy [7, 24–26].

The Denonvilliers' fascia is a thin layer of connective tissue, which does not allow for a discrimination of the embryological two layers. However, it is a clearly distinguishable layer, at least when using the operation microscope [27]. It covers the thin anterior mesorectum. Lindsey et al. [18] reviewed the literature and highlighted that there is no evidence for the so-called posterior layer of the Denonvilliers' fascia. They concluded that TME dissects between the rectal fascia, containing the mesorectum, and the true Denonvilliers' fascia. Our own preparations showed that in adults even histologically there is no longer evidence for two layers of Denonvilliers' fascia, which is in line with Kourambas et al. [9] and Lepor et al. [28]. Good news is that even when the prostatic fascia is accidentally hurt dorsally, there is no risk of extensive nerve damage, because all relevant nerves to the urinary bladder run in the urogenital neurovascular bundle passing the prostate caudalaterally.

The nerve- and ganglia cell density of the neurovascular bundle decreases toward the periphery, because the autonomic nerves to the prostate and urinary bladder branch off at the lateral aspects of the prostate. The remaining fibers penetrate the levator ani and the urogenital diaphragm travelling as cavernous nerve towards the cavernous bodies as well as the urethra [10].

Damage of the inferior hypogastric plexus or the neurovascular bundles may lead to severe disturbances of the urogenital and sexual functions with loss of continence, voiding, erection, and ejaculation, because at this level both sympathetic and parasympathetic efferences intermingle. Injury of the cavernous nerve inevitably results in erectile dysfunction.

From an embryological point of view, it is interesting that the pudendal nerve for the sensory innervation of the clitoris/penis and for the motoric innervation of the

perineal muscles takes an infralevatory pathway together with the pudendal artery, whereas the cavernous nerve runs supralevatorily even though both nerves originate from the same spinal segments, S2–S4.

Conclusions

The complexity of the pelvic autonomous innervations implies a variety of functional losses when injured. However, the anatomically defined planes enable a clear demarcation of the superior hypogastric plexus. Surgery should aim for preservation of autonomous fibers in the most critical area—the neurovascular bundles laterally to Denonvilliers' fascia—where it disperses into multiple thin fascicles. The neurovascular bundle has no clearly demarked fascial covering and runs close to the dissection plane.

Therefore, electrocoagulation has to be used sparsely to avoid broiling this fine neuronal tissue. We recommend careful dissection close to the mesorectum to develop a dissection plane behind the nerves, if oncologically reasonable. The use of bipolar scissors and a fine preparation swab or atraumatic devices, such as the Harmonic® Scalpel or the Hydro-jet® dissector, helps to avoid autonomic nerve injury. Bleedings should be stopped by precise bipolar coagulation.

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