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The role of the longitudinal muscle in the anal sphincter complex: implications for low rectal cancer and anal pathology

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Submitted
Abstract

Background
Intersphincteric resection (ISR) enables radical sphincter-preserving surgery in a subset of low rectal tumours impinging on the anal sphincter complex (ASC). Excellent anatomical knowledge is essential to optimal ISR and treatment of low rectal cancer. It remains unclear how the longitudinal muscle (LM) precisely relates to the intersphincteric plane, and other structures within the ASC. This study describes the role of the LM within the ASC and implications for low rectal and anal pathology and surgery.

Methods
Six human adult en-bloc cadaveric specimens (three males, three females) were obtained from the Leeds GIFT Research Tissue Programme. Paraffin embedded mega blocks containing the ASC were produced and serially sectioned at 250 µm intervals. Whole mount microscopic sections were histologically stained and 3D reconstructions created.

Results
The well-developed LM consisted of smooth muscle fibres and occupied the intersphincteric space to a variable extent. Muscular extensions of the LM gave rise to the formation of the submucosae and corrugator ani muscles by penetrating both the internal and external anal sphincters. Striated muscle fibres from the EAS were identified in the anal submucosa in four of six specimens.

Conclusion
The LM plays a dominant role in the ASC. The high degree of intermingling of smooth and striated muscle fibres, their penetration of major structures and importantly the inconsistency of the LM in the intersphincteric space affects the generation of the surgical intersphincteric plane. The complexity of identifying the correct pathological staging of low rectal cancer invading the ASC is also demonstrated.
Introduction

Low rectal cancer is a challenging disease, particularly when the tumour invades the anal sphincter complex (ASC). The surgical treatment of choice for these tumours is an abdominoperineal excision, though patients requiring this type of surgery end up with a permanent colostomy and have a poorer oncological outcome compared to anterior resection.\(^1\) Surgical damage to the anal sphincters, iatrogenic tumour perforation and incomplete tumour resection are frequently encountered and related to increased local recurrence and decreased survival.\(^2\)

For a select group of patients, in whom low rectal tumours impinge on the anal canal or invade the anal submucosa, an intersphincteric resection (ISR) might be a viable alternative to abdominoperineal excision. ISR is performed in combination with total mesorectal excision and involves transanal division of the low rectum, removal of a part of or all of the internal anal sphincter (IAS), and restoration of bowel continuity by means of a hand-sewn or stapled coloanal anastomosis.\(^3,4\) Although the functional outcomes may be suboptimal, long-term oncological outcomes after ISR are satisfactory.\(^5,6\) Locally advanced tumours might be significantly downsized and downstaged by pre-operative chemoradiation, after which ISR could be justified.\(^9\) Given this advancement, ISR has been more widely accepted in specific cases of low rectal cancers. This surgical approach is potentially attractive in terms of achieving a balance between oncological safety and preserving continence, yet there is an important risk of compromising radical resections.\(^5\)

Hence, excellent anatomical knowledge of the ASC is of paramount importance to obtain good results from ISR. Studies reporting on the surgical technical aspects of an ISR pay little attention to the anatomy of the intersphincteric plane.\(^3,10\) Dissection in this anatomical plane, which is situated between the external anal sphincter (EAS) and IAS and includes the longitudinal muscle (ASC), should enable separation of the IAS and EAS. The LM was studied in the 20\(^{th}\) century, but mainly in the context of benign anal pathology and its function in continence.\(^11-13\) The LM has not effectively been described from the perspective of an oncological approach and it remains unclear how the intersphincteric plane should be generated. Contradicting descriptions of the ASC raise the suspicion that inter-individual differences are present. Although previously suggested,\(^14\) a thorough histological analysis of the whole ASC is needed to further examine this.

We studied the ASC in whole mount microscopic sections of en-bloc cadaveric specimens. Two approaches, high resolution digital scanning and low resolution/cost flat-bed digital scanning, were explored to analyse and three-dimensionally reconstruct the components of the ASC. In particular, the anatomy of the LM and the presence of inter-individual differences were studied, and implications for ISR and other low rectal/anal pathology are described.
Methods

Adult cadaveric specimens
Six human adult specimens were obtained through the Leeds GIFT Research Tissue Programme (www.gift.leeds.ac.uk) from consented donors. 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 three males aged 68, 89 and 99 years and three females aged 63, 64 and 74 years. All female donors had a history of childbirth. All donors were free of pelvic pathology at post mortem examination. The specimens were retrieved during tissue donation autopsies performed at St. James’s University Hospital, Leeds in the prone jack-knife position with a similar technique to that described by Hölm et al. The specimens were essentially pelvic exenterations and comprised the whole en-bloc soft-tissues kept in the bony pelvis including the anal canal with ASC and rectum up to the recto-sigmoid junction. All specimens were fixed in 8% formaldehyde solution for seven days prior to transverse sectioning at one centimetre intervals. 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.

Histological staining
The mega blocks were transversally sectioned in serial 5 µm sections. Whole mount microscopic sections were collected in three different ways to determine an optimal intersectional distance to analyse and reconstruct the ASC. In one male and one female specimen, every 10th section was collected and stained with haematoxylin and eosin (H&E). Extra sections were collected and kept for additional stains. In one male and one female specimen, every 48th, 49th and 50th section was collected and stained with H&E and Masson’s trichrome (MT). One series was kept for additional stains. In one male and one female specimen, every 50th section was taken and a series was alternately stained with H&E and MT. In this way, two series (one male specimen and one female specimen) with a cross-sectional interval of 50 µm and four series (two male specimens and two female specimens) with a cross-sectional interval of 250 µm were created. The extra collected sections were stained with MT, Millers’ elastin (ME) and picrosirius red (PR) to demonstrate elastic fibres and collagen.
Image processing

All glass slides created were digitally scanned with an Aperio XT slide scanner at 20x magnification (Aperio, San Diego, California, USA). These were viewed using Aperio ImageScope version 10.2.2.2319. Two different types of datasets were created. Selected scanned H&E sections from the male and female specimen serially stained at 50 µm cross-sectional intervals were resampled and converted to grayscale with the VIPS image processing system\(^\text{17}\) and IrfanView (Irfan Skiljan, Austria). As the original size of the images exceeded the segmentation software’s limits, resampling was required. Rigid-registration was performed in Fiji software.\(^\text{18}\) Secondly, we selected H&E and MT stained sections from one male and one female specimen to scan with a flat-bed Canoscan 9000F Mark II (Canon, UK) at a resolution of 1200 dpi. The cross-sectional interval of these two datasets was 250 µm. The scanned images were uploaded in customised software and registered using a sequential slice-to-slice image based registration approach as described in detail before.\(^\text{19}\) All four datasets were uploaded in Amira software version 5.3.3 (Amira, Hillsboro, Oregon, USA) and structures of interest were manually segmented. 3D volume rendering and interactive visualization in 3D PDF files was achieved using DeVIDE\(^\text{20}\) software.

Results

The upper limit of the ASC appeared at the anorectal junction where the circular and longitudinal layers of the rectal muscularis propria thickened and continued as the IAS and LM, respectively. Three epithelial zones were recognized downwards consisting of simple columnar (rectal) epithelium, stratified columnar (transitional) epithelium and stratified squamous (anal) epithelium. The dentate line marked the transformation of columnar to squamous epithelium and divided the anal canal into an upper 1/3 and lower 2/3’s components. The upper 1/3 contained muscularis mucosae separating the columnar epithelium from the anal submucosa. The lower 2/3’s component lacked muscularis mucosae, where the tissue underlining the squamous epithelium is referred to as subepithelium. Variations in size were not studied as post-mortem shrinkage and formalin fixation will have affected the specimens in an inconsistent manner. 3D reconstructions were created of which interactive PDF files can be explored online at: http://graphics.tudelft.nl/3danalsphinctercomplex.

External anal sphincter

The EAS extended from the puborectalis muscle, but the exact point where the puborectalis blended into the EAS could not be identified. The EAS could be subdivided into two portions: a cranial and a caudal portion. At the urogenital diaphragm, the cranial portion was not continuous anteriorly, but occupied by the perineal body. The caudal portion of the EAS
showed random defects in one male and one female specimen. The most caudal end of the EAS turned strongly inwards reaching the subepithelium just below the lower limit of the IAS. In two male and two female specimens, a bundle of loose striated muscle fibres reached the subepithelium and ascended within it over a short distance. Some loose striated muscle fibres intermingled in a random manner with the LM in the intersphincteric space (figure 1). The relationship of the EAS with the coccyx was assessable in one male specimen, in whom the caudal EAS was connected to the coccyx in the anococcygeal ligament.

Figure 1
This shows the intermingling of striated muscle fibres of the external anal sphincter (EAS) in a male anal sphincter complex (ASC). Striated fibres are found in the subepithelium (Sub.E; arrow in detail window b) and randomly in the intersphincteric space intermingled with the longitudinal muscle (LM; arrows in detail windows c and d). The star in detail window d shows “true” intersphincteric space. MT: Masson’s trichrome, HE: haematoxylin and eosin, A: anterior, P: posterior, AC: anal canal, IAS: internal anal sphincter, B: bulbospongiosus muscle, STP: superficial transversus perineii muscle. Scale bar overview 7 mm; detail 800 µm.
**Longitudinal muscle**

The LM was a well-developed layer of smooth muscle fibres situated between the IAS and EAS. In all specimens, the LM consisted of merely smooth muscle fibres. At the anorectal junction, extensions from the LM created a strong fixation of the anorectum to the puborectalis (figure 2).

![Figure 2](image)

The conjoint longitudinal muscle is formed by the outer layer of the muscularis propria of the rectum and a small smooth muscular layer covering the medial parts of the levator ani muscle (LAM). The arrows in detail window b show the smooth muscular layer of the LAM connected to the longitudinal muscular layer of the rectum (lowest arrow). ME: Miller’s elastin, A: anterior, P: posterior, AC: anal canal, EAS: external anal sphincter, LM: longitudinal muscle, IAS: internal anal sphincter, C: corpus cavernosum, R: rectourethralis muscle. Scale bar overview 6 mm; detail 2 mm.

There was variability in the extent to which the LM filled the intersphincteric space. The LM was not present anteriorly in the male specimen aged 89 years, whilst other posterolateral parts of the LM were missing in the male specimen aged 68 years. As a general observation, the LM gradually occupied less intersphincteric space in a caudal direction as the thick smooth muscle bundles ended in multiple fibro-elastic septa. There was no specific level identifiable where this transition took place. This suggested that at least some variability was present in the length to which smooth muscle bundles of the LM descended in the intersphincteric space before they turned into fibro-elastic septa. Cranially, the LM was strongly related to the IAS and even by microscopy it was hard to define where the smooth muscle belonged to the LM or the IAS (figure 3). At the level of the anal transition zone, LM fibres penetrated...
the IAS and anchored in the subepithelium to form the submucosae ani muscle, surrounding the superior hemorrhoidal plexus. Multiple fibro-elastic septa from the LM pierced also the caudal portion of the EAS forming the corrugator ani muscle that approached the peri-anal skin and ischiorectal fossa (figure 3). The intersphincteric space also contained blood vessels, nerves and adipose tissue. Blood vessels and nerves were not confined to run on either lateral or medial sides of the LM, but crossed the LM and also the IAS. Towards the anal aperture the amount of adipose tissue increased and Pacinian corpuscles were seen revealing the presence of mechanoreceptors.

**Internal anal sphincter**

The IAS was a direct continuation and thickening of the circular layer of the rectal muscularis propria. At the upper part of the ASC, the IAS was interrupted for a short distance at some points. At the lower part of the ASC, the IAS appeared as a continuous circular smooth muscle layer located directly under the submucosae ani and subepithelium. It did not exceed the lower limit of the EAS and fibro-elastic septa from the LM. Along the length of the ASC, the IAS is penetrated by small blood vessels and nerves (figure 4).
Below the dentate line, fibres of the longitudinal muscle (LM) penetrate the internal anal sphincter (IAS) to form the submucosal ani muscle in the subepithelium (arrows in detail window b indicate the LM bundles containing elastin (black)). Fibro-elastic fibres of the LM penetrate also the caudal external anal sphincter (EAS) as is shown by the arrows in detail window c. The star in detail window b marks the squamous epithelium of the anal canal. ME: Miller’s elastin, A: anterior, P: posterior, PB: perineal body. Scale bar overview 8 mm; detail window b 1 mm; detail window c 2 mm.
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Figure 4
This shows that blood vessels pass through the internal anal sphincter (IAS; arrow in detail window b). Note in detail window b the penetration of fibres of the longitudinal muscle (LM) through the IAS and the very close relation between the LM and the IAS. ME: Miller’s elastin, A: anterior, P: posterior, LM: longitudinal muscle. Scale bar overview 8 mm; detail 2 mm.

Discussion

Until now, anatomical descriptions of the LM in the ASC in the context of an oncological approach are lacking. In ISR, excellent understanding of the ASC is needed to warrant oncological safety and obtain good functional results. By using different histological stains in whole mount microscopic sections from cadaveric exenteration specimens, accurate examination of the intricate anatomy of the ASC was possible. The present work reveals the significant, and previously underappreciated, role of the LM in the formation of the ASC and shows the difficulties surgeons may encounter when generating the intersphincteric plane. Dissection in this plane should enable removal of the anal canal and preservation of the EAS. Surgeons generate this plane in the intersphincteric space between the EAS and IAS. The LM is a well-developed layer taking up nearly the complete intersphincteric space. The usage of the term intersphincteric space is actually misleading and should be avoided to reduce anatomical confusion. As the amount of adipose tissue increases towards the anal aperture, surgeons may prefer to start an ISR by perineal dissection.

Two unique features of the LM complicates the generation of the intersphincteric plane. Firstly, the extent to which the LM occupies the intersphincteric space is likely to vary.
et al.\textsuperscript{11} described the LM to be more densely packed at anterior and posterior sides. On the other hand, we encountered incomplete anterior and posterior parts of the LM in two male specimens, whereas the LM was densely packed throughout the complete intersphincteric space in all female specimens. These are important observations as the intersphincteric plane might be irregular and unpredictable. Secondly, the LM traverses major structures throughout the whole ASC. This must be fully understood as surgeons might create planes on the surface of the IAS rather than the LM reducing the clearance achieved close to superficial low-lying tumours.

This work supports the presence of inter-individual differences in the ASC, as has been previously reported\textsuperscript{14, 22}, and stresses the need to examine this across a population. Even though we studied six en-bloc cadaveric specimens, inconstancies were observed in the caudal EAS and the upper part of the IAS. All female specimens had a history of childbirth, which might explain the muscular defects in the caudal EAS. The defects in the male specimen could be iatrogenic or explained by atrophy. To rule out the effect of age-related degeneration on variability, younger specimens should be studied, including nulliparous female specimens. However, our specimens represented a realistic age group that is encountered daily by rectal surgeons. Age- and gender-related variations of the LM and EAS were described before by endoanal magnetic resonance imaging,\textsuperscript{14} but not confirmed microscopically. The significance of small interruptions of the IAS will be subsequently reported. Also, striated muscle fibres belonging to the EAS were found in the subepithelium in four of six specimens.

This study demonstrates the complexity of identifying the correct pathological staging of low rectal tumours involving the ASC. Primary tumour staging in colorectal cancer is based on penetration through specific anatomical structures, whereas in anal cancer this is based on size. We have shown the intermingling of striated and smooth muscle fibres as they traverse structures. Fibres from the EAS curl around the distal IAS to rest in the subepithelium. Thus, muscle fibre type, i.e. smooth or striated, cannot be used alone without some understanding of the extent of tumour spread that has been required to reach them. What is most appropriate for this complex site? Small blood vessels and nerves cross the IAS suggesting that lymphatics do the same. Any new TNM classification system for low rectal tumours impinging on or invading the ASC should consider these factors when determining its evolution. Further work is needed to understand the lymphatic pathways and how tumour involvement of the different layers of the wall impacts on patient outcome.

Modern anatomical teaching of the ASC is mainly based on the studies of Milligan and Morgan,\textsuperscript{13, 23, 24} who described the ASC from the perspective of anal fistula surgery.\textsuperscript{13, 23, 24} They reported on a trilaminar arrangement of the EAS encompassing a deep, superficial and subcutaneous part.\textsuperscript{25-27} Our results confirm the findings of Fritsch et al.\textsuperscript{27} with the EAS consisting of a caudal part traversed by the LM and a cranial part that is deficient anteriorly. Contrasting earlier reports,\textsuperscript{11, 28} the LM in our current specimens consisted of merely smooth muscle fibres rather
than a mixture of smooth and striated muscle fibres. Kim et al. obtained similar results in Japanese specimens. Previous descriptions of the LM might be caused by misinterpretations of the traversing fibres. Besides, most studies analysed specific parts of the ASC in isolation without integrating the entire ASC.

Additionally, the present work helps to better understand the role of the LM in benign anal pathology. By integrating the EAS and IAS, the LM minimizes deterioration of sphincter function after surgical division and prevents hemorrhoidal and rectal prolapses. The LM divides adjacent tissues into subspaces, which may cause septation of thrombosed external hemorrhoids. Also, LM fibres form potential routes for fistulae extension. Inter-, trans-, supra- and extrasphincteric fistulae can be identified based on their location and relation to the muscles in the ASC. The submucosae ani muscle might reinforce the anal submucosa and support the superior hemorrhoidal plexus. Failure of this suspensory mechanism and anatomical weakness caused by fibres from the LM crossing the planes may be the key to understanding such pathology.

Two approaches were explored to three-dimensionally reconstruct the ASC. Despite the fact that flat-bed scanning saved time and cost in comparison with high resolution digital scanning, accurate reconstruction of the ASC was not feasible. The complexly integrated and intermingled muscular layers of the ASC required reconstruction on high resolution digital images in order to develop solid 3D models.

In conclusion, the LM plays a dominant role in the complexly integrated ASC and presents surgical challenges when generating the intersphincteric plane in ISR. The high degree of complexity is reflected by the intermingling of smooth and striated muscle fibres and their penetration of major structures in the ASC plus the presence of inter-individual differences opening up questions about routes of spread. Future studies should focus on revealing the lymphatic, neural and vascular pathways of the ASC in order to understand the spread of low rectal and anal cancer and further specimens need to be studied to better understand variability across populations induced by age, sex, obstetric trauma and preoperative treatment.
The role of the longitudinal muscle in the anal sphincter complex

Reference List