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Understanding the surgical pitfalls in total mesorectal excision: investigating the histology of the perirectal fascia and the pelvic autonomic nerves

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Abstract

Aim

Excellent understanding of the arrangement of fasciae and nerves surrounding the rectum is necessary for total mesorectal excision (TME). However, the fasciae anterolateral to the mesorectum and surrounding the low-rectum are still poorly understood. We studied the perirectal fascia along the complete length of the rectum in *en-bloc* cadaveric specimens and the University Medical Center Utrecht (UMCU) pelvic dataset, and describe implications for TME.

Methods

Four donated human adult cadaveric specimens (two males, two females) were obtained through the Leeds GIFT Research Tissue Programme. Paraffin-embedded blocks were produced and serially sectioned at 50 and 250 μm intervals. Whole mount sections were stained with haematoxylin & eosin, Masson's trichrome and Millers' elastin. Additionally, the UMCU pelvic dataset including digitalised cryosections of a female pelvis in three axes was studied.

Results

Multiple fascial layers surrounded the upper rectum. In the 'holy plane' of TME, laminae merged with the mesorectal and parietal fascia. Nerves ran directly laterally to the mesorectal fascia. More caudally, the mesorectal fascia approached the longitudinal layer of the rectal muscularis propria with the neurovascular bundles situated anterolaterally. The mesorectal fascia had a variable appearance in terms of thickness and completeness, which was most prominent anterolaterally.

Conclusion

Optimal TME requires dissection on the mesorectal fascia to preserve the nerves. Rectal surgeons are challenged in doing so as the mesorectal fascia varies in thickness and is often absent in some areas. More caudally, a wider excision may be needed to avoid incomplete specimens.

Introduction

Since the late 1990s, total mesorectal excision (TME) has been the golden standard for the surgical treatment of rectal cancer. Dissection in the 'holy plane' between the visceral and parietal fascia enables complete *en-bloc* removal of the diseased rectum, surrounding mesorectum with an *intact* mesorectal fascia and preservation of the autonomic nerves.^{1,2} It has been shown that suboptimal TME leads to a higher risk of tumour involvement of the circumferential resection margin (CRM) affecting the oncological outcome,^{3,4} and iatrogenic damage of the nerves resulting in an impaired functional outcome.⁵⁻⁷ Therefore, excellent anatomical knowledge of the rectum and surrounding structures is essential to perform an optimal TME.

Rectal cancer surgeons mobilize the posterior, lateral and anterior rectum by dissection in an anatomical space, which is bordered by the visceral and parietal fascia. Although there is agreement that the mesorectum is enveloped by the visceral fascia (also known as the mesorectal fascia or fascia propria recti), concepts of the relationship between the hypogastric nerves and fasciae posterolateral to the rectum differ. Kinugasa et al.⁸ clearly illustrated the various descriptions, of which the presence of an extra leaf of the visceral fascia has gained most support throughout recent years. Some argue that the hypogastric nerves are located anterior to this "posterior layer of the visceral fascia",⁹ some believe that they are captured within this "urogenital fascia"¹⁰ or even within the mesorectal fascia,¹¹ whereas others advocate that they run posterior to this "pre-hypogastric nerve fascia".⁸ To make the confusion greater, some support the idea that specific identification of the autonomic nerves is not essential during TME as dissection on the mesorectal fascia would spare the nerves automatically.¹² This is based on the assumption that the mesorectal fascia is a continuous structure. However, one could question if this is really the case.

Moreover, the anatomy of the fasciae and nerves located towards the anterolateral mesorectum and surrounding the low rectum has not been effectively described when compared to the posterolateral rectum. As incomplete mesorectal excisions are still encountered¹³ and tumour involvement of the CRM is most frequently reported in anterior tumours¹⁴ or advanced low rectal tumours,^{15,16} there is a need to elucidate the anatomy of the fasciae and nerves at these sites. We studied the perirectal

fasciae along the whole length of the mesorectum in whole mount microscopic sections of *en-bloc* cadaveric pelvic exenteration specimens and concentrate on the mesorectal fascia and its relation to the autonomic nerves, specifically at the anterolateral mesorectum and towards the pelvic floor. Additionally, the University Medical Center Utrecht (UMCU) pelvic dataset was studied including digitalized transverse cryosections of the whole female pelvis.

Methods

Adult cadaveric specimens

Four human adult *en-bloc* cadaveric specimens were obtained from consented donors through the University of Leeds GIFT Research Tissue Programme (www.gift.leeds.ac.uk). Ethical approval was granted by the Northern and Yorkshire Regional Ethics Committee, Jarrow, UK (unique reference number 11/H0903/6). The donor bodies belonged to two females aged 64 and 74 years and two males aged 68 and 89 years. The donors did not suffer from any pathology in the pelvis. The specimens were retrieved during tissue donation autopsy undertaken at St. James's University Hospital in Leeds, in the prone jack-knife position according to the technique described by Hölm et al.¹⁷ The specimens were essentially *en-bloc* pelvic exenteration specimens and comprised the anal canal and rectum up to the rectosigmoid junction, mesorectum within an intact mesorectal fascia, all surrounding extraperitoneal connective tissues, obturator internus muscle, levator ani muscle, bladder and vagina or prostate. Specimens were fixed in 10% formalin solution for seven days prior to transverse sectioning at one centimetre. After this, the slices were photographed and dissected to fit in Super Mega Cassettes measuring 74.8 x 52.5 x 16.5 mm (CellPath; Powys; UK). The tissues underwent an extended tissue processing cycle in a Leica ASP200 tissue processor as follows: 1 hour (h) in 70% ethanol, 2 h in 80% ethanol, 2 h in 90% ethanol, 3 h in 95 % ethanol, 12 h in 100% ethanol (repeated three times), 12 h in xylene, 24 h in xylene (repeated twice), 24 h in paraffin. All tissues were embedded in paraffin mega blocks.

In addition, the UMCU pelvic dataset was studied containing digitalized cryosections of a pelvis in coronal, transverse and sagittal axes. The pelvis belonged to a female donor body aged 64 years. The spatial resolution of the images was 3,040 x 1,961 pixels and the cross-sectional interval was 75 μm . The production process of the UMCU pelvic dataset has been described elsewhere¹⁸ and can be explored online at: http://www.caskanatomy.info/research/umcu_pelvic_dataset.html.

Histological staining

Each mega block was sectioned at 5 μm . In one male and one female specimen, every 10th section was collected onto glass slides and stained with haematoxylin and eosin (H&E), creating a series with a cross-sectional interval of 50 μm . Additional sections were collected from each mega block. In the other male and female specimens, every 48th, 49th and 50th section were collected, creating three series for each specimen with a cross-sectional interval of 250 μm , of which one series was stained with H&E and one series with Masson's trichrome (MT). The remaining series was reserved for additional stains. Selected sections from all series were stained with MT and Miller's elastin (ME).¹⁹

Image acquisition

The stained glass slides of the cadaveric specimens were digitally scanned in Leeds using an Aperio XT slide scanner (Aperio, San Diego, California, USA) at 20x magnification, creating a resolution of 0.46 microns per pixel. The digital images were compressed with JPEG2000 quality 70 and viewed in Aperio ImageScope (version 10.2.2.2319).

Results

Adult cadaveric specimens

We refer to the different levels of the rectum according to the descriptions in *Gray's Anatomy*²⁰, in which the upper third of the rectum is described to be located at the level of the 3rd sacral vertebra, the mid third of the rectum at the 4th and 5th sacral vertebra, and the lower third of the rectum at the caudal end of the coccyx. The mesorectum was composed of several lobules between which septae were located. In some specimens these septae were more prominent than in others. Towards the pelvic floor, the mesorectum strongly tapered and reduced in volume.

At high microscopic magnification, the mesorectum was enveloped by multiple fascial layers. The innermost fascia was identified as the mesorectal fascia, whereas the outermost and, most frequently thickest fascia was identified as the parietal fascia. The latter covered the presacral space containing the sympathetic chain and the median sacral artery. The upper third of the rectum was surrounded by mesorectum and multiple fasciae (Figure 1). Between the mesorectal and parietal fascia, the 'holy plane' of TME appeared as a compressed avascular area of multiple collagenous laminae. In anterolateral direction, these laminae alternately merged with the mesorectal and parietal fascia. There appeared to be an additional layer of fascia at specific points, but this could not be traced along the mesorectum in either an anterolateral direction or a parietal direction. Due to this, an extra constant fascial layer between mesorectal and parietal fascia was not detected.

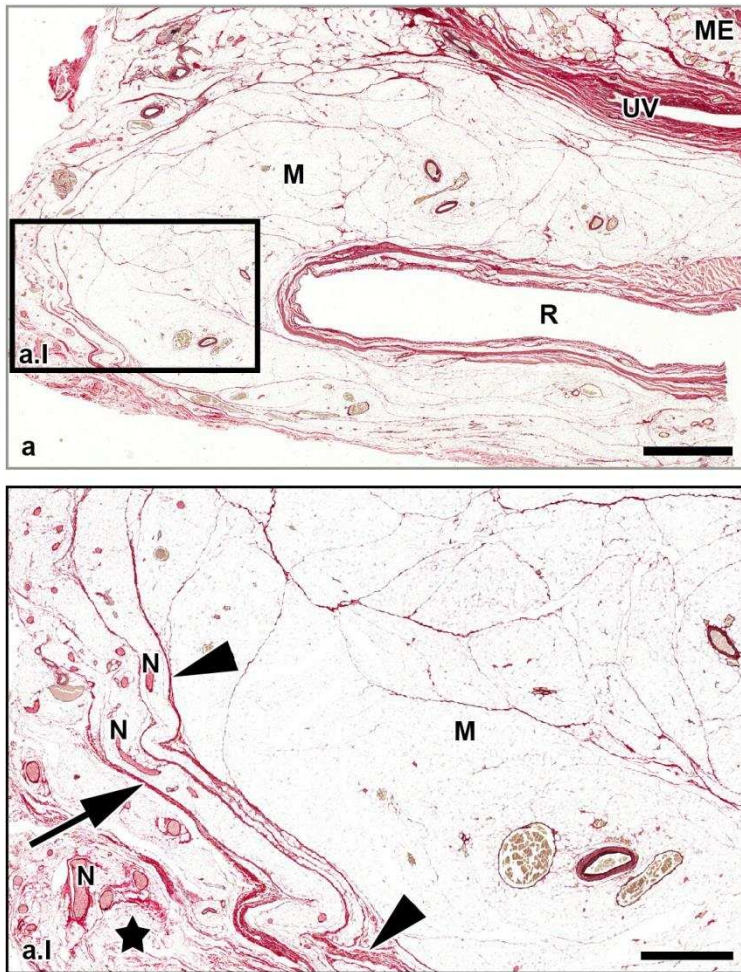


Figure 1

This shows the organization of fasciae posterolateral to the upper rectum (R). The parietal fascia is indicated by the arrow and covers the presacral space (star) in which the pelvic splanchnic nerves are seen. The arrowheads shows the mesorectal fascia, which consists of multiple laminae. Note that the autonomic nerves (N) run both anterior and posterior to these laminae. UV: upper vagina; M: mesorectum; ME: Miller's elastin. Scale bar in window a: 6 mm, window a.l: 2 mm.

The autonomic nerves were located laterally to the mesorectal fascia and ran between the multiple fibrous laminae located posterolaterally to the mesorectum. We did not encounter an augmentation of dense connective tissue as being the lateral ligaments. The lateral ligaments actually represented the entire tract of the inferior hypogastric plexus (IHP) dorsolaterally to the rectum. As the specimens were detached from the bony pelvis, the splanchnic nerves were not examinable in a

consistent manner. Those that were examinable penetrated the parietal fascia and joined the hypogastric nerves to build up the inferior hypogastric plexus (IHP). In all specimens, the IHP was enclosed at the anterolateral mesorectum by a lamina extending from the parietal fascia (Figure 2).

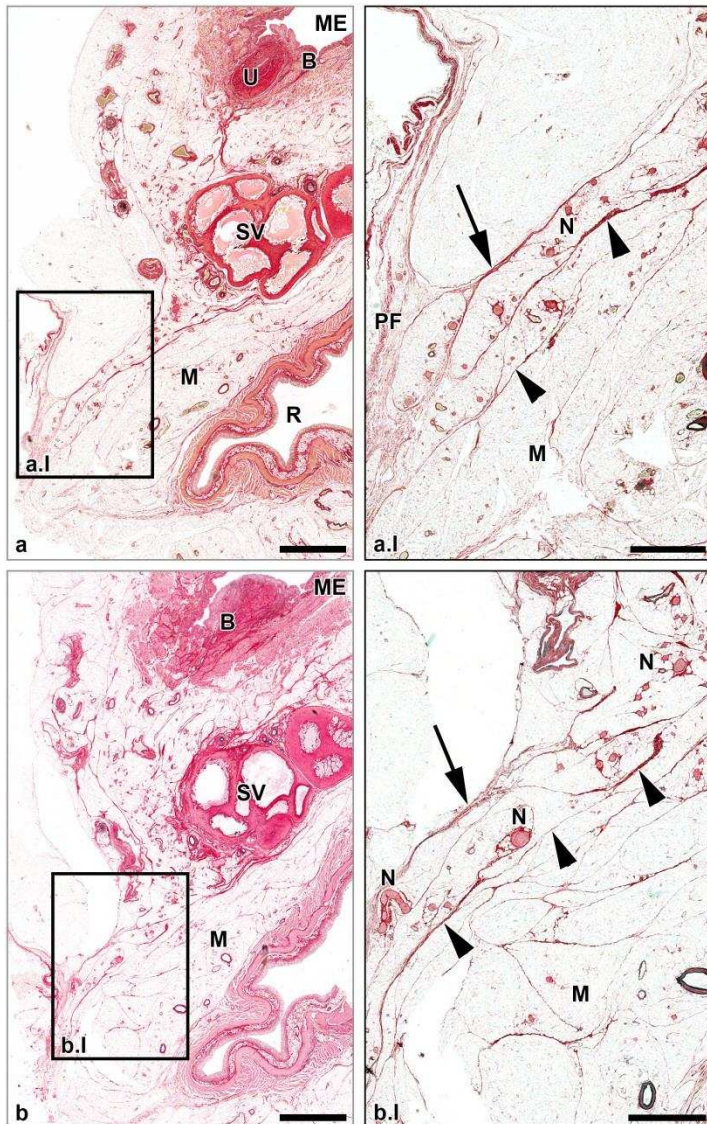


Figure 2

This shows the extra lamina (arrow) that extends from the parietal fascia (PF) to enclose the autonomic nerves (N) at the anterolateral mesorectum (M). Window a and b are successive levels at the insertion of the ureter (U) into the bladder (B), and the seminal vesicles (SV). Note the variable architecture of the mesorectal fascia (arrowheads). R: rectum; ME: Miller's elastin. Scale bar in windows a and b: 6mm, window a.l and b.l: 2 mm.

This lamina proceeded anteriorly, but disappeared in the extraperitoneal adipose tissue between the seminal vesicles or vagina and bladder. More inferiorly, collagenous laminae extending from the parietal fascia attached to the prostatic fascia or formed a continuum with the collagenous vaginal wall. In the middle third of the rectum, inferiorly to the lateral ligaments, the mesorectal fat reduced in volume. The anterior mesorectum was much thinner in comparison with the posterolateral mesorectum and the outer longitudinal layer of the muscularis propria approximated the prostate or vagina at the most caudal level. Here, the mesorectal fascia approached the longitudinal layer of the muscularis propria (Figure 3) and very small non-ganglionated nerves and blood vessels ran on both sites of the mesorectal fascia.

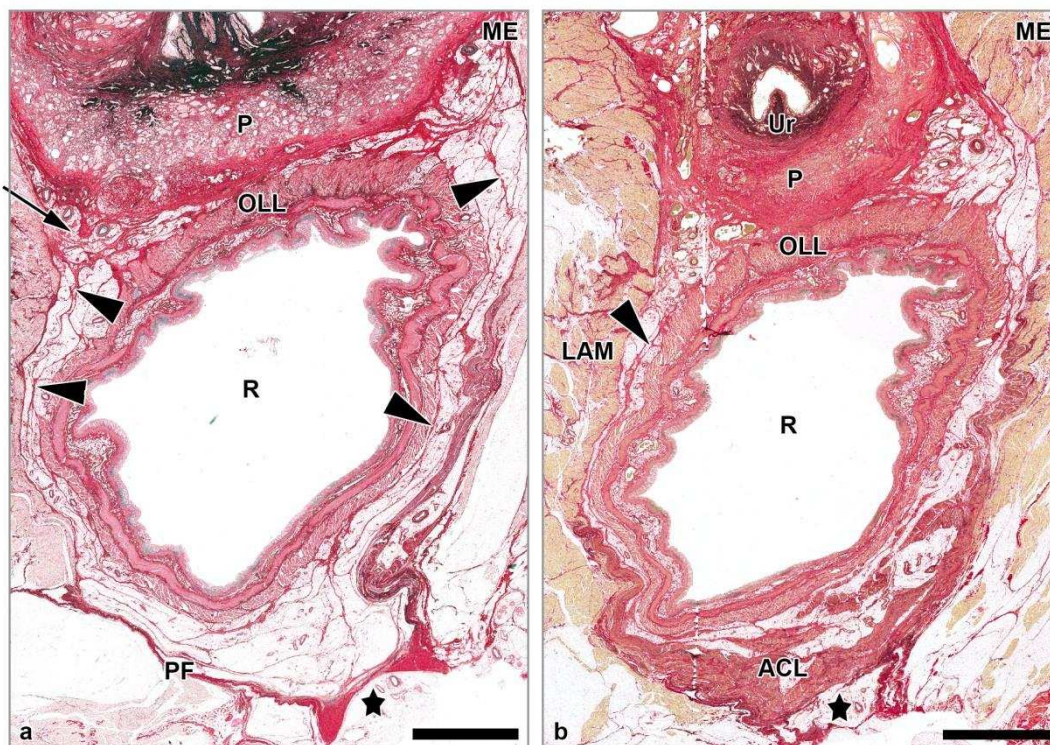


Figure 3

This shows fasciae surrounding the low-rectum (R) at successive inferior levels in a male cadaveric specimen. The mesorectal fascia (arrowheads in window a) approximates the outer longitudinal layer of the muscularis propria (OLL). The neurovascular bundles are located in an angle anterolaterally to the low-rectum (arrow). Note that anterior mesorectum is much thinner and approximates the prostate (P). The star marks the presacral space which is covered by the parietal fascia (PF). More caudally, the anococcygeal ligament (ACL) originates from the PF and the mesorectal fascia continues into the intersphincteric plane (arrowhead in window b). LAM: levator ani muscle, Ur: urethra; ME: Miller's elastin. Scale bars 6 mm.

The parietal fascia was adjacent to the levator ani muscle. Posteriorly, an augmentation of loose connective tissue was visible, which may correspond to the rectosacral fascia (Figure 4).

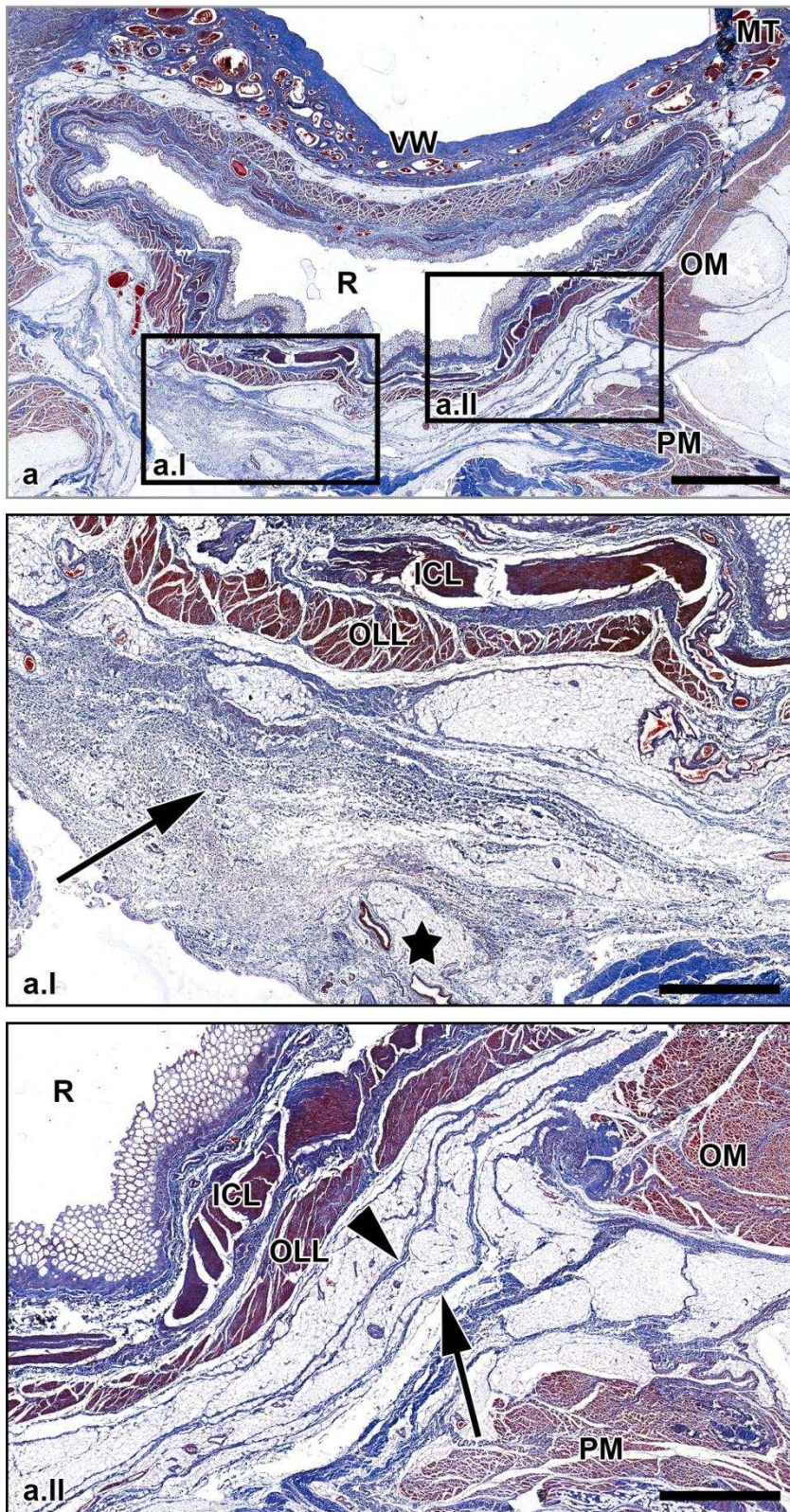


Figure 4

This shows fasciae surrounding the low-rectum (R) in a female cadaveric specimen. Note the amount of densely-packed collagen posteriorly (arrow) in window a.I. The presacral space is marked by the star in this window. The mesorectal fascia (arrowhead) approximates the outer longitudinal layer of the rectal wall (OLL). The parietal fascia (arrow) is closely related to the pelvic side wall and more caudally, to the pelvic floor. VW: posterior vaginal wall; OM: obturator internal muscle; PM: piriformis muscle; ICL: inner circular layer of the rectal wall; MT: Masson's trichrome. Scale bar in windows a: 6mm, window a.I and b.I: 2 mm.

In the lower third of the rectum, only a small strip of mesorectal fat was left beyond the muscularis propria. The neurovascular bundles were situated in an anterolateral angle bounded by mesorectum, the lateral edges of Denonvilliers' fascia and the capsule of the prostate or vaginal wall. In all specimens, the middle rectal artery was identified in this angle. The anococcygeal ligament, which contained a large amount of smooth muscle fibres, originated from the parietal fascia covering the coccyx and proceeded downwards to anchor into the upper limit of the external anal sphincter. At the distal end of the mesorectum, the mesorectal fascia became adjacent to the longitudinal layer of the rectal muscular wall and formed a continuum with the intersphincteric plane.

On a microscopic level, the mesorectal fascia had a variable architecture along the length of the mesorectum. At some sites, it was a thick and well developed fascia, whilst at other sites it was much thinner or even absent. The mesorectal fascia was most prominently variable at the anterolateral mesorectum, where parts of the mesorectal fascia were even lacking in both male and female specimens (Figure 5). Some of the mesorectal septa originated directly from the mesorectal fascia. At the level of the lateral ligaments, small nerve fibres and blood vessels penetrated the mesorectal fascia heading towards the mesorectal fat and rectal muscular wall. The IHP was very closely related to the mesorectal fascia, particularly at the level of the prostate or vagina.

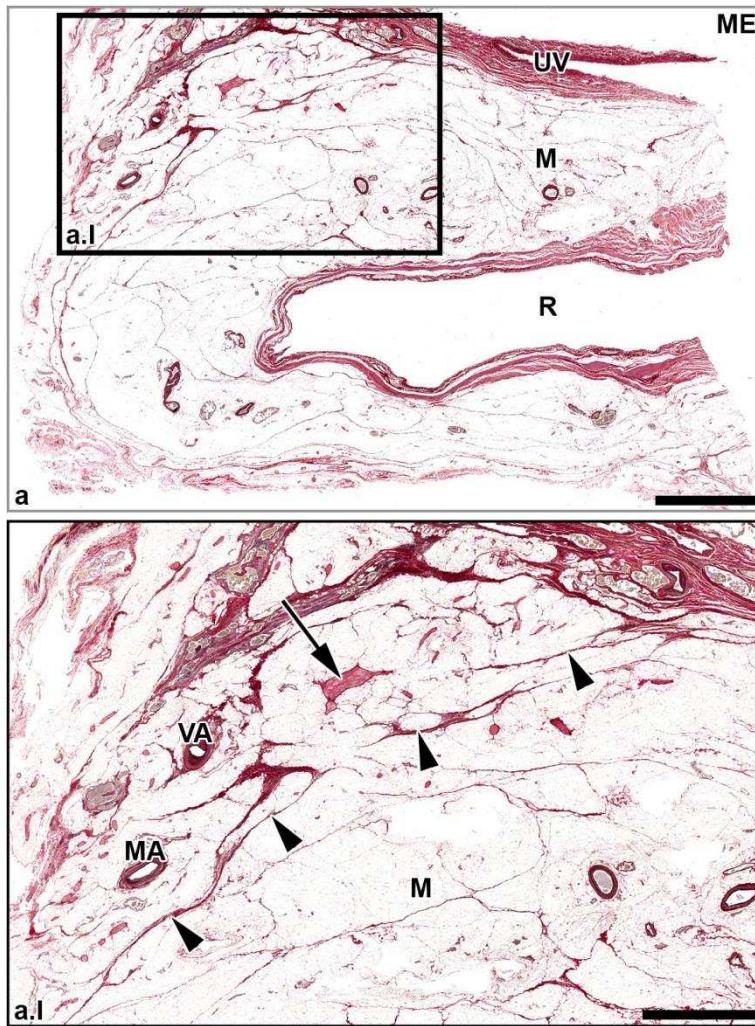


Figure 5

This reveals the variable aspect of the mesorectal fascia (arrowheads) at the anterolateral mesorectum (M) in a female cadaveric specimen. Note that between the second and third arrowhead from left to right, a part of the mesorectal fascia is lacking. The arrow indicates a ganglionated nerve. Between the third and fourth arrowhead, the mesorectal fascia is interrupted and much thinner. UV: upper vagina; R: rectum VA: vaginal artery; MA: middle rectal artery, ME: Miller's elastin. Scale bar in windows a: 6mm, window a.l: 2 mm

The UMCU pelvic dataset

The autonomic nerves were not identifiable in this dataset. We could easily identify the presacral space and mesorectum because the ischiorectal fat, the mesorectal fat and the adipose tissue located in the extraperitoneal compartment had all different colours. The anatomical arrangement of

fasciae surrounding the mesorectum was similar to that in the cadaveric specimens. Multiple laminae enveloped the mesorectum merging and disconnecting with the parietal and mesorectal fascia (Figure 6). The parietal fascia could be easily identified as the thickest fascia covering the presacral space, whilst the mesorectal fascia was much thinner and more difficult to delineate. The lamina that extended from the parietal fascia and enclosed a small zone at the anterolateral mesorectum, similar to the zone containing the IHP in the cadaveric specimens, could also be identified. The mesorectal fascia was more difficult to examine at the mid-rectum and low-rectum, where we could not confirm its course towards the longitudinal layer of the rectal muscular wall. In addition, the architecture of the mesorectal fascia appeared variable, specifically at the anterolateral mesorectum. Finally, blood vessels belonging to the neurovascular bundles were found anterolaterally to the low-rectum.

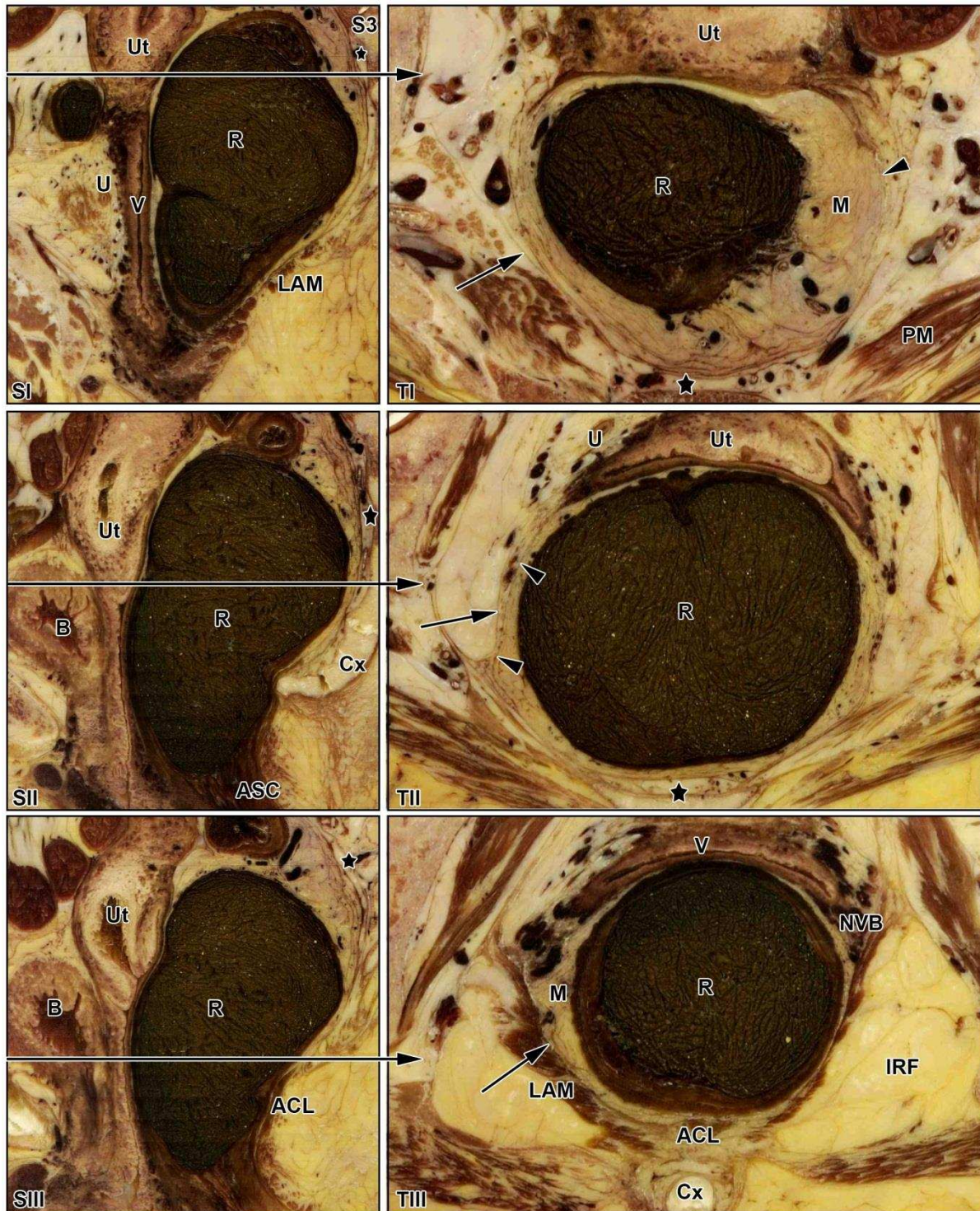


Figure 6

This demonstrates the perirectal fascia at successive inferior levels in the sagittal axis (S) and transversal axis (T). The arrow in windows S1, S2 and S3 correspond to the transverse levels in windows T1, T2 and T3. The presacral space is marked by the star. Note the colour differences in the adipose tissues. Window S1 and T1 show that the upper rectum is enveloped by multiple fasciae. Note that the multi-layered aspect of the perirectal fascia at the upper rectum is more evident in comparison to the middle and lower rectum. In window

TI, the parietal fascia (arrow) can be clearly identified, whereas the mesorectal fascia (arrowhead) is more difficult to delineate. In window TII, the extra laminae that extends from the parietal fascia (arrow) enclosing an anterolateral zone can be seen. The arrowheads in window TII show the mesorectal fascia. More caudally, in window TIII, the mesorectum (M) includes just a small stripe of adipose tissue and the parietal fascia (arrow) is adjacent to the levator ani muscle (LAM). Blood vessels belonging to the neurovascular bundles (NVB) are located anterolaterally to the low-rectum. The origin of the anococcygeal ligament (ACL) can be explored in window SIII. S3: sacral vertebra 3; Ut: uterus; U: ureter; V: vagina; R: rectum; PM: piriformis muscle; B: bladder; Cx: coccyx; ASC: anal sphincter complex; IRF: ischiorectal fossa.

Discussion

Until now, the ongoing discussion surrounding the arrangement of the perirectal fascia and autonomic nerves has hampered defining uniform dissection planes along which the mesorectum should be mobilised during TME. We have microscopically examined the intricate arrangement of the perirectal fascia and autonomic nerves along the complete length of the mesorectum. The most important finding of the present work is that the mesorectal fascia has a variable architecture with regard to its thickness and completeness, most prominently at the anterolateral mesorectum. Besides, in line with previous reports,^{8, 21} we confirm that the upper third of the rectum is enveloped by multiple fascial layers. Extra collagenous laminae are present that merge and disconnect between the mesorectal and parietal fascia, but a constant extra layer of fascia forming a surgical plane could neither be identified in the cadaveric specimens nor in the UMCU pelvic dataset.

The presence of so many different interpretations of the perirectal fasciae in the literature can be easily explained by its multi-layered and variable appearance along the complete length of the rectum. At some points, there is actually an extra fascia⁸⁻¹⁰; autonomic nerves do truly run either anteriorly⁹ or posteriorly⁸ to this fascia; and it seems that the nerves are located within the fascia.¹¹ Many researchers have studied the perirectal fascia through macroscopic dissection.^{10, 22-27} This causes problems as cadaveric or surgical dissections of an area rich in dense connective tissue may easily lead to the creation of artefacts. Radiologic imaging might be useful to study topographical relations, but fail to show enough detail due to limited resolution. Microscopic analysis is a more precise technique to reveal intricate relationships of fasciae and nerves. Ideally, this must

be conducted on large microscopic sections which include the original topographical relationships and thereby enable to follow fascial layers. Caveats in cadaveric analyses are topographical distortions due to fixation and loss of muscle tone.

As this study concerns cadaveric pelvic exenteration specimens and therefore lacks bony landmarks, the parietal fascia cannot be demonstrated unequivocally. However, most microscopic sections included the median sacral artery by which the presacral space and anteriorly situated parietal fascia could be identified. The usage of the UMCU pelvic dataset was of additional value as we could study the perirectal fascia in coronal, transverse and sagittal cryosections of a pelvis including bony landmarks. Scrolling through this dataset allowed us to trace fascial layers and revealed that posterolaterally to the upper third of the rectum no extra constant fascial layer was present which could be used as a potential anatomical or surgical plane. Although a relatively small number of pelvic specimens were studied, the multi-layered variable appearance of the perirectal fascia was detected in all specimens. Hence, the present work tends to reject previous concepts of an extra constant fascial layer along which dissection is said to facilitate complete mesorectal excision and preserve the nerves.⁸⁻¹⁰ The only constant extra layer of fascia is the lamina extending from the parietal fascia enclosing the IHP at the anterolateral mesorectum, which has been reported by Kinugasa et al.⁸ Future anatomical studies involving more specimens may want to focus on relation of gender, age and body mass index to the variable multi-layered appearance of the perirectal fascia.

What does this imply for the rectal surgeon? As stated by Heald,^{1,2} the key factor in TME is to (sharply) dissect on the mesorectal fascia in the “holy plane”. From the mid-rectum downwards, dissection needs to be widened in order to completely remove all resectable tissues surrounding the rectum, avoiding ‘waisted’ specimens. Practically, the excision exceeds the lateral limits of the mesorectal fascia as it thins out and approximates the longitudinal layer of the rectal muscular wall. This is in line with Heald’s descriptions arguing that the “holy plane” continues caudally into the intersphincteric plane.² It is important to preserve the neurovascular bundles at the anterolateral angle. However, (sharp) dissection onto the mesorectal fascia presents surgical challenges.

The mesorectal fascia is not consistently apparent as a laminar structure along the height of the mesorectum. At some points, the mesorectal fascia shows gaps and it varies in thickness. This variability is more pronounced at the anterolateral mesorectum. Bissett et al.²¹ have previously reported a variable thickness in the mesorectal fascia. The surgical plane might easily be lost resulting in either damaging the nerves or ending up dissecting into the lobules of the mesorectal fat. Optimal TME requires excellent understanding of these surgical pitfalls when dissecting in the 'holy plane' along the mesorectal fascia.

In 1950, Hayes published a description of the development of abdominopelvic fasciae.²⁸ He acknowledged two types of fascia: 1) the fusion fascia, which is the result of two fused connective tissue layers that are remnants of peritoneum in which the mesothelium disappears (e.g. the ascending and descending colon and duodenum; 2) the migration fascia, which originates from loose mesenchyme that undergoes morphological changes during growth of abdominopelvic organs. Hayes referred to the perirectal fascia as a migration fascia and stressed that "*...sharp lines of demarcation of this type of fascia are not demonstrable. By the very nature of their source and mode of production, these connective tissue coverings will be continuous everywhere with, and blend into, the general extraperitoneal connective tissue.*"²⁸ Aigner et al. also suggested that fasciae in the pelvis are formed by condensation of loose mesenchyme.²⁹ We fully agree with both descriptions and believe this has played a major role in the various interpretations of fasciae and nerves surrounding the rectum.

Additionally, Hayes' descriptions may also help to understand the ongoing confusion on the anatomy of the rectosacral fascia. Some believe the rectosacral fascia is a pre-existing structure,^{9, 10, 30} whereas others argue it is a surgical artefact.^{8, 21, 26} In our cadaveric specimens, the increased amount of collagen located just cranially to the anococcygeal ligament could correspond to the rectosacral fascia. The nomenclature of the fasciae related to the rectum varies as Waldeyer's fascia may refer to the rectosacral fascia, presacral fascia or all fascia posterior to the rectum.²⁷ Interestingly, Waldeyer first introduced the term "fascia" when he described the pelvic fasciae in general.³¹

In conclusion, the perirectal fascia is an envelope of multiple intermingling fascial layers. The autonomic nerves are located directly lateral to the mesorectal fascia, wherefore proper TME needs to be carried out by (sharp) dissection onto the mesorectal fascia. Rectal surgeons are challenged in doing so as the mesorectal fascia varies in thickness or is even absent at some places. At the low-rectum, excision needs to be widened until the pelvic floor avoiding incomplete “waisted” specimens. Radiologic assessment of tumour involvement of the mesorectal fascia should consider aforementioned notions.

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